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14. ABSTRACT
The purpose of this research is to develop effective hemostatic devices for two difficult types of hemorrhage: (1) traumatic noncompressible (also known as truncal) hemorrhage; and (2) traumatic hemorrhage in the cold coagulopathic subject. The scope of this research will include both military and civilian trauma victims, particularly those suffering from hemorrhagic truncal injury and/or coagulopathic hemorrhage. During the fourth year (Y4) of this project, we continued to generate stocks of two clotting factors (fibrinogen and Factor XIII) needed for the development and manufacture of our hemostatic devices. In addition, we improved our prototypes to deliver foaming technology for the treatment of noncompressible hemorrhage. We performed efficacy testing of the dual (fibrin sealant-alginate) foam technology in our noncompressible model, and we currently are making modifications to both our model system and our foam technology to increase the resultant device utility. We devised an ex vivo adhesion assay to help progress in developing treatments for cold coagulopathic hemorrhage. In addition, we obtained a one year no-cost extension on this award, through 25 Sep 2016. In Y5 we will continue to develop our foam technology for noncompressible hemorrhage, and continue our work on treatments for cold coagulopathic hemorrhage, using both ex vivo tensiometer studies and in vivo procedures with our cold coagulopathic porcine hemorrhage model.

15. SUBJECT TERMS
Trauma, hemorrhage, hemostasis, exsanguination, coagulopathy, hemodilution, liver injury, biologics, recombinant, clotting factors, fibrinogen, biomaterial, bandage, wound, noncompressible, incompressible

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1. INTRODUCTION

The subject of this research project is the treatment of hemorrhagic in two difficult clinical scenarios: (1) traumatic noncompressible (also known as truncal) hemorrhage; and (2) traumatic hemorrhage in the cold coagulopathic subject. The purpose of this research is to advance the technology of hemostasis, and to use preclinical large animal models to test hemostatic technologies on the two difficult types of hemorrhage named above. The scope of this research will include both military and civilian trauma victims, particularly those suffering from hemorrhagic truncal injury and/or coagulopathic hemorrhage, from both penetrating and blunt mechanism. In addition, the technologies under development in this research should be useful in nontrauma surgical procedures, both elective and emergency, in which solid organ, coagulopathic, and/or noncompressible hemorrhage might occur.

3. BODY

Administrative Notes. A one year no-cost extension was granted by the USAMRAA on 18 June 2015 (see Appendix C); the period of performance for this award now will run through 25 September 2016.

Note: The description of the work performed during the past year will be organized according to the Tasks delineated in the project's Statement of Work (SoW), which has been reproduced in the Appendix A. For a list of abbreviations used in this report, see Appendix B.

Task 1: Purification/generation of pd-FI and rFXIIIA2-a.

UNL continues to produce and maintain pre-clinical grade, plasma-derived, fibrin sealant components for study. Ayman Ismail, Weijei Xu and Frank Fabian continued to perform the purification process of fibrinogen using ethanol fractionation to maintain a stock of >100 g of plasma derived fibrinogen for large animal studies at UNMC. Zurima Zaldua is characterizing a substitute recombinant thrombin for future use. UNL continues to maintain >2.5 g of pre-clinical grade recombinant Factor XIII (FXIIIIA2-a) for the study as planned. The activity of FXIIIIA2-a is limited to 3 months under current buffered, non-frozen storage conditions at 4^{o} C. Optimization of liquid fibrin sealant (LFS) and hemostatic recombinant thrombin/FXIII solutions as hemostatic coatings for alginate surgical hemostatic foam use remains ongoing.

Task 2: Generation of ultrafine particles for tamponade carrier foam.

See task 6; the focus of foam formulation has been on the use of alginate derivatives.

Task 3: Testing of candidate tamponade carrier & FS foams.

UNL continues to provide liquid fibrin sealant (LFS) and hemostatic recombinant

thrombin/FXIII solutions as hemostatic coatings for alginate surgical hemostatic foam.

UNL provides TEG analysis of citrated whole blood samples that are taken before the procedure is initiated and at termination of the surgery. The TEG data shows that the administration of biologics (fibrin sealant or recombinant thrombin/Factor XIII via bandage or foam) reduces the time rate (loss) of clotting potential over the course of the surgical procedure relative to control groups. This analysis also indicates that a more profound effect might be observable if the natural hypercoaguable state for normal pig physiology is reduced prior to initiation of the surgical injury. This can be done by controlled hemodilution/blood loss that is calibrated by TEG monitoring of blood samples during the hemodilution phase of the surgical procedure. A lower coagulation level that is similar to humans would be targeted prior to surgical injury. A TEG instrument from Dr. Velander's laboratory would be installed by Dr. Velander's lab personnel in the surgical operating theater at UNMC.

Task 4: Testing of single foams in swine (tamponade carrier & FS foams separately).

Single foam formulations from calcium alginate developed in Y3 underwent further refinement in Y4, as described below.

Alginate foam formulation experiment

Previously a foam formulation with the following composition was used in swine surgeries: 3.8 % sodium alginate (Protanal LF200 FTS pharmaceutical grade produced by FMC Biopolymers - Philadelphia, PA) and 0.6% Tween 20 in DI water. Expanding foam was produced by propelling the formulation out of a canister equipped with a narrow nozzle and pressurized by butane at room temperature. Simultaneously, the exiting expanding foam was mixed with 1.14 M calcium chloride solution in DI water at a flow rate of 84 mL/min to produce rigid foam. This formulation produced a flexible and cohesive foam that performed well in swine surgeries. The foaming process requires the presence of a surfactant in the formulation. The surfactant Tween 20 (polysorbate 20), a non-toxic and stable surfactant commonly used in food products made an excellent choice for this purpose.

Tween 20 is member of a family of surfactants known as polysorbates with three other commonly known members polysorbate 40, polysorbate 60, and polysorbate 80 (Tween 40, 60, and 80 respectively). These surfactants are produced from PEGylated sorbitan (a derivative of sorbital) esterified with fatty acids (monolaurate in tween 20, monopalmitate in tween 40, monostearate in tween 60, and monooleate in tween 80). It was surmised that choice of surfactant used in the foaming formulation can affect the physical characteristics of the resulting foam.

Experimental:

A 6% solution of Tween 20, 40, 60, and 80 in DI water was prepared. These Polysorbates have a nominal density of 1.05 to 1.1 g/mL. Tween 20, 40 and 80 have relatively low viscosity similar to the consistency of thin pancake syrup however Tween 60 is much more viscous with the consistency of crystallized honey. Consequently the solutions produced differ in transparency and viscosity (see Figure 1).

Four compositions of the foaming formulation were prepared only differing in the type

polysorbate surfactant used based on the recipe mentioned above. 200 ml of each formulation was placed in a 500 mL canister and was pressurized with 50 g of butane. The gas and the formulation in each canister was mixed thoroughly and allowed to come to room temperature. Each canister was equipped with a nozzle and discharged while mixing the exiting expanding foam with a stream of calcium chloride solution (1.14 M calcium chloride in DI water at a flow rate of 84 mL/min). The resulting foam was collected in 1 L beakers.

Observation:

The formulation containing Tween 20 was discharged followed by one containing Tween 40, 60, and 80. It was observed that the final volume of foam produced became smaller when comparing the resulting foams in this order. Also the resulting foams became more noodle-like in consistency in the same order. Both smaller volume and more noodle-like nature of the foam could be attributed to increase in viscosity of the foaming formulation due to change of surfactant in the composition. This result became even more evident when the resulting foams were mechanically compressed and showed more firmness in the same order. Overall the formulation consist of Tween 20 produced a foam with the best cohesion and uniformity compared to the other formulations (see Figure 2). These single foam formulations from calcium alginate were combined with fibrin sealant foams, and testing of these formulations being performed during Y4; see description under task 6.

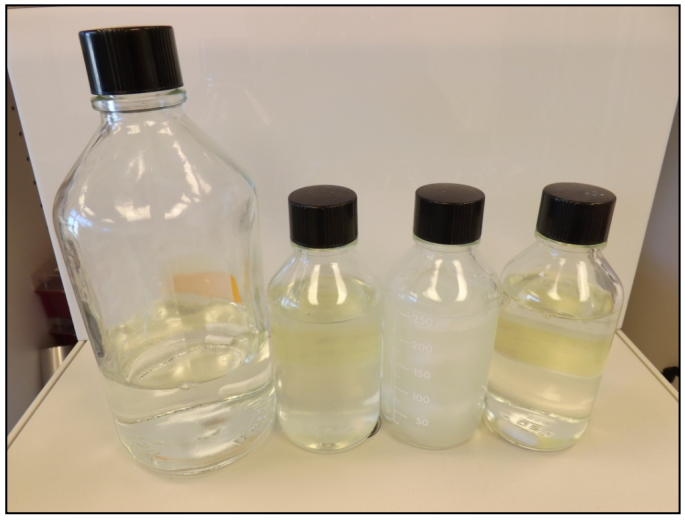

Figure 1. From left to right: 6% solution of polysorbate 20, 40, 60, and 80 in DI water.

Figure 2. From left to right: resulting foam from discharging formulations with Tween 20, 40, 60, and 80.

Task 5: Development & engineering of dual foam candidate devices.

The current applicator nozzle developed in Y2 continues to successfully deliver optimized LFS circumferentially coated alginate foam both *in vitro* and *in vivo*.

Task 6: Testing of dual foam in swine.

A modification of the noncompressible model was developed in order to better discriminate quantitative differences in treatment efficacy. This modification consisted of a lobectomy of the left medial lobe, producing a wound interface which would be oriented anteriorly (i.e., toward the injected foam). Development and use of this modified model was approved by ACURO.

Further testing of dual foam configurations in the swine model of noncompressible hemorrhage was performed in Y4 (data files in Appendix D). We have tested iterations of the dual foam on 180 min subject survival and other endpoints; preliminary results are shown in Table 1. Four experimental combinations were tested: (1) calcium alginate foam (CAF) + fibrin sealant (FS = plasma-derived fibrinogen + recombinant thrombin (rFII) and Factor XIII (rFXIII)); (2) CAF + rFII + rFXIII; (3) CAF alone; or (4) no treatment. The engineered foam injector nozzle produced a dual-phase foam, with CAF constituting the inner bulk, coated with a thin-layer of biologic-based foam. Warm Lactated Ringer's solution was given IV post-injury at 10 mL/min for mean arterial pressure (MAP) <80% of pre-injury MAP. Subjects were monitored (vitals signs and labs) for 3 h or death, followed by necropsy with blood loss measurement.

Table 1. Endpoint assays at the final time point (3 h or death).

Endpoint	CAF + FS	CAF + FII/FXIII	CAF only	No Rx	*p*-value*
Survival (*n*/total)	7/7	6/7	1/4	2/4	**0.031**
SI (MAP/HR)	3.4 ± 1.2	2.2 ± 2.0	7.6 ± 4.3	4.8 ± 3.8	**0.035**
MAP (mmHg)	43 ± 20	59 ± 22	20 ± 19	35 ± 27	0.069
Hb (g/dL)	8.4 ± 2.8	7.8 ± 2.3	7.7 ± 0.7	7.4 ± 2.7	0.920
Base excess (mEq/L)	-6.6 ± 7.3	-1.4 ± 4.6	-3.2 ± 3.6	-9.0 ± 6.8	0.290
EtCO2 (mm Hg)	31 ± 14	30 ± 10	14 ± 14	20 ± 12	0.096
Blood Loss (mL)	1,553 ± 505	1,193 ± 374	1,487 ± 189	1,382 ± 622	0.500

Data are mean ± sd. *ANOVA or Kruskal Wallis for continuous data, chi-square for categorical.

The above preliminary data was submitted and accepted for presentation at the 2016 meeting of the Central Surgical Association (see Appendix J). This preliminary analysis of injectable foam treatments for noncompressible intraabdominal hemorrhage suggested that injured subjects treated with CAF + biologics had improved survival and SI endpoints. These differences were not associated with differences in hematologic, coagulation, blood gas, or other endpoints. These studies are ongoing; we plan on generating an N of 12 in each treatment group for the purpose of further presentation at national meetings and publication, followed by further possible efficacy testing and toxicity testing, with the goal of releasing a prototype for FDA evaluation.

With regards to continuation funding of this work, a Quad Chart and White Paper on the foam technology were submitted to the Air Force Surgeon General during Y4Q3 (see Appendix K). This is in addition to the USAMRMC BAA 15-1 preproposal that was submitted during Y4Q2 (see Appendix M); per a 15 September 2015 letter from the USAMRAA (see Appendix T), this preproposal still is under consideration. Furthermore, a Preproposal and Quad Chart on the foam and bandage technology was submitted to the USSOCOM BAA 15-1 (Appendix L), but this did not receive an invitation for full submission. Supported by the acquisition of our one-year no-cost extension (Appendix C), we will vigorously pursue continuation funding during Y5.

The foam and bandage technology were reviewed and discussed in a May 2015 meeting at the ISR (San Antonio) between the project investigators and the overseeing scientific officers; see Appendices N & O for the slides that were presented at this meeting.

Incidentally, off-protocol work on resuscitation regimens for severe hemorrhagic shock was presented during Y4 at the 2015 meeting of the Central Surgical Association (see Appendix I) and the 2015 Military Health Systems Research Symposium (see Appendices E & F); a related manuscript is under review at *J Trauma Acute Care Surg* (see Appendix Q). In addition, a manuscript is being prepared that describes other off-protocol work on a PCL-bandage prototype (without biologics) for use in the treatment of minor hemorrhage (i.e., for clinical situations that a material like Surgicel® might be used); the target journal of this unfinished manuscript (see Appendix R) will be *PLOS ONE*. Work on this biologic-free PCL bandage was presented at the 2015 Academic Surgical Congress (see Appendix G). Furthermore, extensive off-protocol work in hemostasis (funded by the State of Nebraska Department of Economic Development) during Y4 focused on the development of a nanoengineered PCL bandage combined with biologics for the management of hemorrhage during elective or emergency surgical procedures (e.g., solid organ surgery); see Appendix S for the data files associated with this work.

Task 7: Engineering of firm foams from alginate and alginate derivatives.

Refer to Task 6. Testing of firm foams with and without biologic additives resumed in Y4, with updated results described under task 6. Of note, UNL and LNK continue to share responsibility for the TEG analysis of pig subject blood collected during surgery. These TEG samples are used to analyze the coagulation properties of the pig subject during the surgery.

Task 8: Testing of dual foam in swine noncompressible model (laparotomy with 2˚ closure).

See discussion under Task 6.

Task 9: Delivery of candidate field-ready dual foam device.

This task has yet to start.

Task 10: Testing of dual foam in swine noncompressible model (closed penetrating wound).

The subcontract with TDMI (the actual facility where the Task 10 experiments were to be performed by the UMM investigators) was canceled late in Y3 (described in the Y3 Annual Report). As previously approved by the USAMRAA, the work of Task 10 has been transferred to UNMC, and will be incorporated into tasks 6, 8, and 9.

Task 11: Delivery of report on final recommended product description for dual foam device for treatment of noncompressible hemorrhage.

This task has yet to start.

Task 12: Delivery of resorbable bandage for final preclinical study in hypothermic coagulopathic model.

As described in the Y2 Annual Report, difficulties with bandage adhesion to the wound interface within the hypothermic coagulopathic subject were encountered, and so animal experimentation in this Task was suspended. The strategy was to perform extensive in vitro work to optimize the adhesion phenomena between the synthetic bandage/fibrin sealant device and the surface of liver, in the absence of endogenous clotting factors. Good quantitative experimentation for this purpose would require a tensiometer, with sensitivity down to 0.1 gram-force. Toward this end, in Y3 the project PI applied for and obtained DURIP funding to purchase a $50K Instron tensiometer. This instrument was delivered to the PI's lab at the end of Y4Q1.

Using the new Instron tensiometer, we performed testing of adhesion phenomena at the wound-bandage interface in the absence of endogenous clotting factors during Y4. The basic adhesion assay consists of a peel test, in which a segment of synthetic bandage fabric that has been

glued to a liver strip with fibrin sealant is peeled off by the tensiometer (see Appendix U for explanatory images). During Y4 the parameters of the assay, including conditions such as temperature at which the sealant undergoes reaction, compression time of the bandage and liver, size of the liver strip, and liver surface (capsule vs. parenchyma), were explored and optimized. Preliminary data on the intrinsic strength of liver strips from the pig are shown in Table 2. Of note, these tensile strengths are more than enough needed to perform the peel testing (i.e., the liver will not tear apart prior to the disruption of the glued bandage to the liver surface).

Table 2. Tensile strength of *ex vivo* porcine liver strips.

Liver Region	Orientation	Sample (N)	Mean Peak Stress (N/mm^2)	SD Peak Stress (N/mm^2)
Left lateral lobe	X-axis	20	0.1117	0.0536
Left lateral lobe	Y-axis	20	0.1383	0.0548
Left medial lobe	X-axis	21	0.0791	0.0355
Left medial lobe	Y-axis	20	0.1057	0.0606
Right lateral lobe	X-axis	20	0.0725	0.0313
Right lateral lobe	Y-axis	20	0.0698	0.0301
Right medial lobe	X-axis	20	0.0795	0.0193
Right medial lobe	Y-axis	20	0.0902	0.0378

Strips were 4 cm (length) x 1 cm (width) x 0.5 cm (thickness) Orientation refers to transverse plane (x-axis = length oriented right-left; y-axis oriented anterior-posterior). SD = standard deviation.

Using our optimized conditions, we tested our customized fibrin glue (FG; 0.2 mL; 9 mg/mL plasma-derived fibrinogen, pdFI, + 106 U/mL r-thrombin + 0.36 mg/mL rFXIII) *vs*. commercial FG (0.2 mL Tisseel; Baxter), an FG that contains ~75 mg/mL pdFI and only trace pdFXIII in a liver-bandage peel-test (Appendix U). FG was applied to a 1×2 cm interface between custom electrospun polycaprolactone (PCL) mesh and a fresh porcine liver strip, and the interface was compressed with a 170 g weight for 5 min at 37°C (default setup). A T-peel adhesion test was performed with an Instron 5943 tensiometer with a 10 N load cell. Force vs. displacement data were used to calculate adhesion strength (AS; N/cm), defined as average force during the peel divided by the interface width. AS data were compared with ANOVA ($\alpha<0.05$) and unpaired t-tests ($p<0.05$); see Fig. 2.

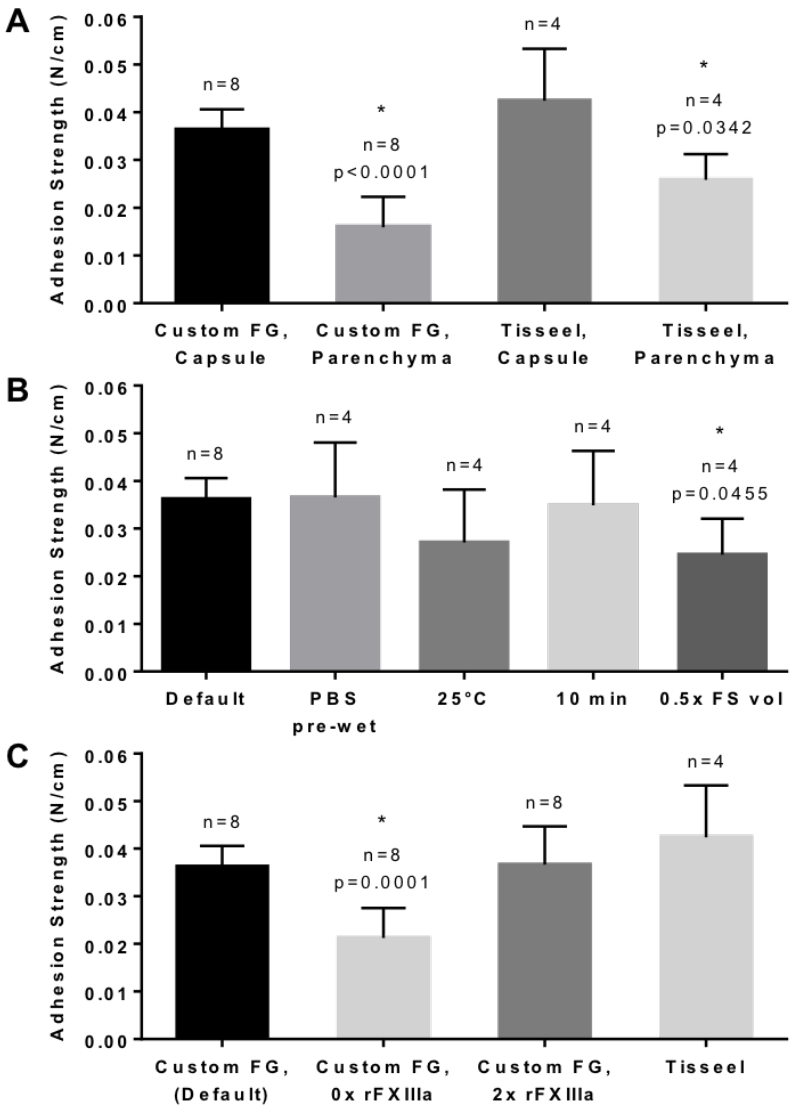

Fig. 2. AS data. *p < 0.05. Customized FG and Tisseel groups in A paired separately. Groups in B compared pair-wise with Default; groups in C compared together via ANOVA.

The data in Fig. 2 were submitted to the 2016 Academic Surgical Congress (Appendix H). AS of custom FG was ~2-fold greater by gluing the PCL mesh to the capsular surface of the liver vs. raw parenchyma (Fig. 2A), so use of the liver capsule was incorporated into the default setup. Neither capsular surface wetness (patted dry vs. pre-wet with PBS) nor prolonged compression time (5 vs. 10 min) affected AS (Fig. 2B). There appeared to be decreased AS with lower temperature during compression (25 vs. 37˚C), but this was not significant (Fig. 2B). Decreasing FG volume by 50% (0.05 vs. 0.1 mL/cm^2) resulted in a lower AS (Fig. 2B). Increasing FG volume beyond 0.1 mL/cm^2 was ineffective secondary to glue spillage during compression. Removing rFXIII from the default setup decreased AS by ~50%, but doubling the [rFXIII] did not increase AS (Fig. 2C). AS of customized FG vs. commercial FG was not different (Fig. 2C).

We concluded that an *ex vivo* adhesion test of synthetic resorbable mesh glued to porcine liver was feasible and produced useful data. The assay had been optimized with respect to liver surface qualities, adhesion compression time and temperature, and FG quantity. AS was augmented by rFXIII in the FG. The customized FG produced AS similar to commercial FG, despite the former having only ~1/8 the pdFI. The AS equivalence between these two FGs likely was a result of the added rFXIII to the customized FG, suggesting that efficacy testing of rFXIII addition to biologic hemostatic devices may be warranted. We will continue with these ex vivo adhesion studies during FY5, with the goal of designing an new/improved bandage + biologics construct for the treatment of severe hemorrhage in a coagulopathic subject.

Task 13: Delivery of fibrin sealant for final preclinical study in hypothermic coagulopathic model.

UNL will continue to generate fibrin sealant so that Tasks 12, 14, and 15 can be completed.

Task 14: Final preclinical study of resorbable bandage for the hypothermic coagulopathic model (swine).

See discussion under Task 12.

Task 15: Delivery of report on final recommended product description for resorbable fibrin sealant bandage for treatment of compressible coagulopathic hemorrhage.

The task has yet to start.

4. KEY RESEARCH ACCOMPLISHMENTS

- Continued refinement of a model of severe noncompressible intraabdominal hemorrhage.
- Ongoing development and testing of a composite alginate/fibrin sealant foam for the treatment of noncompressible intraabdominal hemorrhage.
- Development of an *ex vivo* assay to measure adhesion between liver tissue and synthetic bandage, in the relative absence of endogenous clotting factors.
- Initial characterization of the adhesion phenomena between *ex vivo* liver tissue and synthetic bandage material, including the effect of various exogenous clotting factors.
- Study of crystalloid resuscitation regimens for use during hemorrhagic shock when blood products are not available.
- Development of a stand-alone PCL bandage (no biologics) for the treatment of minor hemorrhage during surgical procedures (related but off-protocol).
- Ongoing development and testing of a PCL bandage supplemented with biologics to treat major hemorrhage during elective or emergency surgical procedures (related but off-protocol).

5. REPORTABLE OUTCOMES

Manuscripts

1. "Fluid resuscitation rate for uncontrolled intraabdominal hemorrhage in pigs." Under review at *J Trauma Acute Care Surg* (Appendix Q).

2. "Comparison of a Synthetic Resorbable Bandage vs. Oxidized Regenerated Cellulose for Treatment of Minor Surgical Hemorrhage in a Porcine Model." Under preparation for *PLOS ONE* (Appendix R).

Abstracts

1. "Effect of Factor XIII in an ex vivo assay of hemostatic bandage adhesion." Submitted to the 2016 Academic Surgical Congress (Appendix H).

2. "Treatment of noncompressible intraabdominal hemorrhage with resorbable foam supplemented with clotting factors." Accepted at the 2016 meeting of the Central Surgical Association (Appendix J).

3. "Effect of crystalloid infusion rate in a porcine model of uncontrolled noncompressible intraabdominal hemorrhage. Accepted at the 2015 meeting of the Military Health Systems Research Symposium (Appendix E).

4. Yanala UR, Noriega S, Spretz R, Ragusa J, Nuñez L, Larsen G, Carlson MA. Synthetic resorbable *vs*. cellulose bandage for minor hemorrhage in a porcine model. *J Surg Res* 2015; in press. (Appendix V).

Posters

1. "Effect of crystalloid infusion rate in a porcine model of uncontrolled noncompressible intraabdominal hemorrhage. Presented at the 2015 meeting of the Military Health Systems Research Symposium (Appendix F).

Presentations

1. "Overview of Porcine Biomedical Research in Omaha"
   Animal Genetics Seminar, UNL Department of Animal Sciences
   Lincoln, NE. October, 2014
   Appendix W

2. "Synthetic Resorbable vs. Cellulose Bandage for Minor Hemorrhage in a Porcine Model"
   2015 Academic Surgical Congress
   Las Vegas, NV. February, 2015
   Appendix G

3. "Effect Of Crystalloid Infusion Rate In A Noncompressible Hemorrhage Model"
   2015 meeting of the Central Surgical Association
   Chicago, IL. March, 2015
   Appendix I

4. "Biomedical Porcine Models at the Omaha VAMC"
   UNMC Department of Surgery Research Forum
   Omaha, NE. June 24, 2015
   Appendix P

Degrees/Education Supported

1. **Yanala, Ujwal**. Dr. Yanala worked as a surgical fellow in Dr. Carlson's laboratory during Y3-Y4. Aided by this experience and background, Dr. Yanala was able to obtain a first-year surgical resident position at UNMC. He began his residency training on 1 July 2015.

2. **Aravind, Shruthi**. Dr. Aravind is a new surgical fellow and the successor to Dr. Yanala. Dr. Aravind is enrolled in the Master's program at UNMC with Dr. Carlson has her advisor.

3. **Remmers, Neeley**. Dr. Remmers worked as a postdoctoral associate in Dr. Carlson's lab during Y3-Y4. Aided by this experience and background, she was accepted into the UNMC School of Medicine, beginning in August of 2015.

4. **Xu, Weijie**. Dr. Xu obtained his PhD while working in Dr. Velander's lab during Y4.

5. **Fabian Sanabria, Frank M**. Mr. Fabian has been working toward his PhD in Dr. Velander's lab.

6. **Ismail, Ayman E. A.** Mr. Ismail has been working toward his PhD in Dr. Velander's lab.

Informatics

1. Porcine model of noncompressible truncal hemorrhage.

2. *Ex vivo* model of bandage adhesion glued to liver tissue.

Funding Applied For

1. "Locoregional Treatment Of (1) Noncompressible Truncal Hemorrhage and (2) Coagulopathic Hemorrhage." Preproposal for USAMRMC W81XWH-BAA-15-1 (Appendix M).

2. "Locoregional Treatment Of (1) Noncompressible Truncal Hemorrhage and (2) Coagulopathic Hemorrhage." Preproposal for W81XWH-USSOCOM-BAA 15-1 (Appendix L).

3. "Prehospital management of torso hemorrhage." Quad chart submitted to the Air Force Surgeon General through the National Strategic Research Institute (Appendix K).

Employment Obtained

1. **Spretz, Ruben.** Dr. Spretz had been an engineer employed at LNK. Aided by this work experience, Dr. Spretz was able to acquire a position in Dr. Carlson's lab.

6. CONCLUSION

Ongoing development and testing of a calcium alginate foam device supplemented with human clotting factors has demonstrated efficacy in a porcine model of noncompressible uncontrolled truncal hemorrhage. This device needs further refinement, including testing in more difficult models of hemorrhage (with a hemodiluted coagulopathic component) in order to optimize its performance under the most severe conditions. The advantages of this treatment are listed below:

1. A completely resorbable configuration (in contrast to published polyurethane foam treatments [*1-6*]).

2. Generation of relatively low intraabdominal pressures (20-30 mm Hg, compared to ~100 mm Hg for published polyurethane foams).

3. Supplementation with human clotting proteins for enhanced hemostatic activity (in contrast to the published polyurethane foams, which do not utilize clotting factors).

With regard to the treatment of hemorrhage in the setting of hemodilutional hypothermic hypocoagulopathy, the institution of an *ex vivo* assay of bandage adhesion is an exciting development for this very-difficult-to-treat clinical problem. Using the data from this adhesion assay, combinations of nanoengineered synthetic bandage with human biologics (clotting factors) will be "intelligently designed" for testing in our established porcine model of hemodilutional hypothermic hemorrhage (Grade V liver injury).

In the coming months we will be aggressively pursuing continuation funding for the above studies, so that we can bring closure to these extraordinarily difficult problems in hemostasis which continue to cause half of early mortality on the modern day battlefield. We have all of the personnel, equipment, assays, and other resources in place to conduct this research, and we do not want to lose the opportunity to make potentially tremendous scientific advances in this arena.

7. REFERENCES

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5. Rago AP, Larentzakis A, Marini J, Picard A, Duggan MJ, Busold R, et al. Efficacy of a prehospital self-expanding polyurethane foam for noncompressible hemorrhage under extreme operational conditions. *J Trauma Acute Care Surg* 2015; 78(2): 324-9. PMID: 25757118.
6. Rago AP, Marini J, Duggan MJ, Beagle J, Runyan G, Sharma U, et al. Diagnosis and deployment of a self-expanding foam for abdominal exsanguination: Translational questions for human use. *J Trauma Acute Care Surg* 2015; 78(3): 607-13. PMID: 25710434.

8. APPENDICES

List of Appendices

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Appendix A

Proposal Title: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"
USAMRMC No. 10091006
Contract No. W81XWH-11-1-0836
PI: Carlson, Mark A.
SoW Version Date: February 13, 2013

STATEMENT OF WORK

No.	Task description	Site	Year	Aim
1	Purification/generation of pd-FI and rFXIIIA2-a	UNL	1-3	1
2	Generation of ultrafine particles for tamponade carrier foam	LNK	1-3	1
3	Testing of candidate tamponade carrier & FS foams	LNK & UNL	1-2	1
4	Testing of single foams in swine (tamponade carrier & FS foams separately)	UNMC	2-3	1
5	Development & engineering of dual foam candidate devices	LNK & UNL	1-2	1
6	Testing of dual foam in swine	UNMC	2-3	1
7	Engineering of firm foams from alginate and alginate derivatives	LNK	2-3	1
8	Testing of dual foam in swine noncompressible model (laparotomy with 2˚ closure)	UNMC	3-4	1
9	Delivery of candidate field-ready dual foam device	UNL & LNK	3	1
10	Testing of dual foam in swine noncompressible model (closed penetrating wound)	UMM	3	1
11	Delivery of report on final recommended product description for dual foam device for treatment of noncompressible hemorrhage	LNK, UNL, UNMC, UMM	4	1
12	Delivery of resorbable bandage for final preclinical study in hypothermic coagulopathic model	LNK	1	2
13	Delivery of fibrin sealant for final preclinical study in hypothermic coagulopathic model	UNL	1	2
14	Final preclinical study of resorbable bandage in for hypothermic coagulopathic model (swine)	UNMC	1	2
15	Delivery of report on final recommended product description for resorbable fibrin sealant bandage for treatment of compressible coagulopathic hemorrhage	LNK, UNL, UNMC	2	2

List of Abbreviations

ACURO	Animal Care and Use Review Office
DCR	damage control resuscitation
DI	deionized
DoD	Department of Defense
DURIP	Defense University Research Instrumentation Program
EHD	electrohydrodynamics
ETCO2	end tidal carbon dioxide
FI	Factor I (fibrinogen)
FIIa	activated Factor II (thrombin)
FN	fibronectin
FS	fibrin sealant
FXIII	Factor XIII (cross-linking factor)
Hb	hemoglobin
HPC	hydroxypropylcellulose
HPSEC	High pressure size exclusion chromatography
IACUC	Institutional Animal Care and Use Committee
ISR	Institute of Surgical Research
IVC	inferior vena cava
IVF	intravenous fluids
LFS	Liquid Fibrin Sealant
LNK	LNKChemsolutions, LLC
LR	Lactated Ringers solution
MAP	mean arterial pressure
PCL	polycaprolactone
pd	plasma derived
PLA	polylactic acid
PT	protime
rFXIIIA2-a	activated recombinant Factor XIII
SBF	simulated body fluid
SDS PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	scanning electron microscopy
SOW	Statement of Work
TCCC	Tactical Combat Casualty Care
TDMI	Thomas D. Morris Institute
TEG	thromboelastography
UMM	University of Maryland Medical Center
UNL	University of Nebraska—Lincoln
UNMC	University of Nebraska Medical Center

Thursday, June 18, 2015 at 12:50:06 PM Central Daylight Time

Subject: W81XWH-11-1-836, MOD1, UNIV OF NEBRASKA MEDICAL CENTER, (10091006) (UNCLASSIFIED)

Date: Wednesday, June 17, 2015 at 2:05:49 PM Central Daylight Time

From: Hebb, Brittany N CIV USARMY MEDCOM USAMRAA (US) <brittany.n.hebb.civ@mail.mil>

To: Sponsored Programs Administration, UNMC <spadmin@unmc.edu>, Carlson, Mark A <macarlso@unmc.edu>

CC: Malloy, Wilbur W CIV USARMY MEDCOM CDMRP (US) <wilbur.w.malloy.civ@mail.mil>, USARMY Ft Detrick MEDCOM CDMRP Mailbox CDMRP Award <usarmy.detrick.medcom-cdmrp.mbx.cdmrp-award@mail.mil>, USARMY Ft Detrick MEDCOM USAMRMC Mailbox USAMRMC RMI-S <usarmy.detrick.medcom-usamrmc.mbx.usamrmc-rmi-s@mail.mil>, USARMY Ft Detrick MEDCOM USAMRMC Other RAA DISTRO TO BUDGET <usarmy.detrick.medcom-usamrmc.other.raa-distro-to-budget@mail.mil>, USARMY Ft Detrick MEDCOM USAMRMC Other RAD PROPOSALS <usarmy.detrick.medcom-usamrmc.other.rad-proposals@mail.mil>, Mckean, Joshua D CIV USARMY MEDCOM USAMRAA (US) <joshua.d.mckean3.civ@mail.mil>, Carrera, Amanda C CIV USARMY MEDCOM USAMRAA (US) <amanda.c.carrera.civ@mail.mil>, Kuhns, Janet P CIV USARMY MEDCOM USAMRAA (US) <janet.p.kuhns.civ@mail.mil>, Hebb, Brittany N CIV USARMY MEDCOM USAMRAA (US) <brittany.n.hebb.civ@mail.mil>

Classification: UNCLASSIFIED
Caveats: NONE

Good Afternoon,

(1) Attached is a fully executed copy of the subject award modification document. Please save or print a copy for your records. Also, forward a copy of this document to your institution's financial office that prepares invoices and other required financial reports.

(2) Please review the award document carefully, as well as the Terms and Conditions incorporated by reference. Links to those Terms and Conditions incorporated by reference are included in the award document. Please pay particular attention to the Request for Payments term as the payments are not automatic.

(3) If you have any questions or concerns, The Contract/Grants Specialist for this award is Mr. Joshua McKean, joshua.d.mckean3.civ@mail.mil, (301) 619-4046.

Ms. Brittany Hebb
U.S. Army Medical Research Acquisition Activity Assistance Agreements Team #3
843 Chandler Street
Fort Detrick, MD 21702-5014
Phone: 301-619-9802
Email: brittany.n.hebb.civ@mail.mil

Classification: UNCLASSIFIED
Caveats: NONE

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 16

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO. (If applicable)
P00001	18-Jun-2015	W23RYX1095N602	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	See Item 6	

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
UNIVERSITY OF NEBRASKA 987835 NEBRASKA MEDICAL CENTER OMAHA NE 68198-6810		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. W81XWH-11-1-0836
	X	10B. DATED (SEE ITEM 13) 26-Sep-2011

CODE 1PPD6 FACILITY CODE

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

☐ C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:

X D. OTHER (Specify type of modification and authority)
IAW USAMRAA General Terms and Conditions

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)

Modification Control Number: jmckean153911

PROJECT TITLE: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"
PRINCIPAL INVESTIGATOR: Dr. Mark Carlson
AWARD AMOUNT: $5,033,983
OBLIGATED AMOUNT: $5,033,983
PERIOD OF PERFORMANCE: 26 September 2011 - 25 September 2016

The purpose of this modification is to extend the period of performance by 12 months at no additional cost to the Government, per the recipient's request dated 21 May 2015. An annual technical report will be due no later than 25 October 2015. The final technical report will be due no later than 25 December 2016. Submission of financial reports (SF425s) shall continue during the extension period.

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) JANET P KUHNS / CONTRACTING OFFICER TEL: 301-619-2827 EMAIL: janet.p.kuhns.civ@mail.mil	
15B. CONTRACTOR/OFFEROR _____ (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY Janet P. Kuhns (Signature of Contracting Officer)	16C. DATE SIGNED 17-Jun-2015

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

The standard size code 500 has been added.
The NAICS code 541712 has been added.
The 'administered by' organization has changed from

US ARMY MEDICAL RESEARCH ACQUISITION ACT
ATTN: DAWN JENNINGS
DAWN.V.JENNINGS@AMEDD.ARMY.MIL
820 CHANDLER STREET
FORT DETRICK MD 21702

to

US ARMY MEDICAL RESEARCH ACQUISITION ACT
DIRECTOR
820 CHANDLER STREET
FORT DETRICK MD 21702-5014

CLIN 0001

The CLIN extended description has changed from:

For Proposal #10091006, Period of Performance: 26 September 2011 - 25 October 2015 (Research ends 25 October 2015).

To:

For Proposal #10091006, Period of Performance: 26 September 2011 - 25 September 2016..

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC
POP 26-SEP-2011 TO 25-OCT-2015	N/A	USA MED RESEARCH MAT CMD TMED AND ADV TECH RSRCH CTR TATRC 504 SCOTT STREET FORT DETRICK MD 21702-5012 FOB: Destination	W90ERG

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC

POP 26-SEP-2011 TO 25-SEP-2016	N/A	USA MED RESEARCH MAT CMD TMED AND ADV TECH RSRCH CTR TATRC 504 SCOTT STREET FORT DETRICK MD 21702-5012 FOB: Destination	W90ERG

The following have been modified:
PI NAME & TITLE
Principal Investigator: Dr. Mark Carlson

Proposal Title: "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"

Grants Administration:
Joshua McKean, Grant Specialist
USAMRAA- Assistance Branch 4
820 Chandler Street
Fort Detrick, MD 21702
Phone: 301-619-4046
Email: joshua.d.mckean3.civ@mail.mil

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

A. This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's statement of work and budget dated 11 March 2011, for the proposal dated 11 March 2011 are incorporated herein by reference. The Catalog of Federal Domestic Assistance number relative to this award is CFDA 12.420.

B. ACCEPTANCE OF AWARD. The recipient is not required to countersign this assistance award. In case of disagreement, the recipient shall notify the Grants Officer and not assess the award any costs until such disagreement(s) is resolved.

C. MAXIMUM OBLIGATION (SEP 2006) (USAMRAA)

The maximum obligation for support of the project will not exceed the amount specified in the award, as amended. USAMRAA does not amend assistance agreements to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits and other costs.

D. TERMS AND CONDITIONS: The recipient agrees to the General Terms and Conditions of the Federal Demonstration Partnership, Phase V, dated July 1, 2008 and Department of Army – Agency Specific Requirements. Modifications to the General Terms and Conditions dated July 1, 2008 are modified as indicated below.

1. PATENTS AND INVENTIONS (DEC 2001) (USAMRAA)

a. The recipient shall use the Interagency Edison through the National Institutes of Health Commons (http://www.iedison.gov/) for filing of Patent Application and Invention Disclosure. Negative reports are required and shall be submitted on a DD Form 882 to the Grants Officer. (DD Form 882 can be located on web site http://www.usamraa.army.mil).

b. Invention reports are due annually and at the end of the period of the award. Annual reports are due 30 days after the anniversary date of the award and final reports are due 30 days after the expiration of the award. The award will NOT be closed out until all invention reporting requirements are met.

2. TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)

PROGRAMMATIC LINE REVIEW (PLR)

a. The reporting requirements for Telemedicine and Advanced Technology Research Center (TATRC) include quarterly, annual and final reports and the Principal Investigator's (PI's) participation in at least one programmatic line review (PLR) for this project each year of the project's period-of-performance.

b. The PI shall prepare for and participate in at least one PLR for this project for each year of the project's term, at the Grants Officer's Representative's (GOR's) request. The invitation and format for the programmatic review will be provided by TATRC at least 90 days prior to the meeting. The meetings will generally be held in the Fort Detrick, Maryland, area, but may occur elsewhere in the U.S. Participation in the PLR will be in lieu of submitting next scheduled Quarterly report required under the award.

QUARTERLY REPORTS

a. Quarterly reports are the most immediate and direct contact between the Principal Investigator (PI) and the Grants Officer's Representative (GOR). The reports provide the means for keeping this Command advised of developments and problems as the research effort proceeds. The quarterly reports also provide a measure against which decisions on release of funding and on requests for supplements are made.

b. In accordance with Section C., a Quarterly Report shall be submitted for each three-month period beginning with the effective date of the assistance agreement. This requirement includes all three-month periods of the assistance agreement.

c. Copies of each report shall be submitted in the quantities indicated to the addresses shown below within fifteen (15) days after the end of each quarter. Internal Government distribution will be made by those offices (electronic submission preferred).

(1) One (l) copy of the report to:

Email: wilbur.w.malloy.civ@mail.mil

(2) One (1) copy of the report to:

Email: joshua.d.mckean3.civ@mail.mil

d. The Quarterly Report sample (See following Quarterly Report Format) shall serve as the format. Each item of the report format shall be completed.

QUARTERLY REPORT FORMAT

1. Award No. _____2. Report Date _____

3. Reporting period from _____ to _____

4. PI _____ 5. Telephone No. _____

6. Institution_____

7. Project Title_____
_____
_____

8. Current staff, with percent effort of each on project.

_____ _____% _____ _____%

_____ _____% _____ _____%

9. Award expenditures to date (as applicable):

	This Qtr/Cumulative		**This Qtr/Cumulative**
Personnel	_____/_____	Travel	_____/_____
Fringe Benefits	_____/_____	Equipment	_____/_____
Supplies	_____/_____	Other	_____/_____

	This Qtr/Cumulative
Subtotal	_____/_____
Indirect Costs	_____/_____
Fee	_____/_____
Total	_____/_____

10. Comments on administrative and logistical matters.

_____

_____

_____

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this assistance agreement.

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter.

TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)

Format Requirements for Annual/Final Reports

a. Annual reports must provide a complete summary of the research accomplishments to date with respect to the approved Statement of Work. Journal articles can be substituted for detailed descriptions of specific aspects of the research, but the original articles must be attached to the report as an appendix and appropriately referenced in the text. The importance of the report to decisions relating to continued support of the research can not be over-emphasized. An annual report shall be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the Grants Officer, then an annual report must still be submitted within 30 days of the anniversary date of the award. A final report will be due upon completion of the extended performance date that describes the entire research effort.

b. A final report summarizing the entire research effort, citing data in the annual reports and appended publications shall be submitted at the end of the award performance period. The final report will provide a complete reporting of the research findings. Journal publications can be substituted for detailed descriptions of specific aspects of the research, but an original copy of each publication must be attached as an appendix and appropriately referenced in the text. All final reports must include a bibliography of all publications and meeting abstracts and a list of personnel (not salaries) receiving pay from the research effort.

Although there is no page limitation for the reports, each report shall be of sufficient length to provide a thorough description of the accomplishments with respect to the approved Statement of Work. Submission of the report in electronic format (PDF or Word file only), shall be submitted to https://ers.amedd.army.mil.

All reports shall have the following elements in this order.

FRONT COVER: Sample front cover provided at https://mrmc.amedd.army.mil/rrpindex.asp. The Accession Document (AD) Number should remain blank.

STANDARD FORM 298: Sample SF 298 provided at https://mrmc.amedd.army.mil/rrpindex.asp. The abstract in Block 13 must state the purpose, scope, major findings and be an up-to-date report of the progress in terms of results and significance. Subject terms are keywords that may have previously assigned to the proposal abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Please count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers should be typed: please do not hand number pages.

TABLE OF CONTENTS: Sample table of contents provided at https://mrmc.amedd.army.mil/rrpindex.asp.

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the

Army Grants Officer's Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

> manuscripts, abstracts, presentations; patents and licenses applied for and/or issued; degrees obtained that are supported by this award; development of cell lines, tissue or serum repositories; infomatics such as databases and animal models, etc.; funding applied for based on work supported by this award; employment or research opportunities applied for and/or received based on experience/training supported by this award.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science*, *Military Medicine*, etc.).

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Pages shall be consecutively numbered throughout the report. DO NOT RENUMBER PAGES IN THE APPENDICES.

Mark all pages of the report which contain proprietary or unpublished data that should be protected by the U.S. Government. REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE. It is the responsibility of the Principal Investigator to advise the U.S. Army Medical Research and Materiel Command when restricted limitation assigned to a document can be downgraded to Approved for Public Release. DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS.

3. PAYMENTS

ADVANCE PAYMENTS AND FULL FUNDING (DEC 2008) (USAMRAA)

a. Payments. Advance payments will be made to the recipient. Questions relative to payment issues involving Defense Finance and Accounting Service shall be directed to Usarmy.detrick.medcom-usamraa.mbx.aa4@mail.mil.

b. Electronic Funds Transfer. All advance payments to the recipient will be made by electronic funds transfer (EFT) to the recipient's financial institution account listed in the Central Contractor Registry (CCR). Failure to update CCR may result in nonpayment.

c. If the recipient fails to perform, the Grants Officer shall notify DFAS in writing to withhold payments.

d. Advance Payment Schedule: $5,033,983.00

Year 1: $1,471,211

Amount	On or About
$367,802.75	26 September 2011
$367,802.75	26 December 2011
$367,802.75	26 March 2012
$367,802.75	26 June 2012

Year 2: $1,474,452

Amount	On or About
$368,613	26 September 2012
$368,613	26 December 2012
$368,613	26 March 2013
$368,613	26 June 2013

Year 3: $1,675,306

Amount	On or About
$418,826.50	26 September 2013
$418,826.50	26 December 2013
$418,826.50	26 March 2014
$418,826.50	26 June 2014

Year 4: $413,014

Amount	On or About
$103,253.50	26 September 2014
$103,253.50	26 December 2014
$103,253.50	26 March 2015
$103,253.50	26 June 2015

e. Financial Reporting Requirements:

Federal Financial Report (SF 425): Quarterly and Final Reports (For reporting individual assistance agreements)

Reporting period end dates fall on the end of the calendar quarter for quarterly reports (3/31, 6/30, 9/30, 12/31) and the end date of the assistance agreement period of performance for the final report. Reports are due 30 days after the reporting period end date for quarterly reports and 90 days after the end date of the assistance agreement for the final report.

The SF425 and instructions for completion can be obtained from https://usamraa.army.mil. All SF425's shall be submitted electronically to https://www.usamraa.army.mil/pages/sf425. The award number assigned by USAMRAA, W81XWH-11-1-0836 shall be included in the subject line of the electronic submission.

NOTE: The SF425 is a single form that consolidates and replaces the Federal Cash Transaction Report (SF272.SF272A) and the Financial Status Report (SF269/SF269A)

f. Interest Bearing Account. Unless exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (CMIA) (31 U.S.C. 3335), the recipient shall deposit all advance payments in an interest bearing account. Interest over the amount of $250 per year shall be remitted annually to the Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, MD 20852. A copy of the transmittal letter stating the amount of interest remitted shall be sent to the U.S. Army Medical Research Acquisition Activity, ATTN: MCMR-AAA-T, 820 Chandler Street, Fort Detrick, MD 21702-5014.

4. PROHIBITION OF USE OF HUMAN RESEARCH (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Research under this award involving the use of human subjects, to include the use of human anatomical substances and/or human data, may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human subjects under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the recipient. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

5. PROHIBITION OF USE OF LABORATORY ANIMALS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Notwithstanding any other provisions contained in this award or incorporated by reference herein, the recipient is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the US Army Medical Research and Materiel Command, Animal Care and Use Office (ACURO). The recipient will receive written approval to begin research under the applicable protocol proposed for this award from the US Army Medical Research and Materiel Command, ACURO, under separate letter. A copy of this approval will be provided to the US Army Medical Research and Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in the termination of the award.

6. PROHIBITION OF USE OF HUMAN CADAVERS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS****

Research under this award using human cadavers may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human cadavers under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the recipient. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

7. SUPPORTING INFORMATION (APR 2008) (USAMRAA)

Information such as subawards, consultant agreements, vendor quotes, and personnel work agreements may be required in order to support proposed costs or to determine the employment status of personnel under the assistance agreement. The Government’s receipt of this information does not constitute approval or acceptance of any term or condition included therein. The terms and conditions of the assistance agreement take precedence over any term or condition included in supporting information.

8. TRAFFICKING VICTIMS PROTECTION ACT (May 2008) (USAMRAA)

Trafficking in persons.

a. Provisions applicable to a recipient that is a private entity.

l. You as the recipient, your employees, subrecipients under this award, and subrecipients' employees may not--

i. Engage in severe forms of trafficking in persons during the period of time that the award is in effect;
ii. Procure a commercial sex act during the period of time that award is in effect; or
iii. Use forced labor in the performance of the award or subawards under the award.

2. We as the Federal awarding agency may unilaterally terminate this award, without penalty, if you or a subrecipient that is a private entity--

i. Is determined to have violated a prohibition in paragraph a.1 of this award term; or
ii. Has an employee who is determined by the agency official authorized to terminate the award to have violated a prohibition in paragraph a.1 of this award term through conduct that is either--
A. Associated with performance under this award; or
B. Imputed to you or the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR 180, "OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement)," as implemented by our agency at 2 CFR part 1125.

b. Provision applicable to a recipient other than a private entity. We as the Federal awarding agency may unilaterally terminate this award, without penalty, if a subrecipient that is a private entity--

l. Is determined to have violated an applicable prohibition in paragraph a.1 of this award term; or

2. Has an employee who is determined by the agency official authorized to terminate the award to have violated an applicable prohibition in paragraph a.1 of this award term through conduct that is either--

i. Associated with performance under this award;
ii. Imputed to the subrecipient using the standards and due process for imputing the conduct of an individual to an organization that are provided in 2 CFR part 180, "OMB Guidelines to Agencies on Governmentwide Debarment and Suspension (Nonprocurement)," as implemented by our agency at 2 CFR part 1125.

c. Provision applicable to any recipient.

l. You must inform us immediately of any information you receive from any source alleging a violation of a prohibition in paragraph a.1 of the award term.

2. Our right to terminate unilaterally that is described in paragraph a.2. or b. of this section:

i. Implements section 106(g) of the Trafficking Victims Protection Act of 2000 (TVPA), as amended (22 U.S.C. 7104(g)), and
ii. Is in addition to all other remedies for noncompliance that are available to us under this award.

3. You must include the requirements of paragraph a.1 of this award term in any subaward you make to a private entity.

d. Definitions. For the purpose of this award term:

l. "Employee" means either:

i. An individual employed by you or a subrecipient who is engaged in the performance of the project or program under this award; or

ii. Another person engaged in the performance of the project or program under this award and not compensated by you including, but not limited to, a volunteer or individual whose services are contributed by a third party as an in-kind contribution toward cost sharing or matching requirements.

2. "Forced labor" means labor obtained by any of the following methods: the recruitment, harboring, transportation, provision, or obtaining of a person for labor or services, through the use of force, fraud, or coercion for the purpose of subjection to involuntary servitude, peonage, debt bondage, or slavery.

3. "Private entity":

i. Means any entity other than a State, local government, Indian Tribe, or foreign public entity, as those terms are defined in 2 CFR 175.25.

ii. Includes:

A. A nonprofit organization, including any nonprofit institution of higher education, hospital, or tribal organization other than one included in the definition if Indian Tribe at 2 CFR 175.25(b).

B. A for-profit organization.

4. "Severe forms of trafficking in persons," "commercial sex act," and "coercion" have the meanings given at section 103 of the TVPA, as amended (22 U.S.C. 7102).

9. REQUIREMENTS FOR FEDERAL FUNDING ACCOUNTABILITY AND TRANSPARENCY ACT (2 CFR 170)

Appendix A to Part 170--Award Term

I. Reporting Subawards and Executive Compensation

A. Reporting of first-tier subawards.

1. Applicability. Unless you are exempt as provided in paragraph D. of this award term, you must report each action that obligates $25,000 or more in Federal funds that does not include Recovery funds (as defined in section 1512(a)(2) of the American Recovery and Reinvestment Act of 2009, Pub. L. 111-5) for a subaward to an entity (see definitions in paragraph e. of this award term).

2. Where and when to report.

i. You must report each obligating action described in paragraph a.1. of this award term to http://www.fsrs.gov.

ii. For subaward information, report no later than the end of the month following the month in which the obligation was made. (For example, if the obligation was made on November 7, 2010, the obligation must be reported by no later than December 31, 2010.)

3. What to report. You must report the information about each obligating action that the submission instructions posted at http://www.fsrs.gov specify. must report the information about each obligating action that the submission instructions posted at http://www.fsrs.gov specify.

B. Reporting Total Compensation of Recipient Executives.

1. Applicability and what to report. You must report total compensation for each of your five most highly compensated executives for the preceding completed fiscal year, if--

i. the total Federal funding authorized to date under this award is $25,000 or more;

ii. in the preceding fiscal year, you received—

(A) 80 percent or more of your annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) $25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

iii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at http://www.sec.gov/answers/execomp.htm.)

2. Where and when to report. You must report executive total compensation described in paragraph b.1. of this award term:

i. As part of your registration profile at http://www.ccr.gov.

ii. By the end of the month following the month in which this award is made, and annually thereafter.

C. Reporting of Total Compensation of Subrecipient Executives.

1. Applicability and what to report. Unless you are exempt as provided in paragraph d. of this award term, for each first-tier subrecipient under this award, you shall report the names and total compensation of each of the subrecipient's five most highly compensated executives for the subrecipient's preceding completed fiscal year, if--

i. in the subrecipient's preceding fiscal year, the subrecipient received--

(A) 80 percent or more of its annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) $25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts), and Federal financial assistance subject to the Transparency Act (and subawards); and

ii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at http://www.sec.gov/answers/execomp.htm.)

2. Where and when to report. You must report subrecipient executive total compensation described in paragraph c.1. of this award term:

i. To the recipient.

ii. By the end of the month following the month during which you make the subaward. For example, if a subaward is obligated on any date during the month of October of a given year (i.e., between October 1 and 31), you must report any required compensation information of the subrecipient by November 30 of that year.

D. Exemptions. If, in the previous tax year, you had gross income, from all sources, under $300,000, you are exempt from the requirements to report:

i. Subawards, and

ii. The total compensation of the five most highly compensated executives of any subrecipient.

E. Definitions. For purposes of this award term:

1. Entity means all of the following, as defined in 2 CFR part 25:

i. A Governmental organization, which is a State, local government, or Indian tribe;

ii. A foreign public entity;

iii. A domestic or foreign nonprofit organization;

iv. A domestic or foreign for-profit organization;

v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

2. Executive means officers, managing partners, or any other employees in management positions.

3. Subaward:

i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.

ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ---- .210 of the attachment to OMB Circular A-133, ``Audits of States, Local Governments, and Non-Profit Organizations'').

iii. A subaward may be provided through any legal agreement, including an agreement that you or a subrecipient considers a contract.

4. Subrecipient means an entity that:

i. Receives a subaward from you (the recipient) under this award; and

ii. Is accountable to you for the use of the Federal funds provided by the subaward.

5. Total compensation means the cash and noncash dollar value earned by the executive during the recipient's or subrecipient's preceding fiscal year and includes the following (for more information see 17 CFR 229.402(c)(2)):

i. Salary and bonus.

ii. Awards of stock, stock options, and stock appreciation rights. Use the dollar amount recognized for financial statement reporting purposes with respect to the fiscal year in accordance with the Statement of Financial Accounting Standards No. 123 (Revised 2004) (FAS 123R), Shared Based Payments.

iii. Earnings for services under non-equity incentive plans.This does not include group life, health, hospitalization or medical reimbursement plans that do not discriminate in favor of executives, and are available generally to all salaried employees.

iv. Change in pension value. This is the change in present value of defined benefit and actuarial pension plans.

v. Above-market earnings on deferred compensation which is not tax- qualified.

vi. Other compensation, if the aggregate value of all such other compensation (e.g. severance, termination payments, value of life insurance paid on behalf of the employee, perquisites or property) for the executive exceeds $10,000.

End of clause

10. FINANCIAL ASSISTANCE USE OF UNIVERSAL IDENTIFIER AND CENTRAL CONTRACTOR REGISRATION (2 CFR Part 25)

Appendix A to Part 25--Award Term

I. Central Contractor Registration and Universal Identifier Requirements

A. Requirement for Central Contractor Registration (CCR). Unless you are exempted from this requirement under 2 CFR 25.110, you as the recipient must maintain the currency of your information in the CCR until you submit the final financial report required under this award or receive the final payment, whichever is later. This requires that you review and update the information at least annually after the initial registration, and more frequently if required by changes in your information or another award term.

B. Requirement for Data Universal Numbering System (DUNS) Numbers. If you are authorized to make subawards under this award, you:

1. Must notify potential subrecipients that no entity (see definition in paragraph C of this award term) may receive a subaward from you unless the entity has provided its DUNS number to you.

2. May not make a subaward to an entity unless the entity has provided its DUNS number to you.

C. Definitions. For purposes of this award term:

1. Central Contractor Registration (CCR) means the Federal repository into which an entity must provide information required for the conduct of business as a recipient. Additional information about registration procedures may be found at the CCR Internet site (currently at http://www.ccr.gov).

2. Data Universal Numbering System (DUNS) number means the nine-digit number established and assigned by Dun and Bradstreet, Inc. (D&B) to uniquely identify business entities. A DUNS number may be obtained from D&B by telephone (currently 866-705-5711) or the Internet (currently at http://fedgov.dnb.com/webform).

3. Entity, as it is used in this award term, means all of the following, as defined at 2 CFR part 25, subpart C:

a. A Governmental organization, which is a State, local government, or Indian Tribe;

b. A foreign public entity;

c. A domestic or foreign nonprofit organization;

d. A domestic or foreign for-profit organization; and

e. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

4. Subaward:

a. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.

b. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ----.210 of the attachment to OMB Circular A-133, ``Audits of States, Local Governments, and Non-Profit Organizations").

c. A subaward may be provided through any legal agreement, including an agreement that you consider a contract.

5. Subrecipient means an entity that:

a. Receives a subaward from you under this award; and

b. Is accountable to you for the use of the Federal funds provided by the subaward.

End of Clause

(End of Summary of Changes)

I. OVERVIEW
Date: October 03, 2014
Swine no: 241
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Cavanaugh, Siford

II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male
Date swine received from UNL Mead: 09/29/2014
Pre-procedure wt: 34.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS

- HR: 108
- MAP: 114
- Temp: 37.4
- EtCO2: 44

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 318.5 gm
LR (22°C) infused after splenectomy: 955 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 318.5 + 20 + 22 = 360.5 mL
- LR (22°C) infused (spleen replacement + incidental): 955 + 50 = 1055 mL

Pre-injury VS

- HR: 94
- MAP: 105
- Temp: 37.0
- EtCO2 : 40

III. INJURY & TREATMENT PHASE

Time of injury: 08:49 AM
Injury type: Hepatic left medial lobectomy, non anatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 79
Resuscitation fluid: warm LR, 1.7 L preset maximum (50 mL/kg), given at constant rate of 9.6 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:50AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:00 AM

10 min post-injury VS
- HR: 109
- MAP: 22
- Temp: 36.4
- EtCO2: 22

Blood draw no. 3: (30 min post-injury): 09:20 AM

30 min post-injury VS
- HR: 91
- MAP: 31
- Temp: 35.6
- EtCO2:

Blood draw no. 4: (60 min post-injury): 09:50 AM

60 min VS
- HR: 92
- MAP: 43
- Temp:34.9
- EtCO2: 36

Blood draw no. 5: (90 min post-injury): 10:20 AM

90 min post-injury VS
- HR: 87
- MAP: 44
- Temp: 34.7
- EtCO2: 35

Blood draw no. 6: (120 min post-injury): 10:50 AM

120 min post-injury VS
- HR: 82
- MAP: 47
- Temp: 34.1
- EtCO2: 35

Blood draw no. 7: (150 min post-injury): 11:20 AM

150 min post-injury VS
- HR: 78
- MAP: 60
- Temp: 34.3
- EtCO2: 33

Blood draw no. 8: (180 min post-injury): 11:50 AM

180 min post-injury VS
- HR: 76
- MAP: 46
- Temp: 34.1
- EtCO2: 33

Survival at 180 min? YES
Target MAP attained ? No
Time of death: 11:50 AM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 730.8 mL (suction) + 462.3 mL (clot + lap pads) = 1193.1 mL
- IV fluid given: LR (37°C): 1900 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering the surface of the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.

Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 141.5 (resected LM lobe) + 718.3 (remaining liver) = 859.8 g

Tissue harvested: IVC

VI. COMMENTS

N = 3 of no-treatment controls for LM lobe resection mechanism of noncompressible hemorrhage model. Subject was quite hypotensive early on, but recovered, and survived the 3 h period easily with 1.2 L blood loss. We are restricting LR in the 3 h period to a volume max of 50 mL/kg. So we are now at 1 survivor, 2 deaths.

VII. PLAN

Continue generating no-Rx controls; next subject will be on Fri Oct 10th.

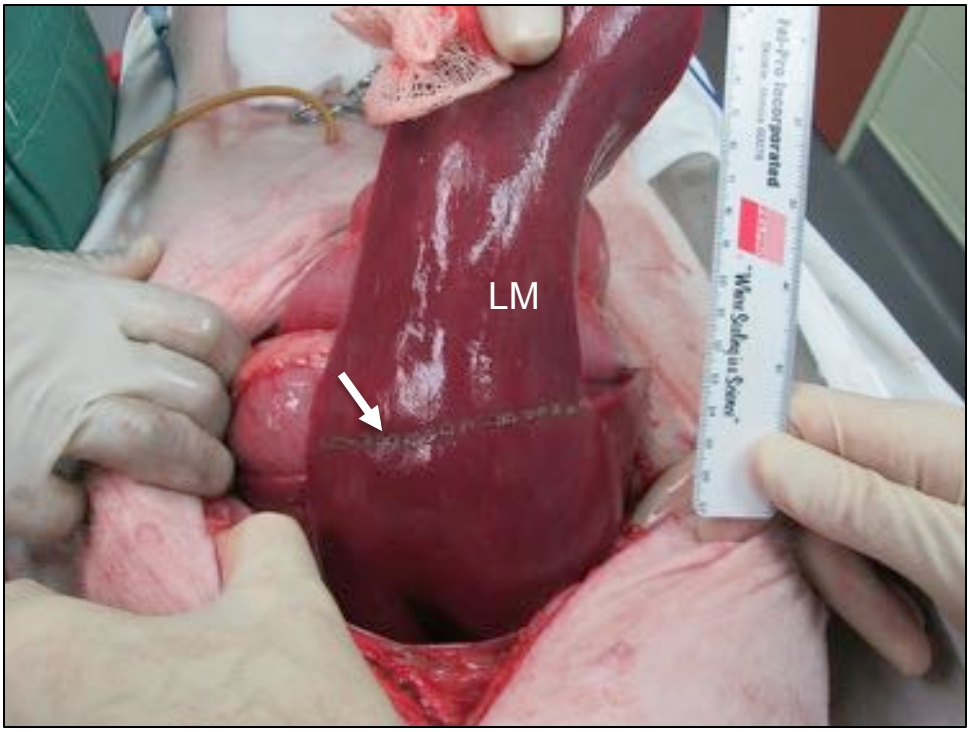

Figure 1, swine 241. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).

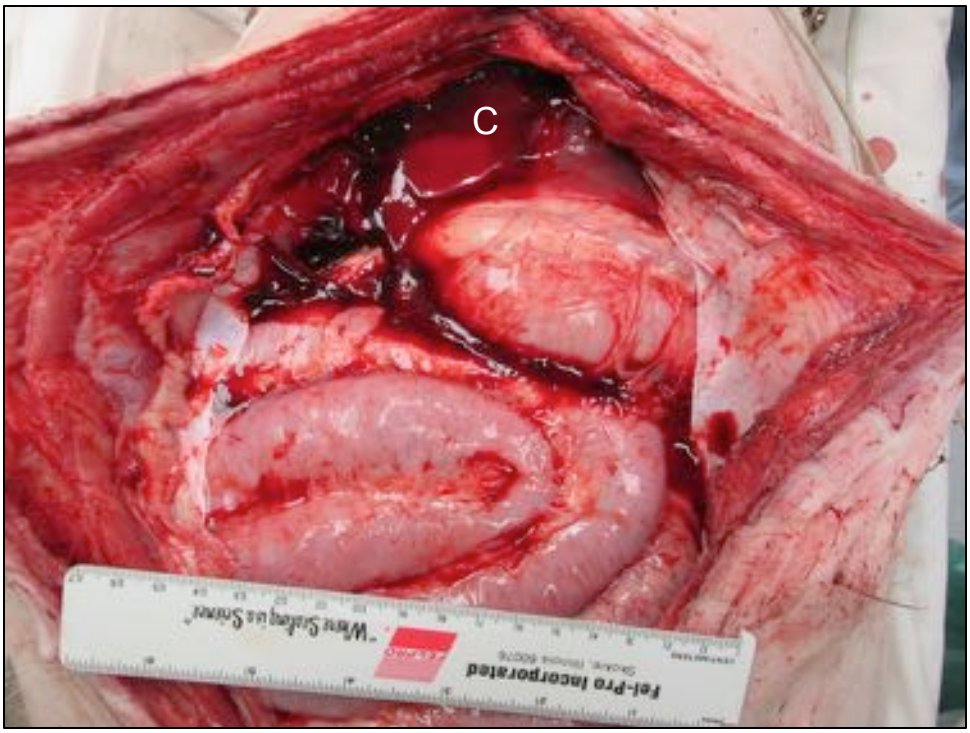

Figure 2, swine 241. Site of injury, after death of subject. A large clot (C) overlies the injury site (latter not visible). Loops of intestine are visible inferior to the clot. Cephalad is at top of image.

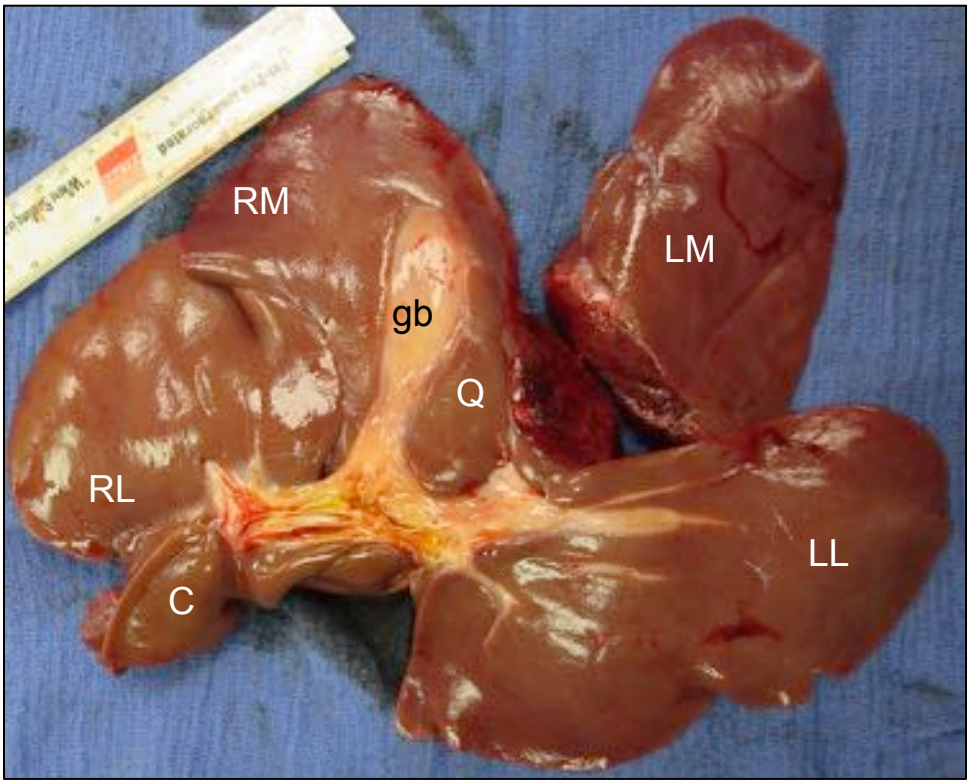

Figure 3, swine 241. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Anterior is at top of image.

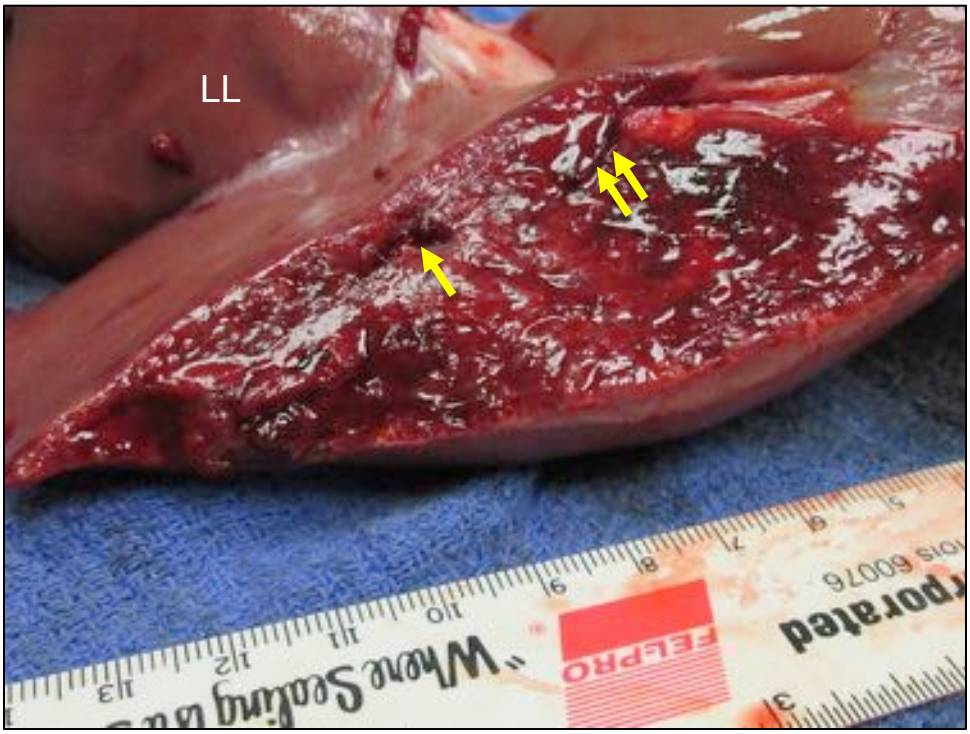

Figure 4, swine 241. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). A thin layer of clot overlies the cut liver surface. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates transected hepatic vein to LM lobe; double arrow indicates site of transected PV branch to LM lobe. These transected veins were all occluded at the time of necropsy.

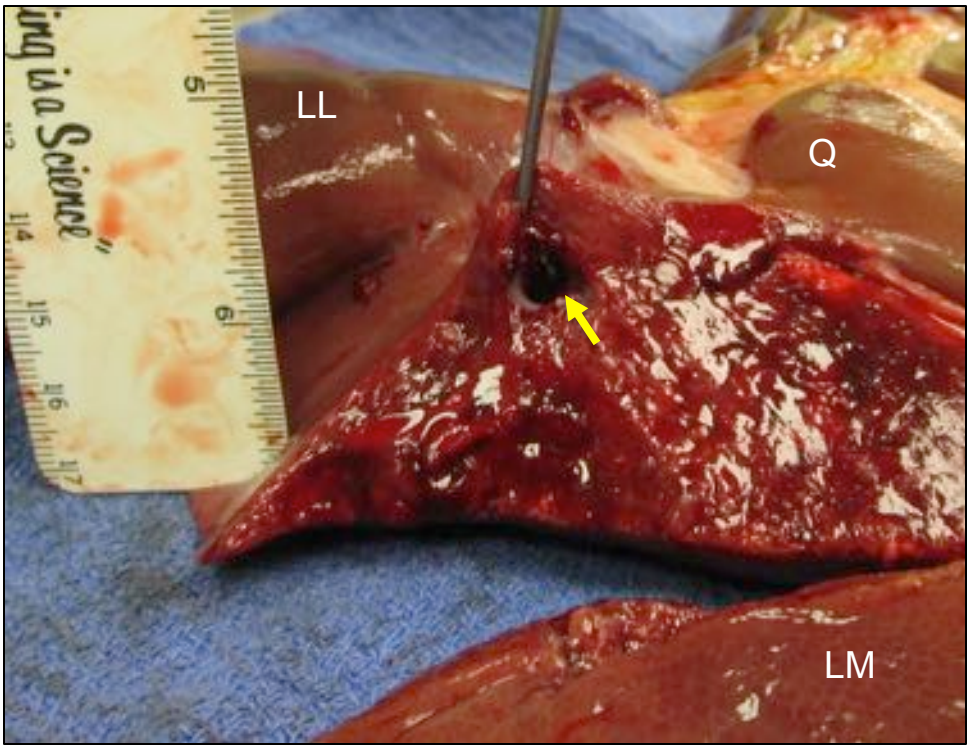

Figure 5, swine 241. Liver *ex vivo*, close-up of injury/lobectomy site, similar view as in Fig. 4. The HV to the LM lobe has been reopened with a forceps, showing size of lumen (arrow).

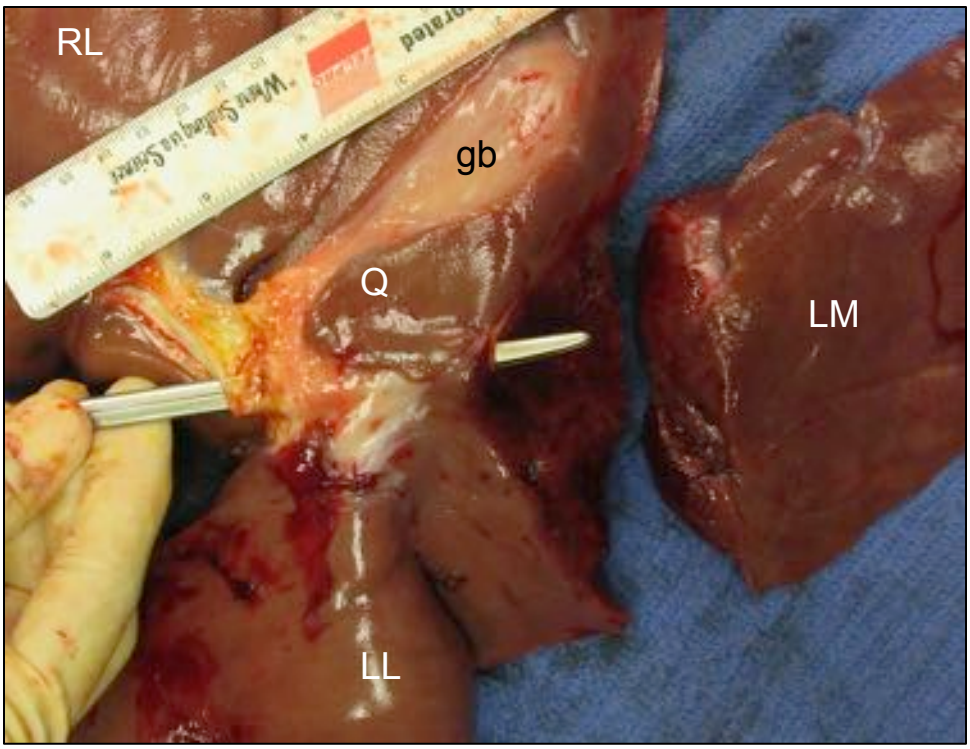

Figure 6, swine 241. Liver *ex vivo*, inferior surface, similar view as in Fig. 3. The scissors has been inserted into the main portal vein, and the tips emerge through the transected branch of the PV to the LM lobe. The LL lobe has been reflected medially/posteriorly.

I. OVERVIEW
Date: October 10, 2014
Swine no: 243
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Cavannaugh, Siford.

II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: male
Date swine received from UNL Mead: 09/29/2014
Pre-procedure wt: 32.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS

- HR: 128
- MAP: 86
- Temp: 38.2
- EtCO2: 42

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:38 AM
Spleen wt: 205.5 gm
LR (22°C) infused after splenectomy: 616.5 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 205.5 + 20 + 22.7 = 248.2 mL
- LR (22°C) infused (spleen replacement + ear vein): 616.5 + 100 = 716.5 mL

Pre-injury VS

- HR: 127
- MAP: 90
- Temp: 37.8
- EtCO2 : 51

III. INJURY & TREATMENT PHASE

Time of injury: 08:54 AM
Injury type: Hepatic left medial lobectomy, non anatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. The left medial lobe in this subject was hypoplastic, about one-third the typical size. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 72
Resuscitation fluid: warm LR, 1.64 L preset maximum (50 mL/kg), given at constant rate of 9.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:55AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:05 AM

10 min post-injury VS
- HR: 135
- MAP: 66
- Temp: 37.2
- EtCO2: 32

Blood draw no. 3: (30 min post-injury): 09:25 AM

30 min post-injury VS
- HR: 128
- MAP: 77
- Temp: 36.8
- EtCO2: 33

Blood draw no. 4: (60 min post-injury): 09:55 AM

60 min VS
- HR: 121
- MAP: 78
- Temp:36.6
- EtCO2: 33

Blood draw no. 5: (90 min post-injury): 10:25 AM

90 min post-injury VS
- HR: 111
- MAP: 81
- Temp: 36.3
- EtCO2: 33

Blood draw no. 6: (120 min post-injury): 10:55 AM

120 min post-injury VS
- HR: 106
- MAP: 88
- Temp: 36.2
- EtCO2: 33

Blood draw no. 7: (150 min post-injury): 11:25 AM

150 min post-injury VS
- HR: 102
- MAP: 97
- Temp: 36.2
- EtCO2: 33

Blood draw no. 8: (180 min post-injury): 11:55 AM

180 min post-injury VS
- HR: 109
- MAP: 99
- Temp: 36.2
- EtCO2: 34

Survival at 180 min? YES
Target MAP attained? Yes at 9:20 (25 min from injury).
Time of death: 11:55 AM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 100 mL (suction) + 101.4 mL (clot + lap pads) = 201.4 mL
- IV fluid given: LR (37°C): 250 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, minimal amounts of blood and clot around injury site (see Figs). A large clot was attached to the surface of the injury site (see Figs).
Heart: not examined.

Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1, to LM lobe
Other: none
Ex vivo liver wt: 44.7 (resected LM lobe) + 923.3 (remaining liver) = 968.0 g

Tissue harvested: None

VI. COMMENTS

N = 4 of no-treatment controls for LM lobe resection mechanism of noncompressible hemorrhage model. This subject had a small, hypoplastic LM lobe, and so the vessels transected were also small. This subject probably should not be counted in the no-treatment control group 2˚ to this observation. Blood loss was minimal (~200 mL), and subject survived quite easily.

VII. PLAN

Continue generating no-Rx controls; next subject will be on Fri Oct 24th.

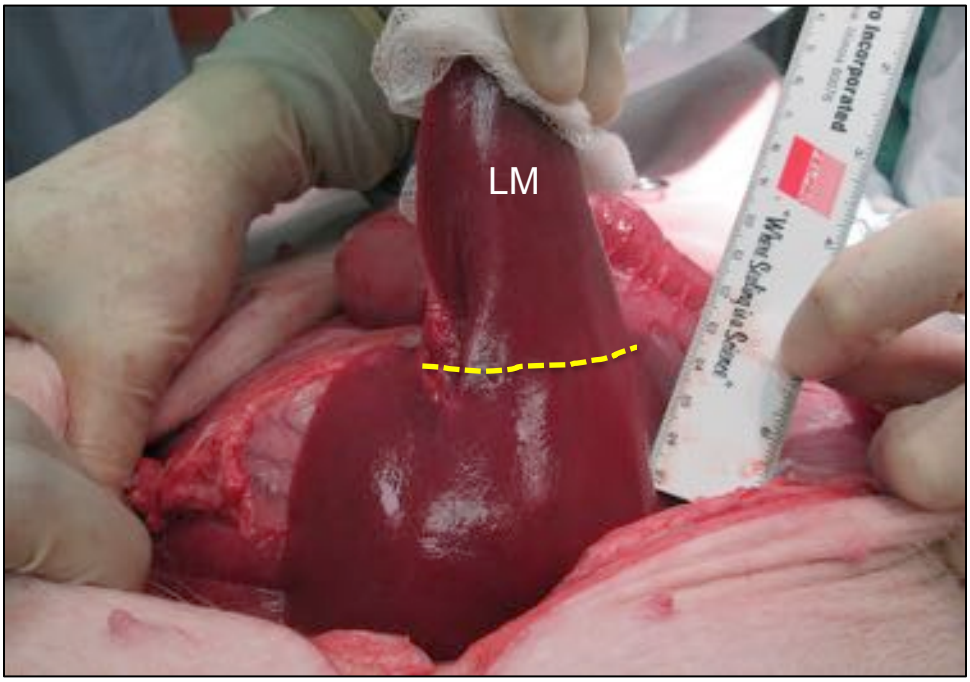

Figure 1, swine 243. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The LM lobe was hypoplastic in this subject, about 1/3 of the typical size. A dashed yellow line indicates the planned line of transection on this lobe. View from the head down to the hindlimbs (superior view).

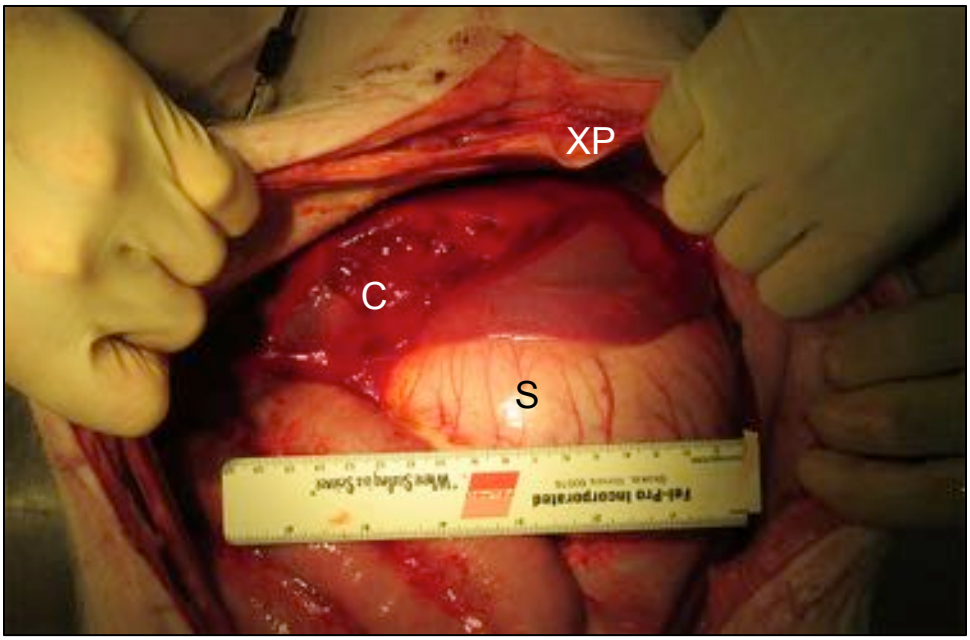

Figure 2, swine 243. Site of injury, after 60 min obsevation; subject alive and well. A large clot (C) overlies the injury site (latter not visible). Loops of intestine are visible inferior to the clot; S = stomach; XP = xiphoid process. Cephalad is at top of image.

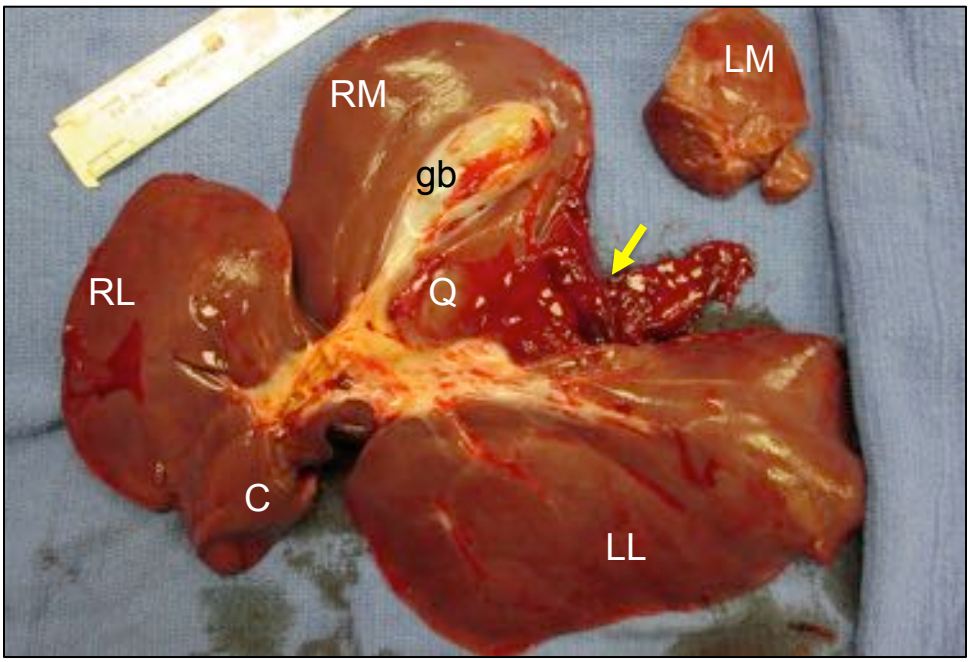

Figure 3, swine 243. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position, note relative small size of this hypoplastic lobe); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Arrow = large clot overlying surface of injury. Anterior is at top of image.

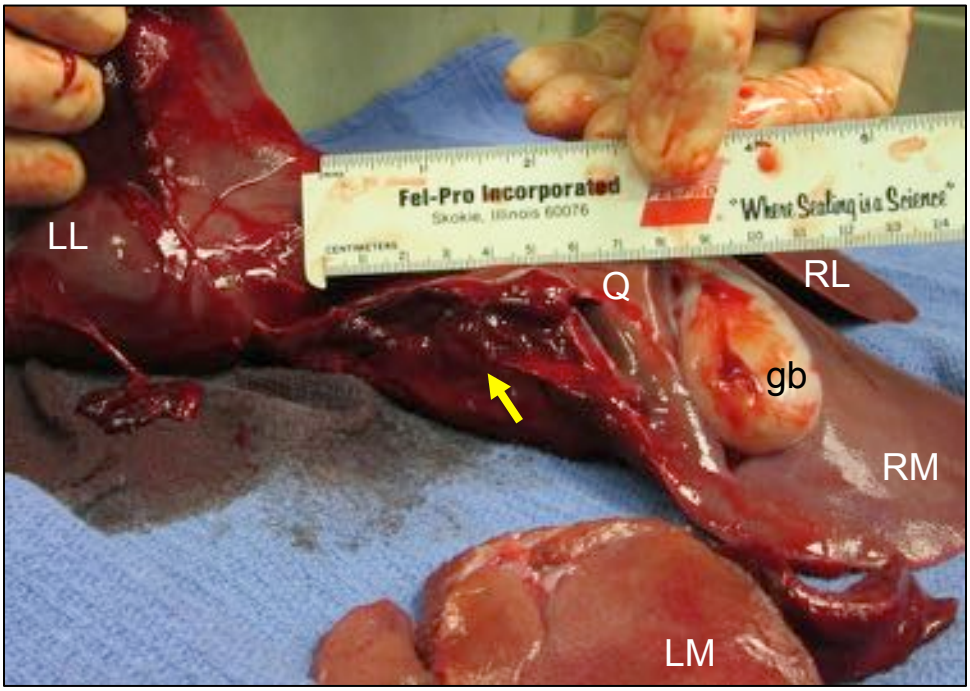

Figure 4, swine 243. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). Clot overlies the cut liver surface (arrow). Injury mechanism was complete transection of LM lobe near its base.

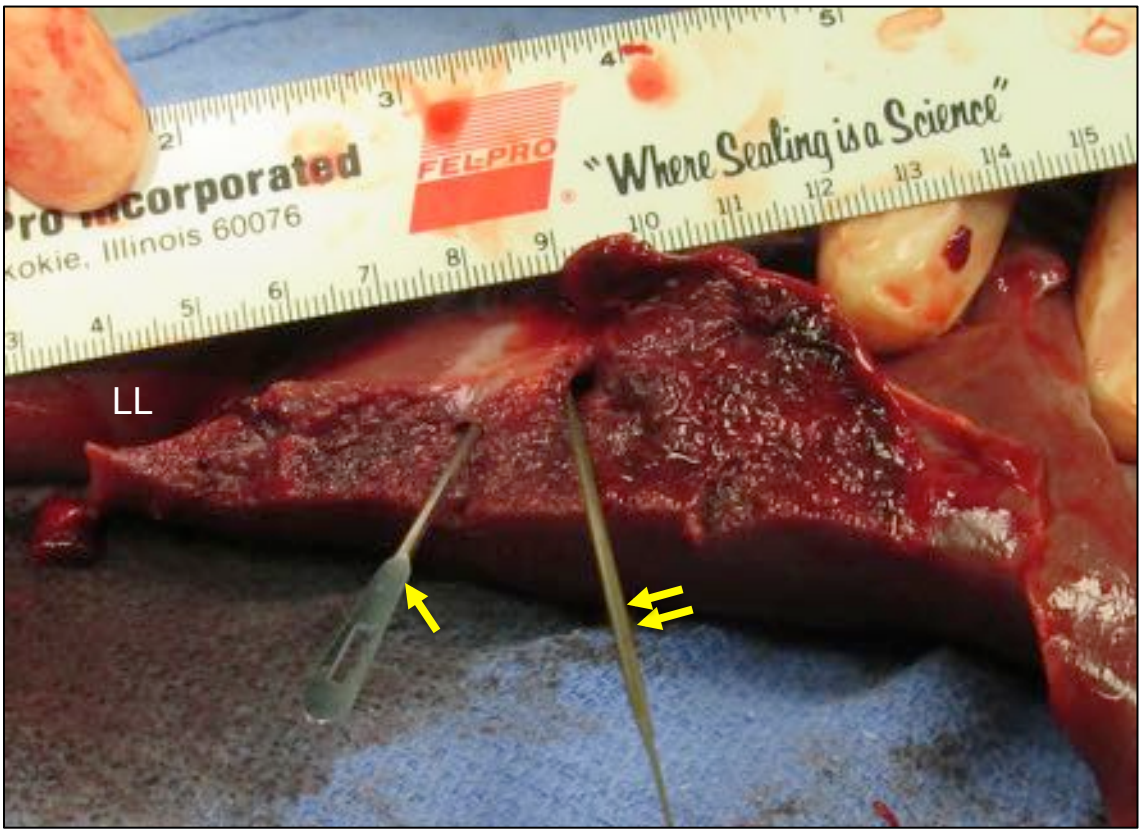

Figure 5, swine 243. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). The clot has been removed from the cut liver surface. Injury mechanism was complete transection of LM lobe near its base; this subject had a hypoplastic LM lobe. Single arrow indicates metal probe inserted in orifice of transected hepatic vein to LM lobe; double arrow indicates metal probe inserted in orifice of transected PV branch to LM lobe. These transected veins were all occluded by clot at the time of necropsy.

I. OVERVIEW
Date: October 24, 2014
Swine no: 246
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 10/17/2014
Pre-procedure wt: 36.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS

- HR: 102
- MAP: 80
- Temp: 38.4
- EtCO2: 51

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:15 AM
Spleen wt: 254.9 gm
LR (22°C) infused after splenectomy: 765 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 254.9 + 20 + 0 = 274.9 mL
- LR (22°C) infused (spleen replacement + incidental): 765 + 200 = 965 mL

Pre-injury VS

- HR: 103
- MAP: 97
- Temp: 37.1
- EtCO2 : 47

III. INJURY & TREATMENT PHASE

Time of injury: 08:27 AM
Injury type: portal/hepatic vein injury, cut across base of left medial lobe, see figs; (Left medial lobe lobectomy). Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was pulled out from the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR, 3.6 L preset maximum (50 mL/kg), given at constant rate of 9.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:28 AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:40 AM

10 min post-injury VS
- HR: 108
- MAP: 80
- Temp: 36.7
- EtCO2: 40

Blood draw no. 3: (30 min post-injury): 09:00 AM

30 min post-injury VS
- HR: 94
- MAP: 58
- Temp: 36.7
- EtCO2: 38

Blood draw no. 4: (60 min post-injury): 09:30 AM

60 min VS
- HR: 92
- MAP: 63
- Temp:35.8
- EtCO2: 39

Blood draw no. 5: (90 min post-injury): 10:00 AM

90 min post-injury VS
- HR: 81
- MAP: 73
- Temp: 35.3
- EtCO2: 38

Blood draw no. 6: (120 min post-injury): 10:30 AM

120 min post-injury VS
- HR: 81
- MAP: 85
- Temp: 34.3
- EtCO2: 38

Blood draw no. 7: (150 min post-injury): 11:00 AM

150 min post-injury VS
- HR: 78
- MAP: 75
- Temp: 34.4
- EtCO2: 38

Blood draw no. 8: (180 min post-injury): 11: 30 AM

180 min post-injury VS
- HR: 114
- MAP: 68
- Temp: 34.9
- EtCO2: 28

Survival at 180 min? Yes
Target MAP attained ? Yes, briefly, on and off
Time of death: 11:30 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 326.6 mL (suction) + 118.9 mL (clot + lap pads) + 160 (phlebotomies)= 605.5 mL
- IV fluid given: LR (37°C): 1450 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and some clots are seen (see Figs). A large clot was covering the surface of the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none

Ex vivo liver wt: 144.1 (resected LM lobe) + 987.3 (remaining liver) = 1131.4g

Tissue harvested: Pancreatic duct.

VI. COMMENTS

Noncompressible injury, LM lobectomy, no Rx, 3 h observation (N = 5 of this group). Easy survival to 3 h. Based on results so far, probably should generate N = 10 prior to using foam.

VII. PLAN

Next procedure will be week of Nov 17th.

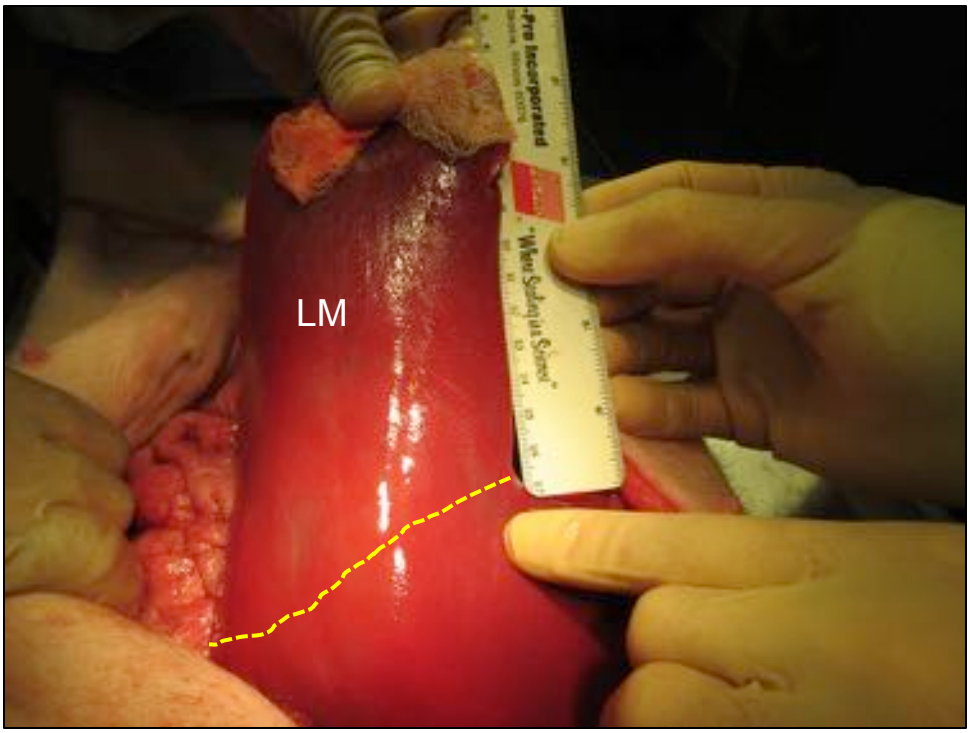

Figure 1, swine 246. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. A dashed yellow line indicates the planned line of transection on this lobe. View from the head down to the hindlimbs (superior view).

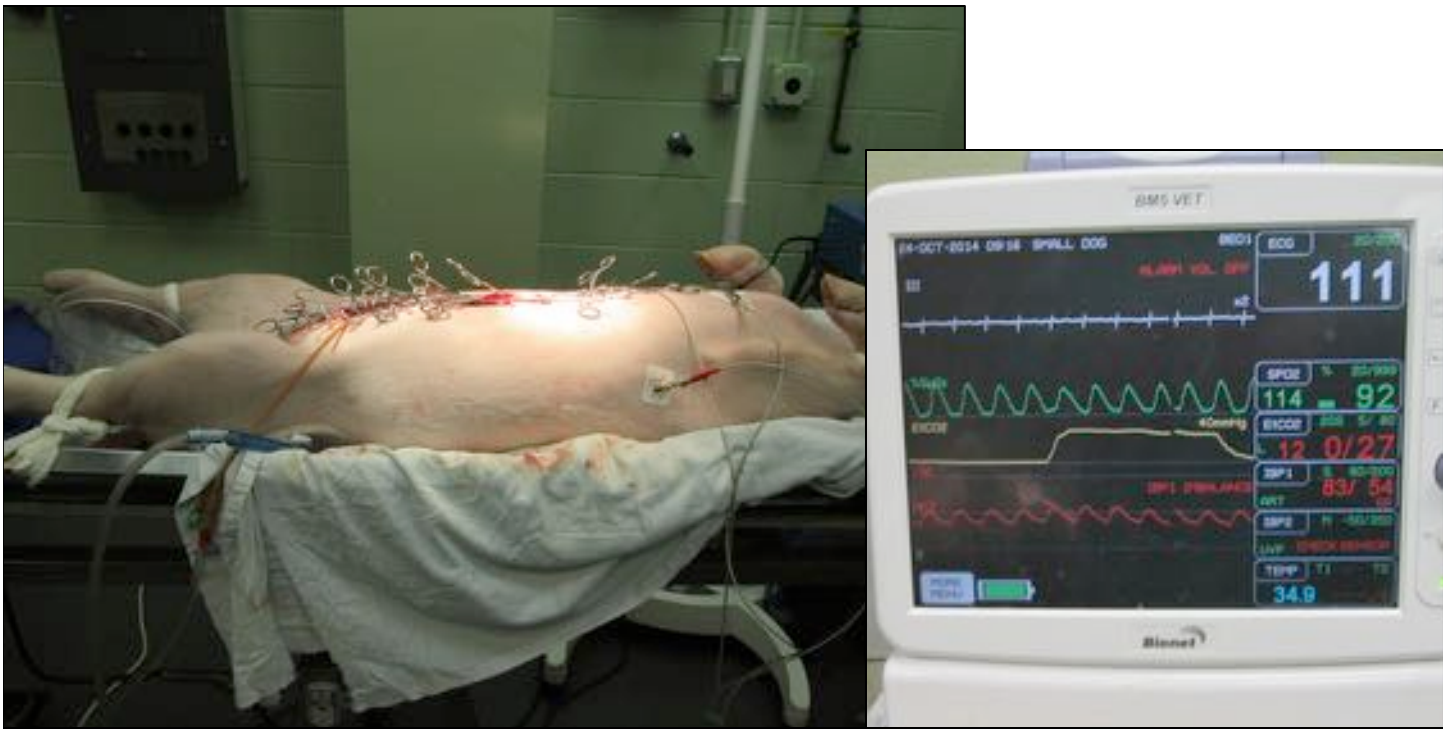

Figure 2, swine 246. Lateral view of swine after completion of 3 h observation period. Subject alive & well, with pulse = 111, MAP = 68, O2 sat = 92%, EtCO2 = 27, temp = 34.9˚C. Abdomen nondistended.

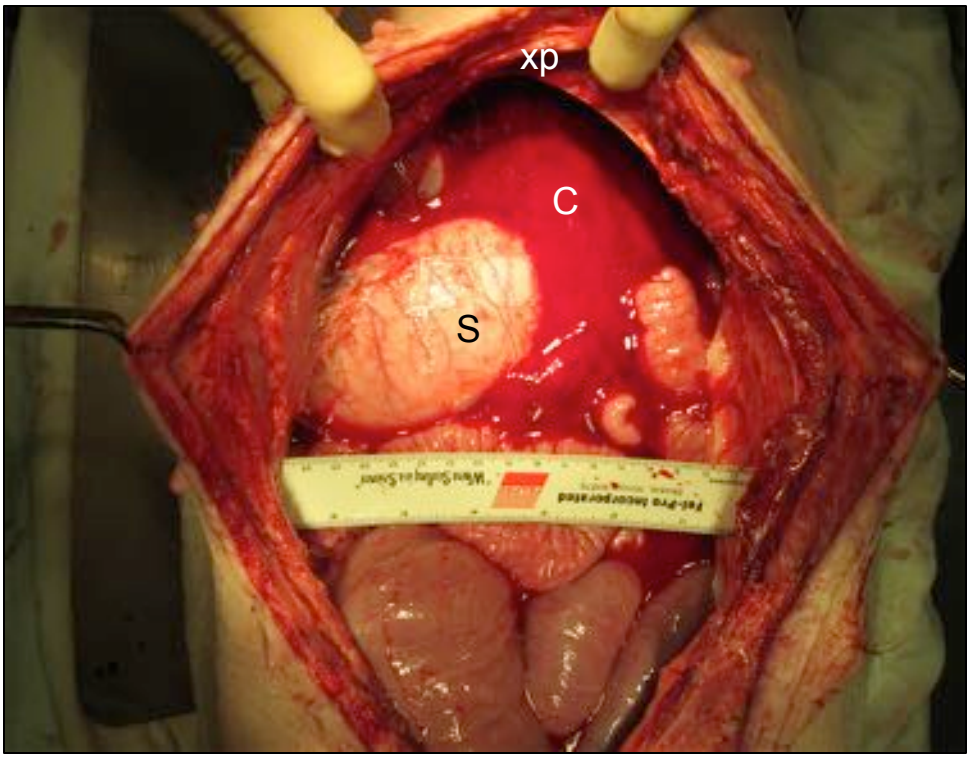

Figure 3, swine 246. Site of injury, after 180 min observation; subject alive and well. A large clot (C) overlies the injury site (latter not visible). Loops of intestine are visible inferior to the clot; S = stomach; XP = xiphoid process. Cephalad is at top of image.

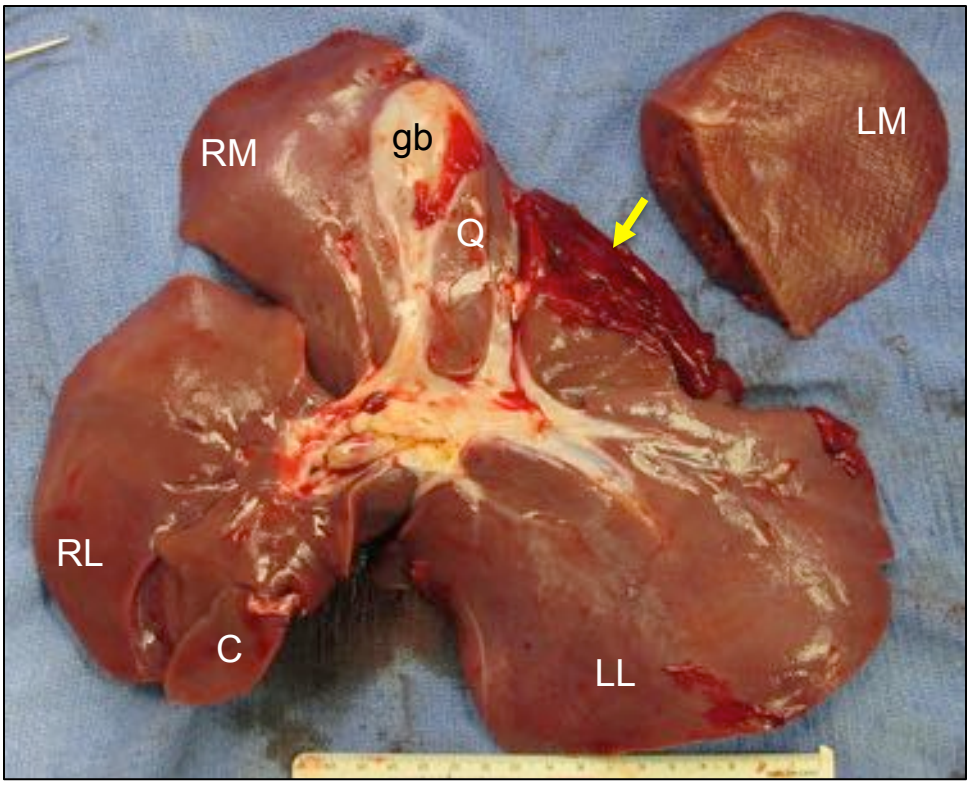

Figure 4, swine 246. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Arrow = large clot overlying surface of injury. Anterior is at top of image.

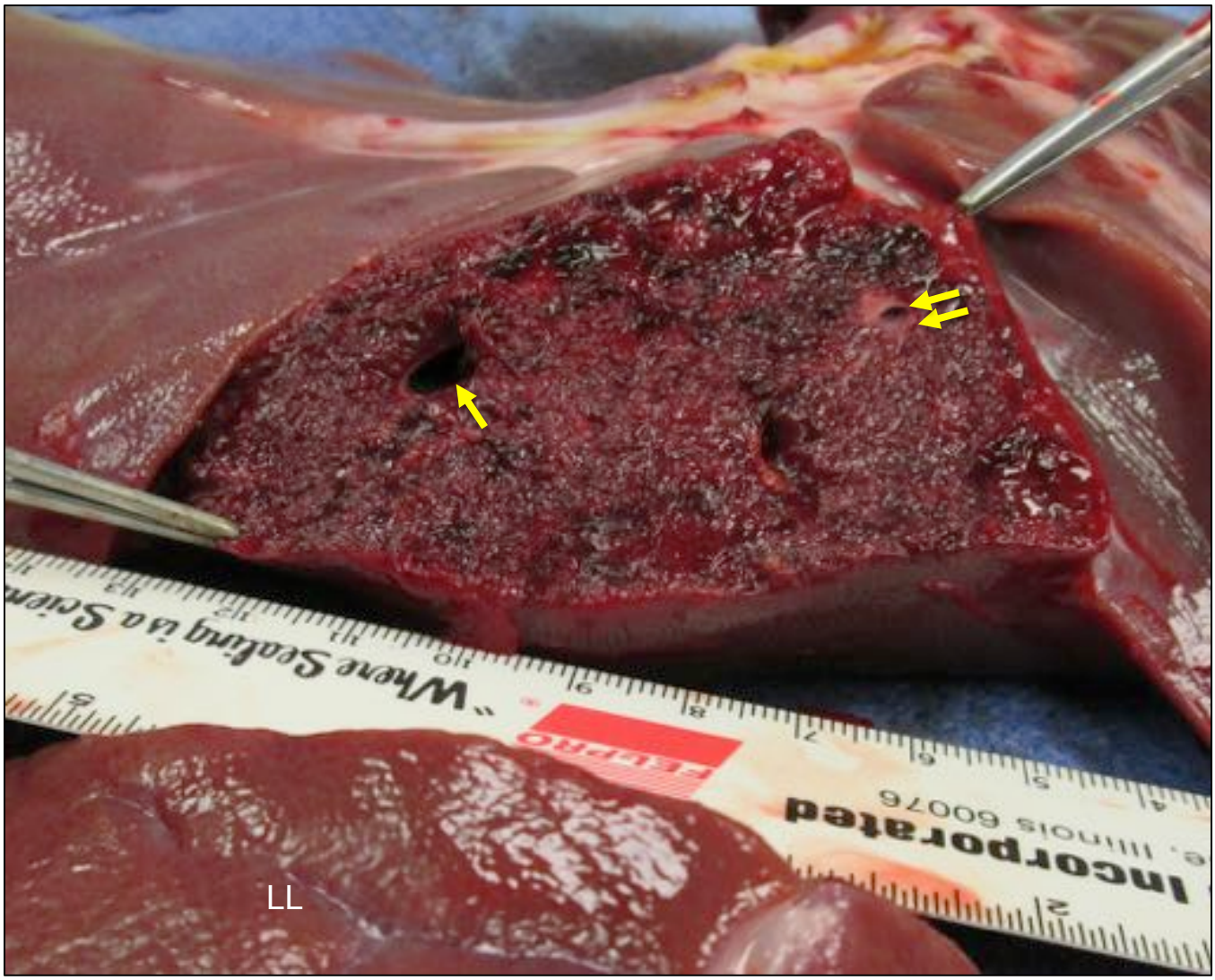

Figure 5, swine 246. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). The clot has been removed from the cut liver surface. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates orifice of transected hepatic vein to LM lobe; double arrow indicates orifice of transected PV branch to LM lobe. These transected veins were all occluded by clot at the time of necropsy.

I. OVERVIEW
Date: November 20, 2014
Swine no: 248
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury (LLL hemistransection)
Treatment: None (No treatment control)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 08:45 AM
Swine sex: male
Date swine received from UNL Mead: 11/14/2014
Pre-procedure wt: 36.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS

- HR: 108
- MAP: 118
- Temp: 38.0
- EtCO2: 45

Blood draw no. 1 (initial): 9:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:45 AM
Spleen wt: 417.5 gm
LR (22°C) infused after splenectomy: 1252.5 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 417.5 + 20 + 423.5 = 861.0 mL
- LR (22°C) infused (spleen replacement + incidental): 1252.5 + 1400 = 2652.5 mL

Pre-injury VS

- HR: 99
- MAP: 100
- Temp: 36.4
- EtCO2 : 48

III. INJURY & TREATMENT PHASE

Time of injury: 10:15 AM
Injury type: portal/hepatic vein injury, cut across base of left lateral lobe (i.e., LLL hemitransection; see Figs). The scissors were applied in the cleft between the LM and LL lobes, but directed to the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 80
Resuscitation fluid: warm LR, 3.7 L preset maximum (100 mL/kg), given at constant rate of 150 mL/min, continuously during the entire 60 min observation period, or until animal expires.
Time resuscitation fluid began: 10:15AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 10:30 AM

15 min post-injury VS
- HR: 158
- MAP: 34
- Temp: 37.1
- EtCO2: 36

Blood draw no. 3: (Final, 38 min post-injury): 10:53 AM

Final VS:
- HR: 0
- MAP: 0
- Temp: 36.6
- EtCO2: 0

Survival at 60 min? No
Target MAP attained ? No
Time of death: 10:53 AM
Cause of death: exsanguination from injury
Interval from injury to death: 38 min

Post-treatment fluid data:
- Blood loss: 1812.4 mL (suction) + 859.1 mL (clot + lap pads) + 40 mL (phlebotomies) = 2711.5mL
- IV fluid given: LR (37°C): 3850 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen mildly distended. Upon re-opening abdomen, large amounts of blood and clots are seen (see Figs). No clot was covering the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe.
Number of portal veins lacerated: 2 branches, to LL lobe
Other: none
Ex vivo liver wt: 808.5 g

Tissue harvested: None

VI. COMMENTS

This was a no-treatment control for the LLL hemitransection model. We originally did 10 of these subjects, which were used in the recent *PLOS ONE* paper. The reason we are revisiting this injury mechanism is that we are required to submit a manuscript to the journal *Surgery* to go along with the abstract that Ujwal will be submitting to the CSA meeting in March of 2015 (see accompanying attachment). Since the comparator group of swine in this abstract (LLLH injury, no treatment, rapid fluid resuscitation) was the group of 10 subjects described in the PLOS ONE paper, we will need to supplement this group with additional subjects to avoid dual-publication issues when the *Surgery* manuscript is submitted. I suppose 2-3 additional subjects would be adequate for this purpose. So today's swine was an additional subject for this purpose.

Today's subject had some hypotension during the preparatory phase, but this responded to some extra fluid and ventilator adjustments, so by the time of injury the MAP was acceptable (~100 mm Hg). Though I was a little too aggressive with the scissors today (there was a near-complete amputation of the LLL; see Figures), the number of vessels injured still was consistent with previous subjects. As expected this subject, resuscitated with 100 mL/kg of crystalloid given at 150 mL/min, died from exsanguination prior to completion of the 60 min observation period.

VII. PLAN

Accrue several more control subjects for the purpose of the above *Surgery* submission. Next subject was scheduled for Tue Nov. 25th, but this subject had to be euthanized in our facility on Nov 22nd because of a joint infection. So next subject will not be until after Thanksgiving, on Tue Dec 2nd.

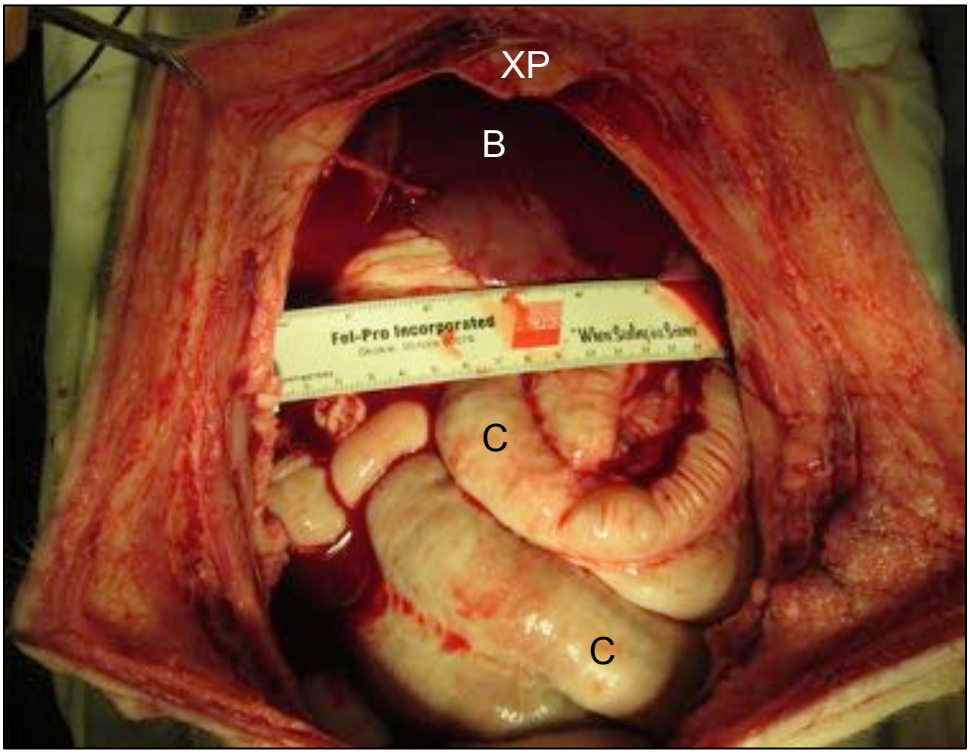

Figure 1, swine 248. Overhead view of abdomen, reopened after death by exsanguination at 38 min. Large amount of unclotted blood (B) present superficially. C = loops of colon; XP = xiphoid process.

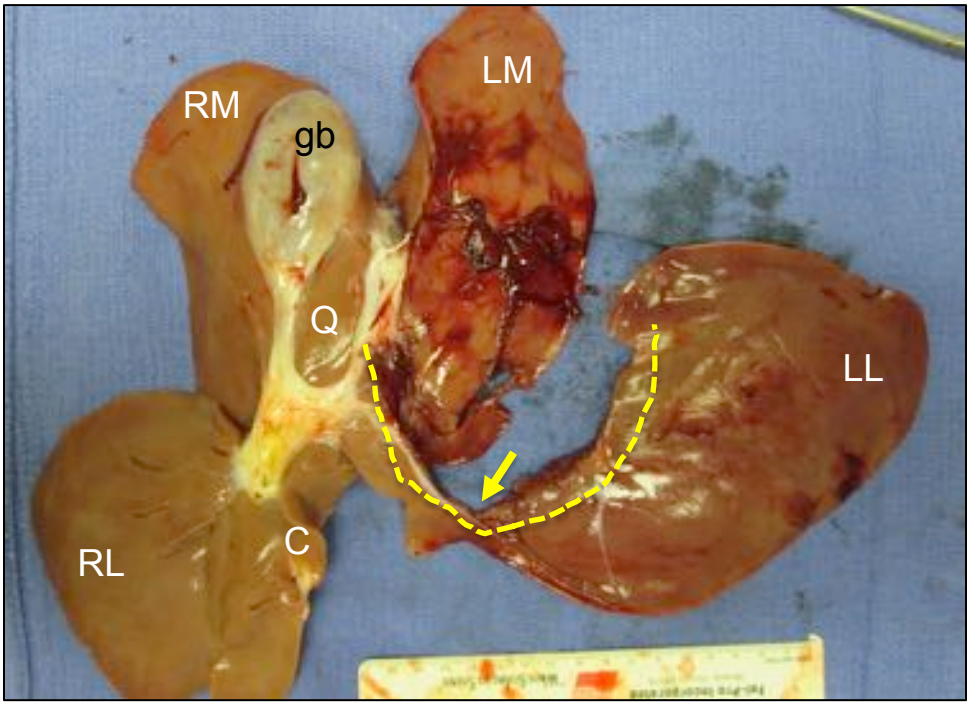

Figure 2, swine 248. Liver *ex vivo*, inferior surface. The injury mechanism was hemitransection of the LL lobe near its base. In this subject, the hemitransection was nearly a complete transection; the LL lobe remained attached to the rest of the liver by just a small strand of parenchyma (arrow). Dashed yellow line = gap induced by hemitransection. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial; LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Anterior is at top of image.

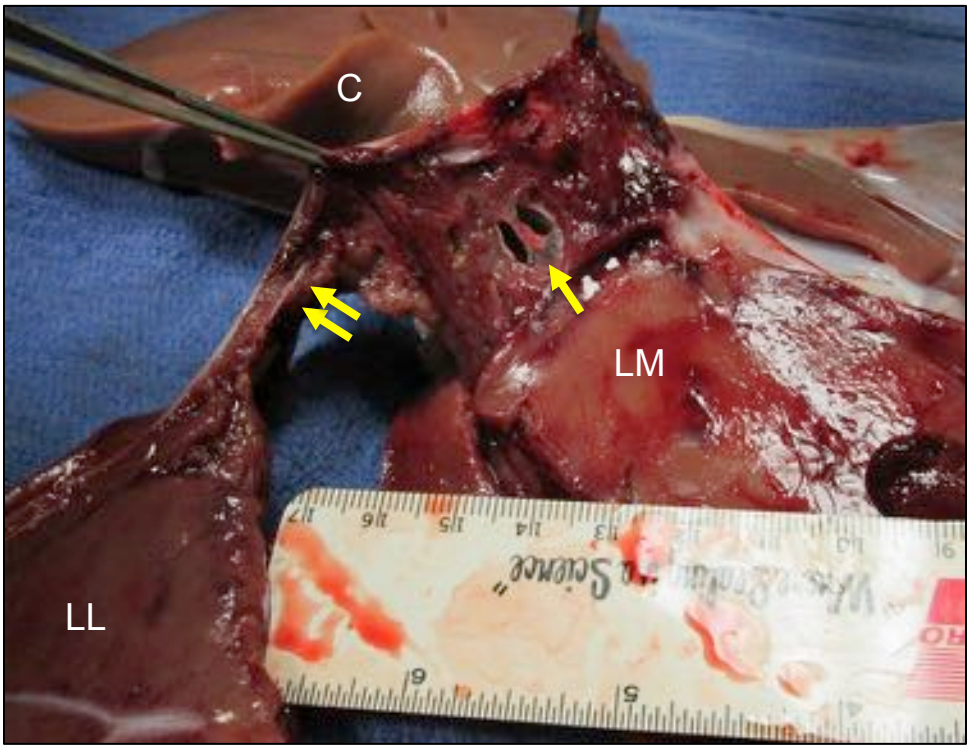

Figure 3, swine 248. Liver *ex vivo*, close-up of injury site (left lateral aspect, inferior is toward top of image). Single arrow indicates lumen of transected hepatic vein to LL lobe. HV appears to have been transected at a branch point, as indicated by a septum running through the lumen. Double arrow indicates thin strand of parenchyma by which LL lobe is still attached to the main body of the liver.

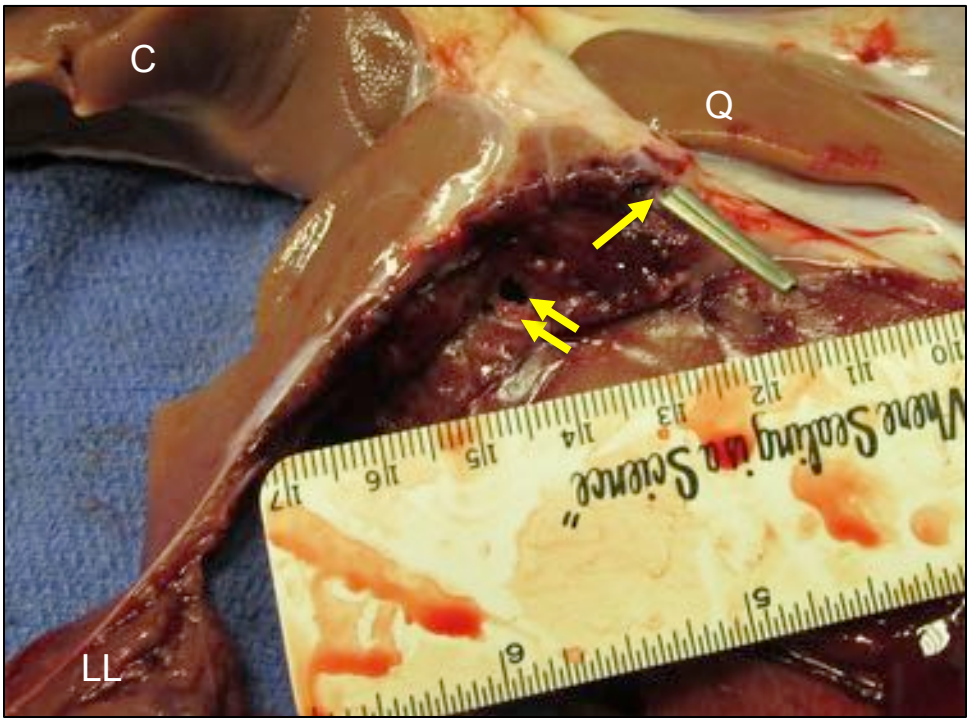

Figure 4, swine 248. Liver *ex vivo*, close-up of injury site, similar view as in Fig. 3. Single arrow indicates transected branch of portal vein to LL lobe (with tip of forceps emerging). Double arrow indicates transected HV seen in Fig. 3.

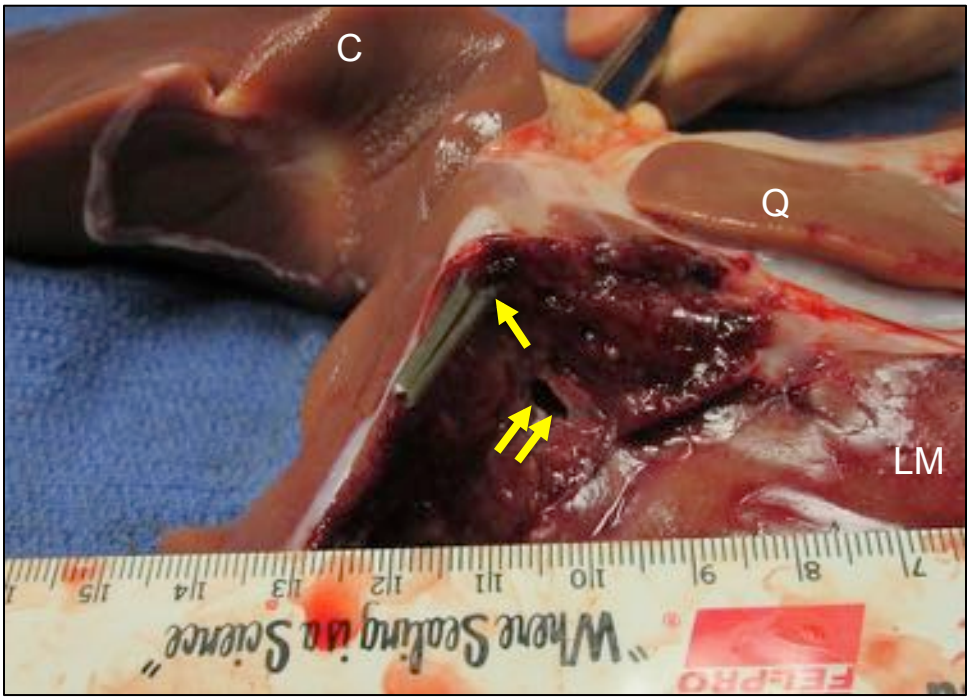

Figure 5, swine 248. Liver *ex vivo*, close-up of injury site, similar view as in Fig. 3. Single arrow indicates a second transected branch of portal vein to LL lobe (with tip of forceps emerging); i.e., there were two PV branches transected in this subject. Double arrow again indicates transected HV seen in Fig. 3.

I. OVERVIEW
Date: December 02, 2014
Swine no: 250
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury (LLLH)
Treatment: None (No treatment control); high-rate LR resuscitation
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 11/25/2014
Pre-procedure wt: 34.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 86
- MAP: 115
- Temp: 37.3
- EtCO2: 44

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:40 AM
Spleen wt: 549.4 gm
LR (22°C) infused after splenectomy: 1114.5 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 549.5 + 20 + 0 = 569.5 mL
- LR (22°C) infused (spleen replacement + incidental): 1114.5 + 100 = 1214.5 mL

Pre-injury VS
- HR: 82
- MAP: 130
- Temp: 36.7
- EtCO2 : 45

III. INJURY & TREATMENT PHASE

Time of injury: 09:52 AM
Injury type: portal/hepatic vein injury, cut across base of left lateral lobe, see figs; (i.e., the LLLH, or left lateral lobe hemitransection for the noncompressible hemorrhage model). The scissors were applied in the cleft between the LM and LL lobes, but directed to the base of the LLL. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 105
Resuscitation fluid: warm LR, 3.5 L preset maximum (100 mL/kg), given at constant rate of 150 mL/min, continuously during the entire 60 min observation period, or until animal expires.
Time resuscitation fluid began: 08:53AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 10:07 AM

15 min post-injury VS
- HR: 84
- MAP: 103
- Temp: 36.4
- EtCO2: 39

Blood draw no. 3: (Final, 60 min post-injury): 09:53 AM

Final VS:
- HR: 81
- MAP: 41
- Temp: 36.4
- EtCO2: 33

Survival at 60 min? Yes
Target MAP attained ? Briefly.
Time of death: 09:53 AM
Cause of death: exsanguination from euthanasia
Interval from injury to death: 60 min

Post-treatment fluid data:
- Blood loss: 767.5 mL (suction) + 806.3 mL (clot + lap pads) + 40 mL (phlebotomies) = 1613.8 mL
- IV fluid given: LR (37°C): 3610 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen mildly distended. Upon re-opening abdomen, large amounts of clotted blood was seen (see Figs). Clots were covering the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe (small)
Number of portal veins lacerated: 1 branch, to LL lobe (small)
Other: none
Ex vivo liver wt: 682.1 g

Tissue harvested: skin strips and liver strips

VI. COMMENTS

Subject number 12 in noncompressible hemorrhagic model using the LLLH injury mechanism with the "rapid" (150 mL/min) infusion protocol. With this subject, N = 12 for the LLLH mechanism and the rapid infusion protocol.

VII. PLAN

We now will obtain 2 more subjects in the slow infusion protocol in order to get N = 12 in that group, analyze the data, write the manuscript, and then submit this to *Surgery* (an obligation for having our abstract on this topic accepted to the March meeting of the Central Surgical Association).

I. OVERVIEW
Date: December 5, 2014
Swine no: 251
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury (LLLH)
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 11/25/2014
Pre-procedure wt: 37.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 97
- MAP: 89
- Temp: 39.1
- EtCO2: 57

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 359.5 gm
LR (22°C) infused after splenectomy: 1078.5 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 359.5 + 20 + 44.9 = 424.4 mL
- LR (22°C) infused (spleen replacement + incidental): 1078.5 + 40 = 1118.5 mL

Pre-injury VS
- HR: 91
- MAP: 101
- Temp: 37.9
- EtCO2 : 60

III. INJURY & TREATMENT PHASE

Time of injury: 08:53 AM
Injury type: portal/hepatic vein injury, cut across base of left lower lobe (i.e., the left lateral lobe hemitransection injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 80
Resuscitation fluid: warm LR, 3.7 L preset maximum (100 mL/kg), given at constant rate of 20 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (100 mL/kg) ÷ 180 min; begin at time of injury and continue for 180 min or until subject expires.
Time resuscitation fluid began: 08:54AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:05 AM

10 min post-injury VS
- HR: 122
- MAP: 19
- Temp: 37.2
- EtCO2: 21
- IAP: 0

Blood draw no. 3: (30 min post-injury): 09:25 AM

30 min VS
- HR: 87
- MAP: 15
- Temp: 36.8
- EtCO2: 13

Blood draw no. 4: (60 min post-injury): 09:55 AM

60 min VS
- HR: 106
- MAP: 23
- Temp: 36.3
- EtCO2: 32

Blood draw no. 5: (90 min post-injury): 10:25 AM

90 min VS
- HR: 123
- MAP: 27
- Temp: 35.8
- EtCO2: 38

Blood draw no. 6: (120 min post-injury): 10:55 AM

120 min VS
- HR: 114
- MAP: 28
- Temp: 35.4
- EtCO2: 31

Blood draw no. 7: (150 min post-injury): 11:25 AM

150 min VS
- HR: 93
- MAP: 29
- Temp: 35.2
- EtCO2: 30

Blood draw no. 8: (180 min post-injury): 11:55 AM

180 min VS
- HR: 90
- MAP: 33
- Temp: 35.0
- EtCO2: 32

Survival at 180 min? Yes
Target MAP attained ? No.
Time of death: 11:55 AM
Cause of death: intentional exsanguination of euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 826.7 mL (suction) + 906.4 mL (clot + lap pads) = 1733.1 mL
- IV fluid given: LR (37°C): 3800 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, somewhat tense (see Figs). Upon re-opening abdomen, it was found that the abdominal distension was secondary to distension of the stomach and intestines, but not really from blood (see Figs). There was a moderate amount of clotted blood overlying the liver (see Figs), with unclotted blood in both colonic gutters.

Heart: not examined.
Number of hepatic veins lacerated: 1, to LL lobe (large).
Portal vein injury: 1 branch, to LL lobe (large)
Other: left medial lobe was hypotrophic, and fused to the quadrate and right medial lobes (see Fig. 3). The left lateral lobe (injury lobe) was hypertrophic (see Figs).
Ex vivo total liver wt: 944.4 g

Tissue harvested: skin strips, rectus sheath, and pancreas

VI. COMMENTS

LLLH noncompressible model, no treatment control with slow intravenous infusion rate, N = 11 for this group, need N = 12. Even though he was markedly hypotensive throughout the 180 min observation period, subject survived. The MAP did slowly improve over time. Subject's relatively good outcome may have been secondary to possible tamponade from his abdominal distension, which was called by gas-filled stomach & intestines. Cause of gastrointestional distension is not clear. Intestine became more distended as time progressed, suggesting that there was a continual insufflation of air into the GI tract from some source Endotracheal tube? But subject's lungs obviously were well-ventilated, indicated that ET tube was in correct position.

VII. PLAN

Obtain one more subject in this group for the CSA *Surgery* manuscript. I am out of town next week. Next subject in this protocol will be Dec 19th.

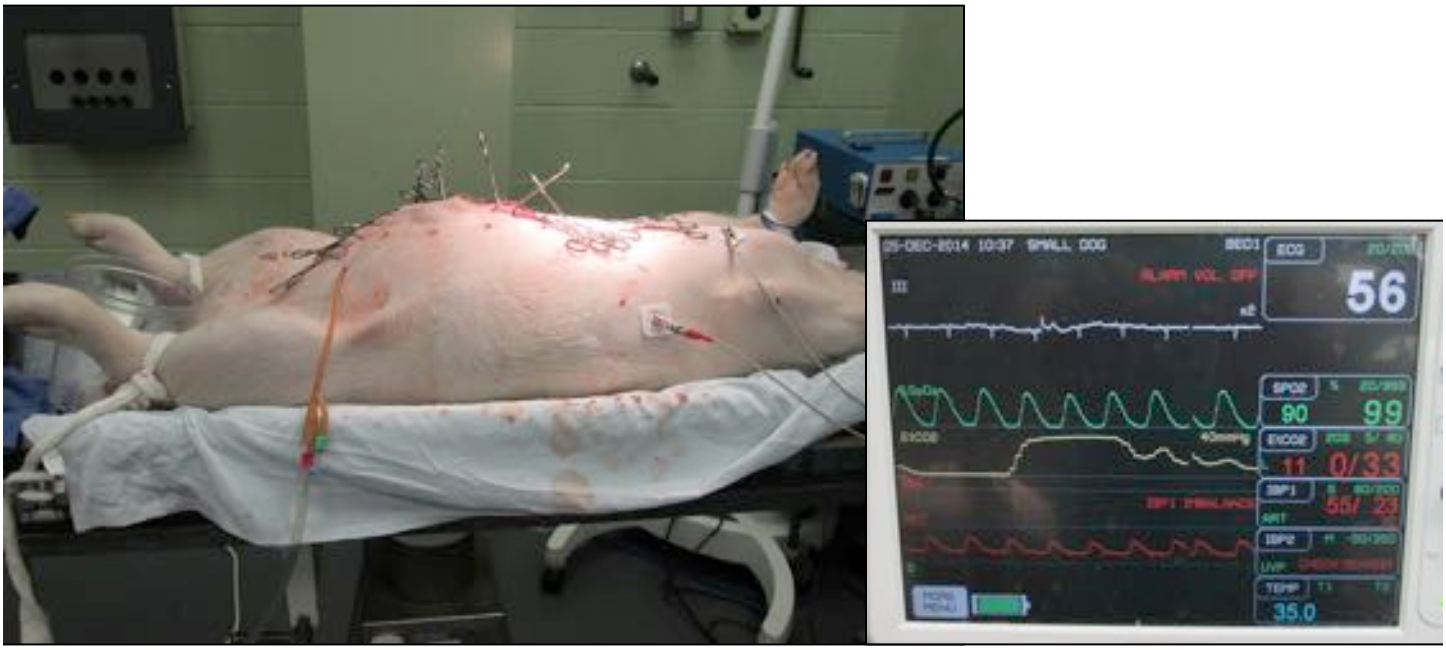

Figure 1, swine 251. Side view of subject at end of 3 h observation. Subject alive but hypotensive with MAP = 32; marked abdominal distension.

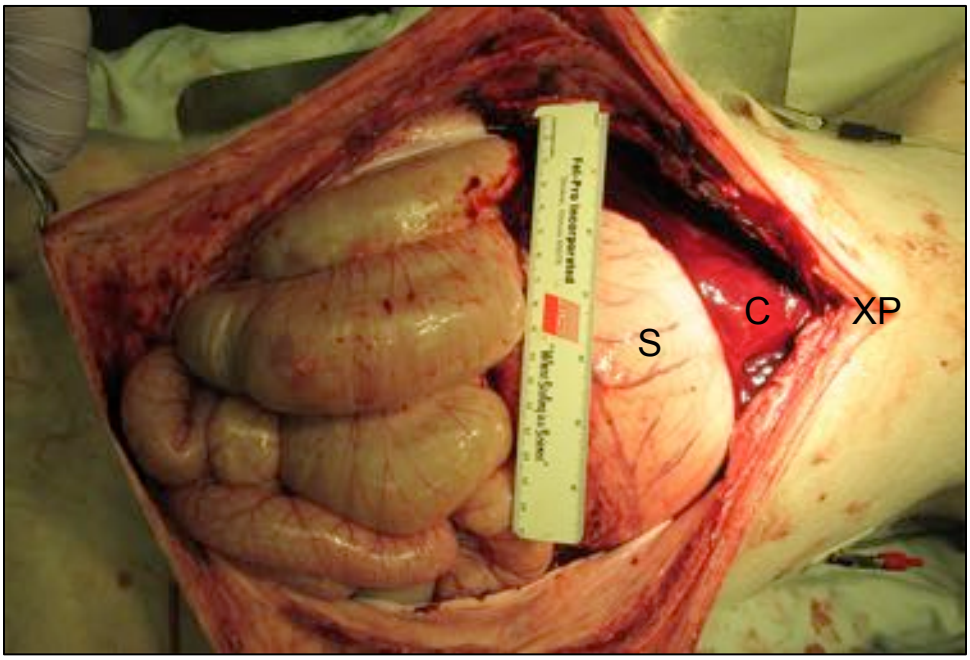

Figure 2, swine 251. Overhead view of abdomen, reopened after 3 h observation. Subject still alive. Large clot (C) overlying liver, but most of abdominal distension due to intestines, full of air. XP = xiphoid process; S = stomach; cephalad is at right of image.

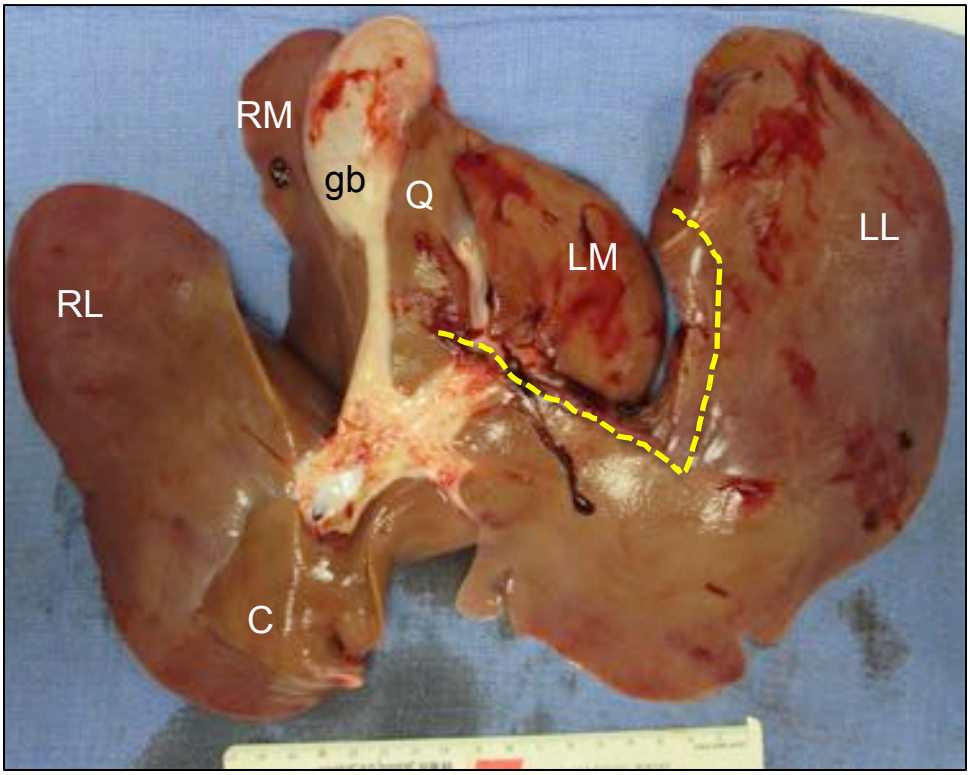

Figure 3, swine 251. Liver *ex vivo*, inferior surface. The injury mechanism was hemitransection of the LL lobe near its base. Dashed yellow line = gap induced by hemitransection. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (hypotrophic); LL = left lateral (hypertrophic); Q = quadrate; C = caudate; gb = gallbladder. Anterior is at top of image.

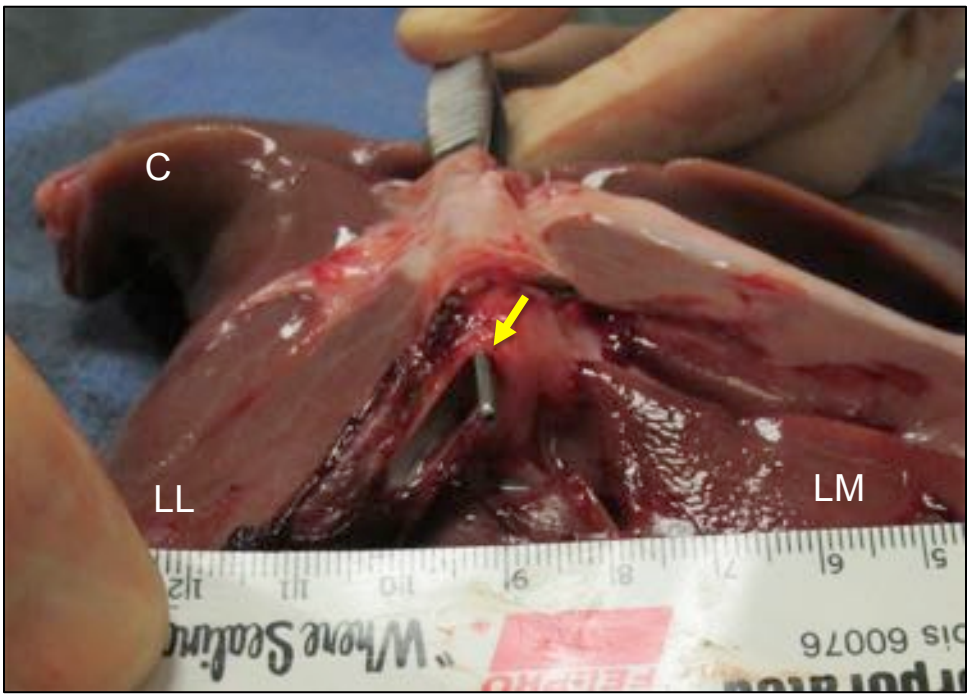

Figure 4, swine 251. Liver *ex vivo*, close-up of inferior side of injury site, left lateral view. Arrow indicates transected branch of portal vein to LL lobe (with tip of forceps emerging); this branch was large in this subject. Anterior at right of image.

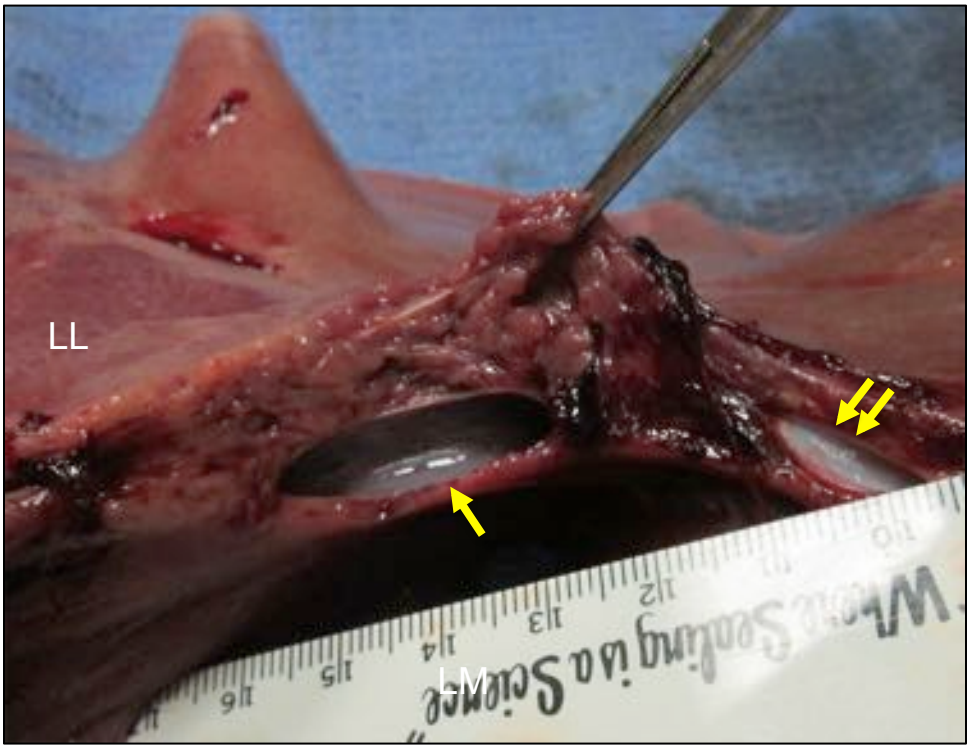

Figure 5, swine 251. Liver *ex vivo*, close-up of injury site, similar view as in Fig. 4. Single arrow indicates transected hepatic vein to LL lobe, large in this subject. Double arrow indicates transected PV branch seen in Fig. 4.

I. OVERVIEW
Date: December 19, 2014
Swine no: 253
Model: swine, normothermic, normovolemic noncompressible hemorrhage; PV + HV injury
Treatment: None (No treatment control using restricted IVF rate)
Personnel: Carlson, Yanala, Hansen, Siford.

II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male
Date swine received from UNL Mead: 12/12/2014
Pre-procedure wt: 34.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 70
- MAP: 78
- Temp: 36.8
- EtCO2: 37

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:40 AM
Spleen wt: 316 gm
LR (22°C) infused after splenectomy: 948 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 316 + 20 + 10 = 346.0 mL
- LR (22°C) infused (spleen replacement + incidental): 948 + 350 = 1298 mL

Pre-injury VS
- HR: 68
- MAP: 86
- Temp: 35.0
- EtCO2 : 36

III. INJURY & TREATMENT PHASE

Time of injury: 08:57 AM
Injury type: portal/hepatic vein injury, cut across base of left lateral lobe (i.e., the "standard" injury for the noncompressible model). The scissors were applied in the cleft between the LM & LL lobes. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips.
Treatment formulation: No treatment.
Clotting factors: None
Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left lateral) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the injured liver lobe was dropped back into the abdomen, and the upper half of the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 68
Resuscitation fluid: warm LR, 3.44 L preset maximum (100 mL/kg), given at constant rate of 19.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. This is a "hypotensive resuscitation" protocol.
Formula for IVF rate in hypotensive resuscitation protocol: (Subject wt in kg) × (100 mL)/180 min = mL/min
Time resuscitation fluid began: 08:58AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-injury): 09:10 AM

10 min post-injury VS
- HR: 88
- MAP: 15
- Temp: 34.9
- EtCO2: 12

Blood draw no. 3: (30 min post-injury): 09:30AM

30 min VS
- HR: 138
- MAP: 25
- Temp: 34.1
- EtCO2: 27

Blood draw no. 4: (60 min post-injury): 10:00 AM

60 min VS
- HR: 126
- MAP: 28
- Temp: 34.0
- EtCO2: 22

Blood draw no. 5: (90 min post-injury): 10:30 AM

90 min VS
- HR: 103
- MAP: 33
- Temp: 33.6
- EtCO2: 27

Blood draw no. 6: (120 min post-injury): 11:00 AM

120 min VS
- HR: 91
- MAP: 35
- Temp: 33.1
- EtCO2: 32

Blood draw no. 7: (150 min post-injury): 11:30 AM

150 min VS
- HR: 78
- MAP: 38
- Temp: 32.7
- EtCO2: 33

Blood draw no. 8: (180 min post-injury): 12:00 PM

180 min VS
- HR: 72
- MAP: 48
- Temp: 32.8
- EtCO2: 34

Survival at 60 min? Yes
Target MAP attained ? No
Time of death: 12:00 PM
Cause of death: intentional exsanguination (euthanasia) after completion of 3 h observation
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 1097.4 mL (suction) + 885.8 mL (clot + lap pads) = 1982.9 mL
- IV fluid given: LR (37°C): 3500 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, large amounts of blood and clots present in upper abdomen (see Figs). A large clot was covering the surface of the injury site (see Figs).
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe
Portal vein injury: 1 large branch, to LM lobe

Other: none
Ex vivo liver wt: 885.5 g
Tissue harvested: None

VI. COMMENTS

LLLH noncompressible model, no treatment control with slow intravenous infusion rate, N = 12 for this group, which completes this group. We now have the numbers we need to submit a manuscript on this topic (slow vs. rapid fluid resuscitation) to *Surgery*.

As observed before with the slow-infusion subjects, this subject was markedly hypotensive early on, but slowly improved during the 180 min observation period. This is in contrast with the rapid infusion subjects, who for the most part did not exihibit this recovery.

VII. PLAN

Write up *Surgery* manuscript, which is a companion piece to the Central Surgical Association abstract that will be presented at the CSA meeting in Chicago this March.

At this point we will return to the lobectomy-type injury for the noncompressible model, with new procedures to be scheduled for the month of January.

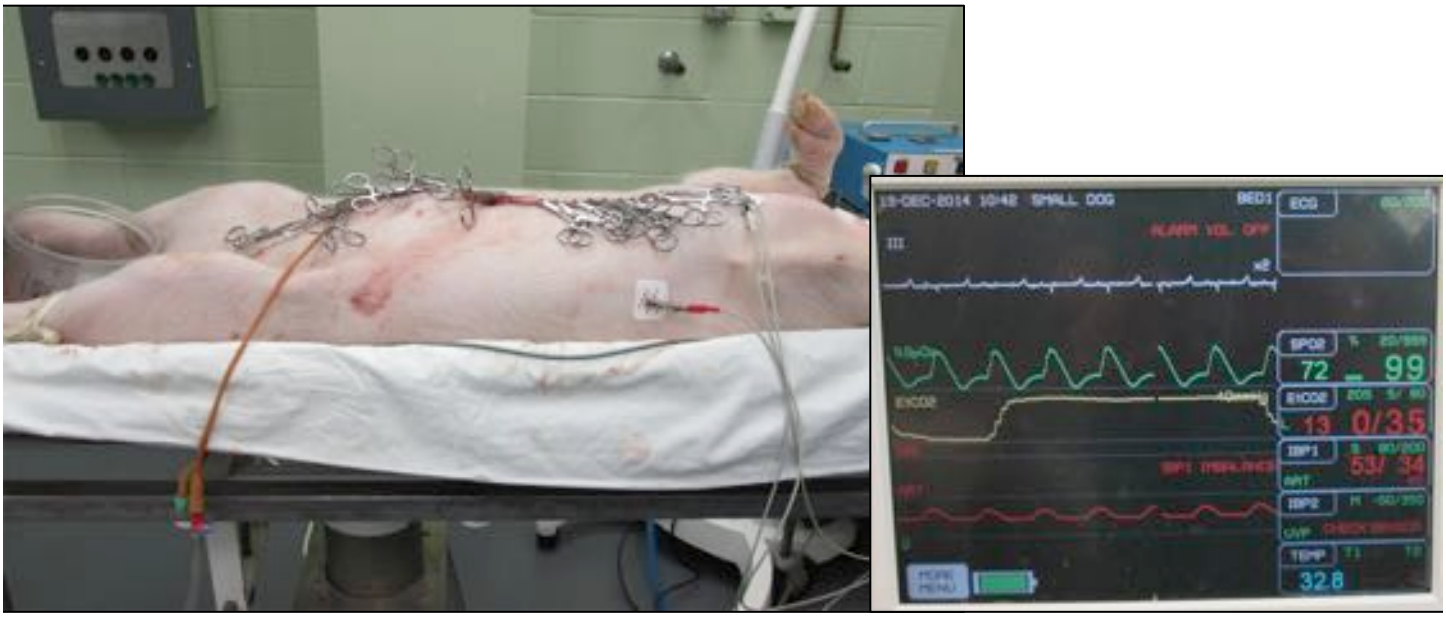

Figure 1, swine 253. Side view of subject at end of 3 h observation. Subject alive with pulse = 72, MAP = 40, temp = 32.8, EtCO2 = 35, O2 Sat = 99%.

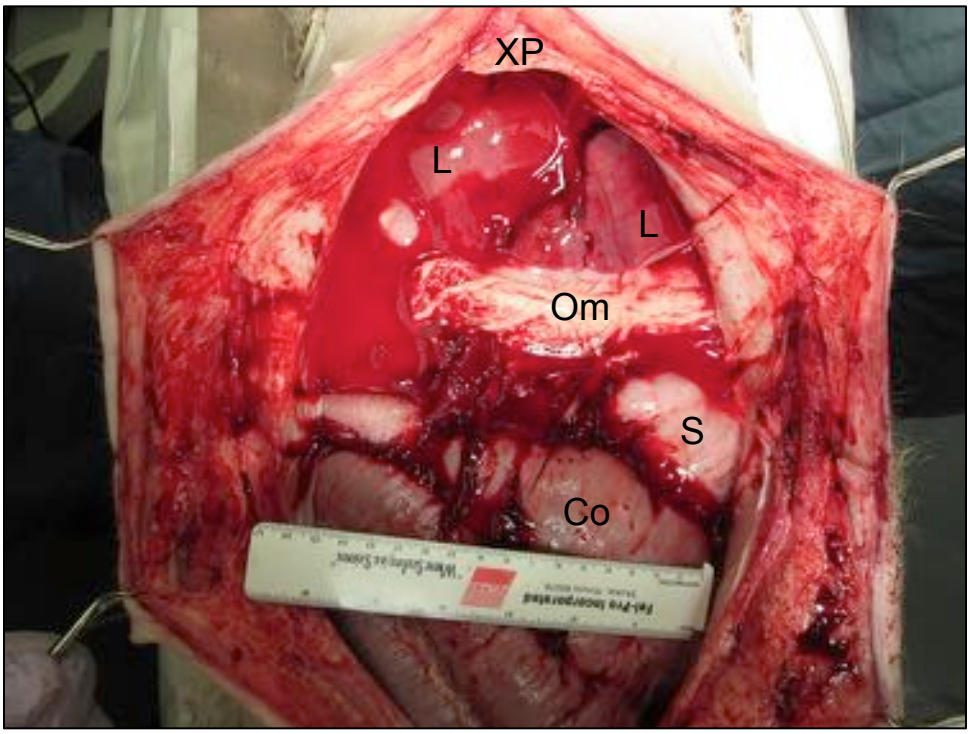

Figure 2, swine 253. Overhead view of abdomen, reopened after 3 h observation. Subject still alive. Liver (L) shown surrounded by liquid blood and clot. XP = xiphoid process; S = stomach; Om = omentum; Co = colon; cephalad is at top of image.

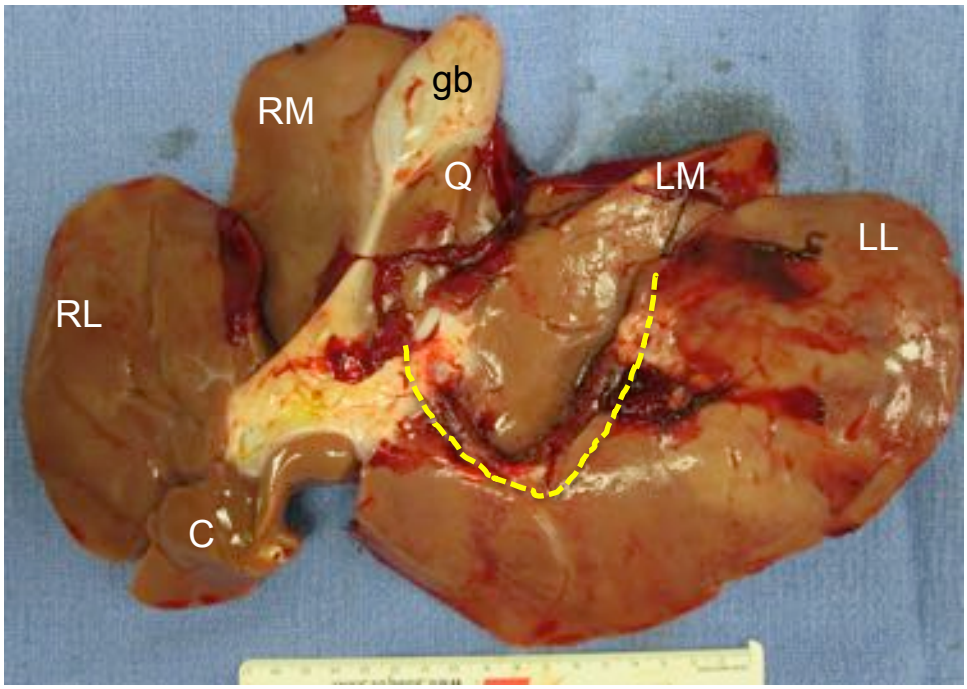

Figure 3, swine 253. Liver *ex vivo*, inferior surface. The injury mechanism was hemitransection of the LL lobe near its base. Dashed yellow line = gap induced by hemitransection. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial; LL = left lateral; Q = quadrate; C = caudate; gb = gallbladder. Anterior is at top of image.

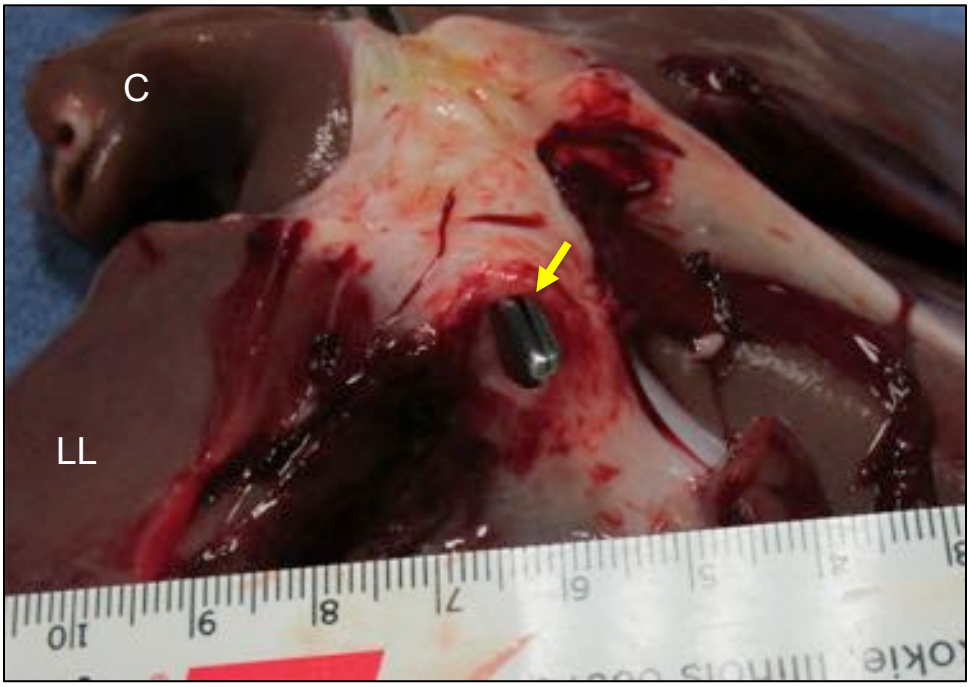

Figure 4, swine 253. Liver *ex vivo*, close-up of inferior side of injury site, left lateral view. Arrow indicates transected branch of portal vein to LL lobe (with tip of forceps emerging); this branch was large in this subject. Anterior at right of image.

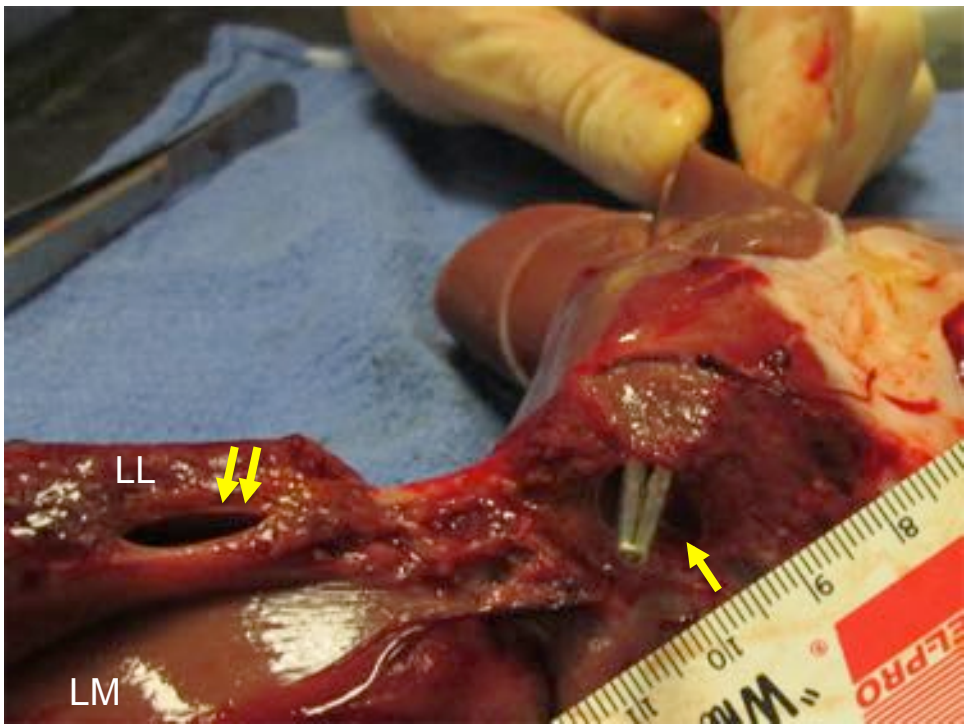

Figure 5, swine 253. Liver *ex vivo*, close-up of injury site, similar view as in Fig. 4. Single arrow indicates transected hepatic vein (proximal end) to LL lobe, large in this subject. Double arrow indicates distal end of this vein on the hemistransected lobe.

I. OVERVIEW
Date: March 10, 2014
Swine no: 262
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (pdFI, FII, FXIII)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian.

II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male
Date swine received from UNL Mead: 03/06/2015
Pre-procedure wt: 38.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 134
- MAP: 115
- Temp: 38.6
- EtCO2: 35

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:40 AM
Spleen wt: 429.1 gm
LR (22°C) infused after splenectomy: 1290 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 429.1 + 20 + 23.3 = 472.4 mL
- LR (22°C) infused (spleen replacement + incidental): 1290 + 50 = 1340 mL

Pre-injury VS

- HR: 151
- MAP: 113
- Temp: 37.2

- EtCO2 : 52
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:12 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 84 mL/min of 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: 810 mg Fibrinogen, 32.4 mg Factor XIII, 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around the upper quadrants of the abdomen.

Total mass injected: 518.5 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.9 L preset maximum (50 mL/kg), given at constant rate of 10.5 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in this hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:13AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:25 AM

10 min post-injury VS

- HR: 175
- MAP: 40
- Temp: 3 7. 0
- EtCO2: 36
- IAP: 4

Blood draw no. 3: (30 min post-injury): 09:45 AM

30 min post-injury VS

- HR: 144
- MAP: 49
- Temp: 36.6

- EtCO2: 40
- IAP: 3

Blood draw no. 4: (60 min post-injury): 10:15 AM

60 min VS
- HR: 154
- MAP: 56
- Temp:36.2
- EtCO2: 40
- IAP: 4

Blood draw no. 5: (90 min post-injury): 10:45 AM

90 min post-injury VS
- HR: 163
- MAP: 60
- Temp: 35.9
- EtCO2: 38
- IAP: 5

Blood draw no. 6: (120 min post-injury): 11:15 AM

120 min post-injury VS
- HR: 155
- MAP: 50
- Temp: 35.6
- EtCO2: 38
- IAP: 7

Blood draw no. 7: (150 min post-injury): 11:45 AM

150 min post-injury VS
- HR: 147
- MAP: 51
- Temp: 35.4
- EtCO2: 38
- IAP: 8

Blood draw no. 8: (180 min post-injury): 12:15 PM

180 min post-injury VS
- HR: 155
- MAP: 51
- Temp: 35.2
- EtCO2: 36
- IAP: 7

Survival at 180 min? YES
Target MAP attained ? No.
Time of death: 12:15 PM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 1252.3 mL (suction) + 17.5 mL (clot + lap pads) + 140 (phlebotomies) = 1409.8 mL
- IV fluid given: LR (37°C): 2100 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~ 7 mm Hg). Upon re-opening abdomen, Foam mixed with blood and moderate amounts of unclotted blood and some clots are seen (see Figs). Ischemic areas are noted over small intestine and stomach (see Figs). A large clot was covering the surface of the injury site (see Figs).
Volume foam recovered: 768.2 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 157.0 (resected LM lobe) + 948.8 (remaining liver) = 1105.8 g

Tissue harvested: None

VI. COMMENTS

First subject with LM resection mechanism for noncompressible model that was treated with Ca alginiate + biologics. Easy 3 h survivor with ~1.4 L blood loss. IAP increases were close to 20 mm Hg for a few brief moments, but then went down to <10 mm Hg. Large clot covering injury site. Lots of surface changes to intestines, stomach and liver, presumably from free $CaCl_2$. This side effect will need continual monitoring and tweaking, because we will not be able to push this device into clinical use if this degree of toxicity is present.

VII. PLAN

Repeat procedure on Fri Mar 13th.

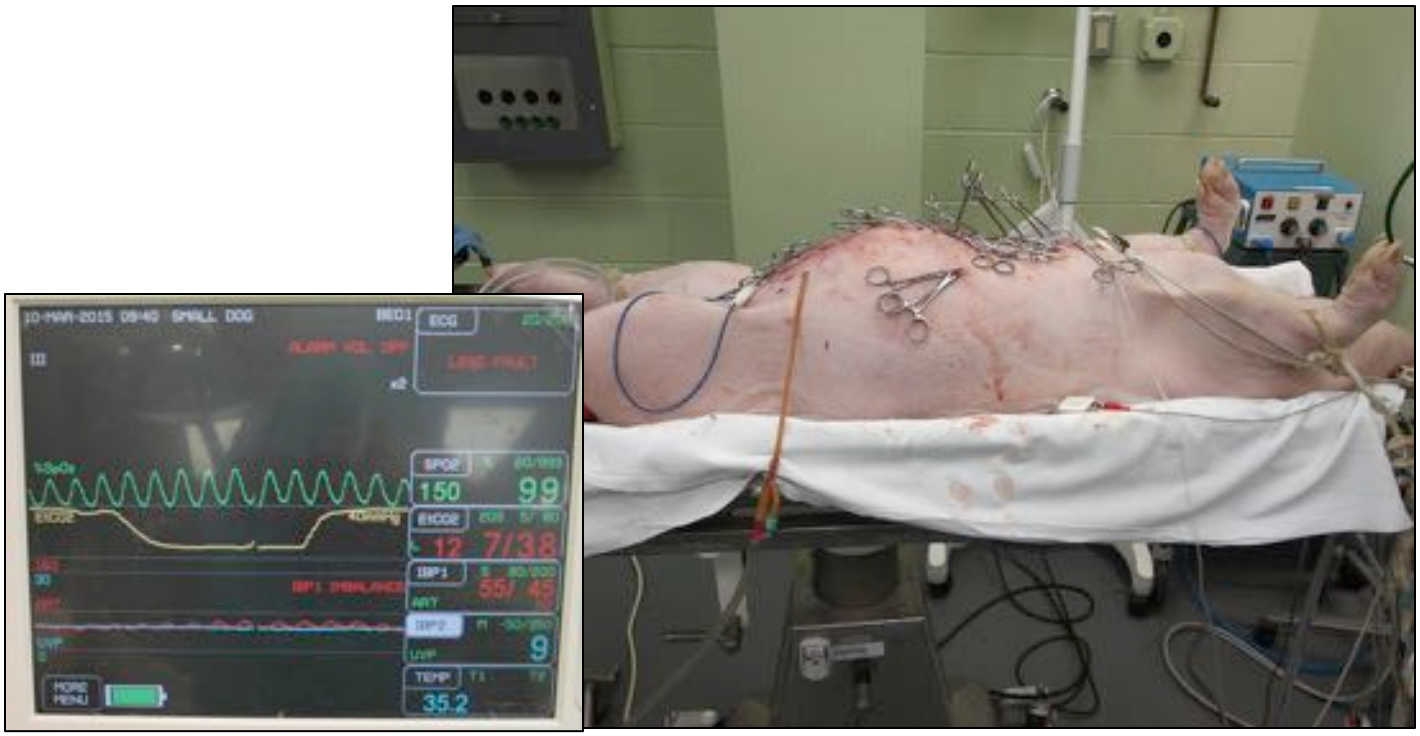

Figure 1, swine 262. Lateral view of swine after completion of 3 h observation period. Subject alive & well, with pulse = 150, MAP = 50 mm Hg, O2 sat = 99%, EtCO2 = 38 mm Hg, temp = 35.2˚C, IAP = 9 mm Hg (see monitor inset).

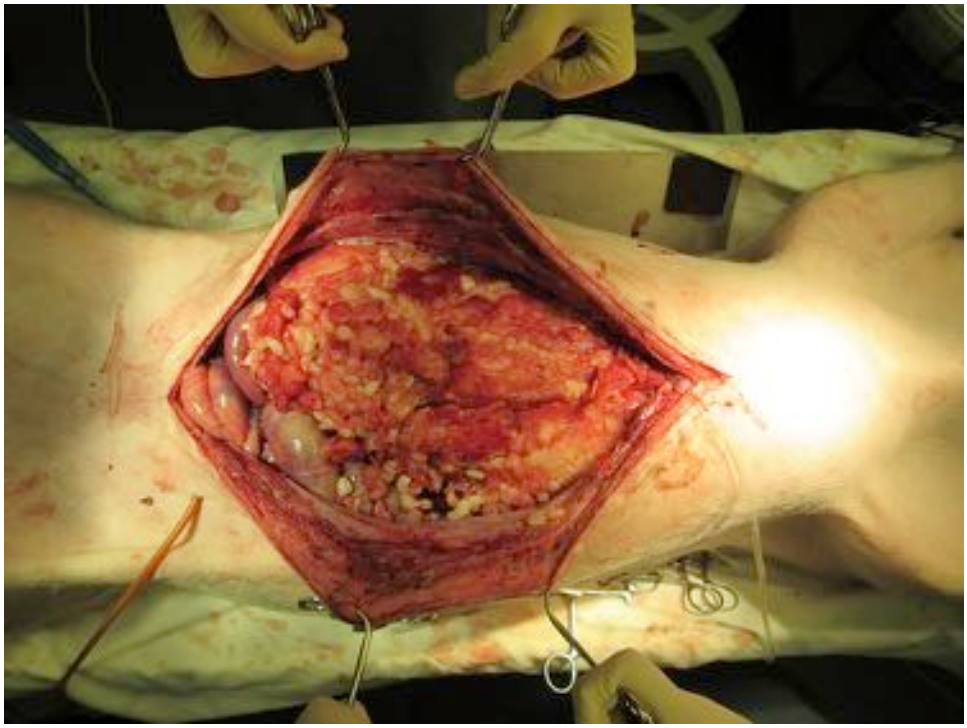

Figure 2, swine 262. Overhead view of re-opened abdomen immediately after Fig. 1 image. Subject alive & well. Cephalad is to right. Foam is overlying the intestines. No clot or blood present superficially.

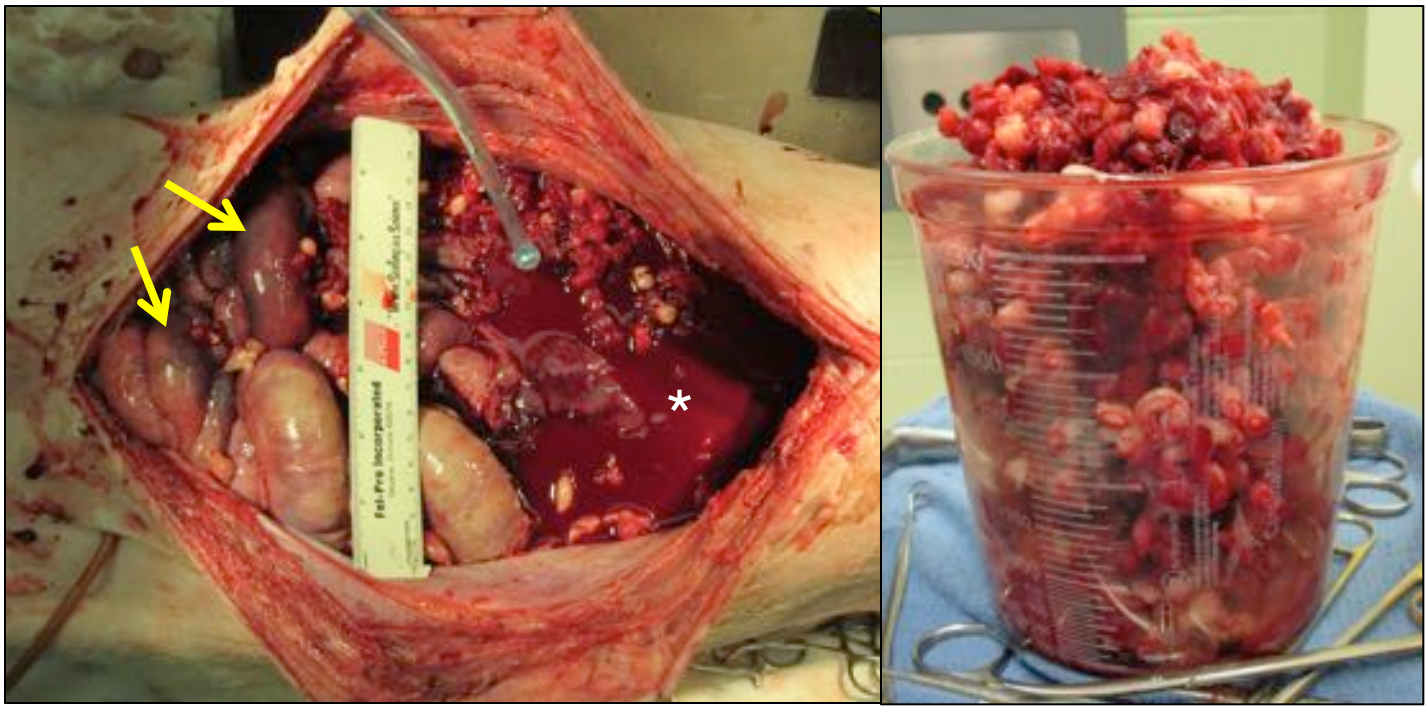

Figure 3, swine 262. Left: overhead view of re-opened abdomen immediately after Fig. 2 image; foam has been removed. Subject alive & well. Cephalad is to right. Unclotted blood present in upper abdomen (asterisk*). Note ischemic-appearing loops of small bowel (arrows). Right: evacuated foam placed into 2 L plastic canister.

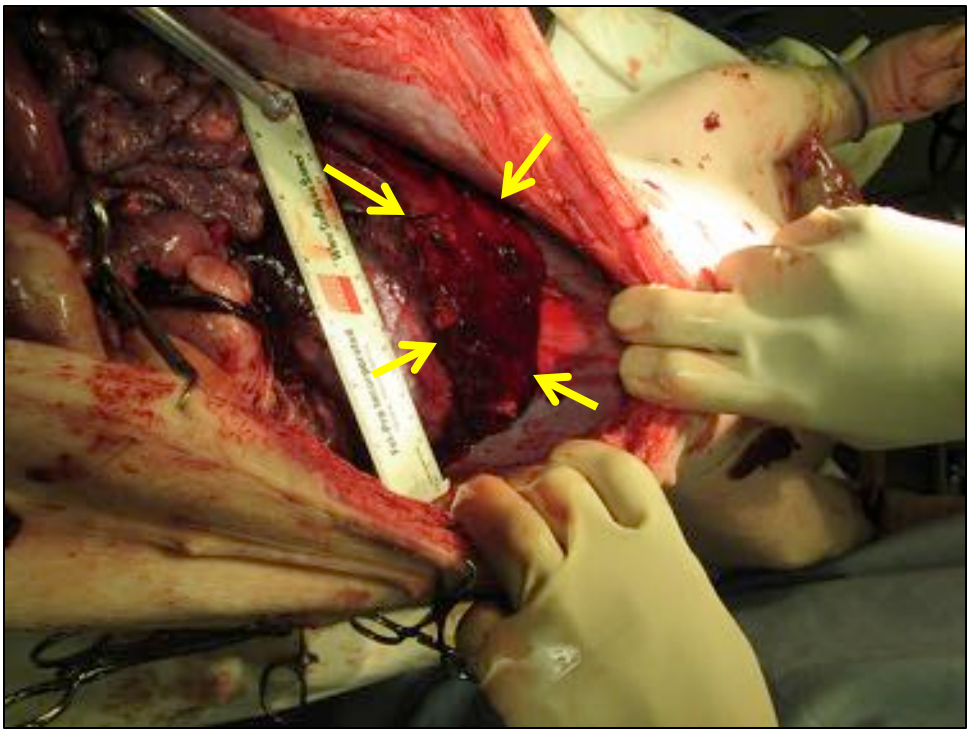

Figure 4, swine 262. View of superior abdomen immediately after Fig. 3 image; unclotted blood has been suctioned out. Subject alive & well. Cephalad is to right. Large clot overlying injury site (arrows) with no active bleeding.

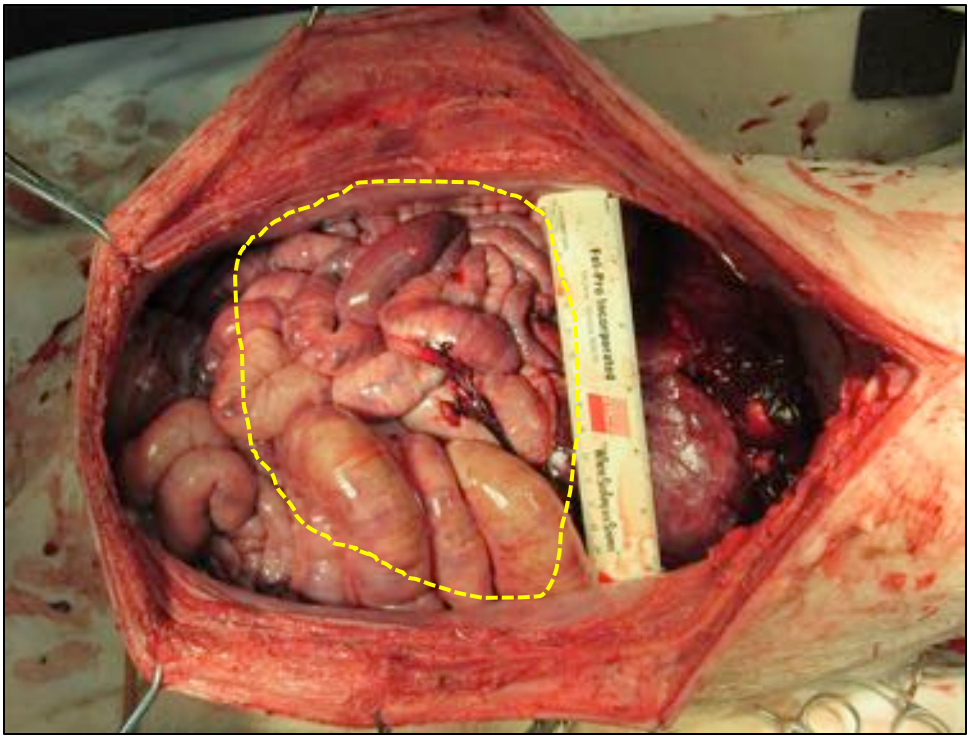

Figure 5, swine 262. Overhead view of abdomen immediately after euthanasia (IVC transection), showing surface changes to intestines (encircled with dashed yellow line).

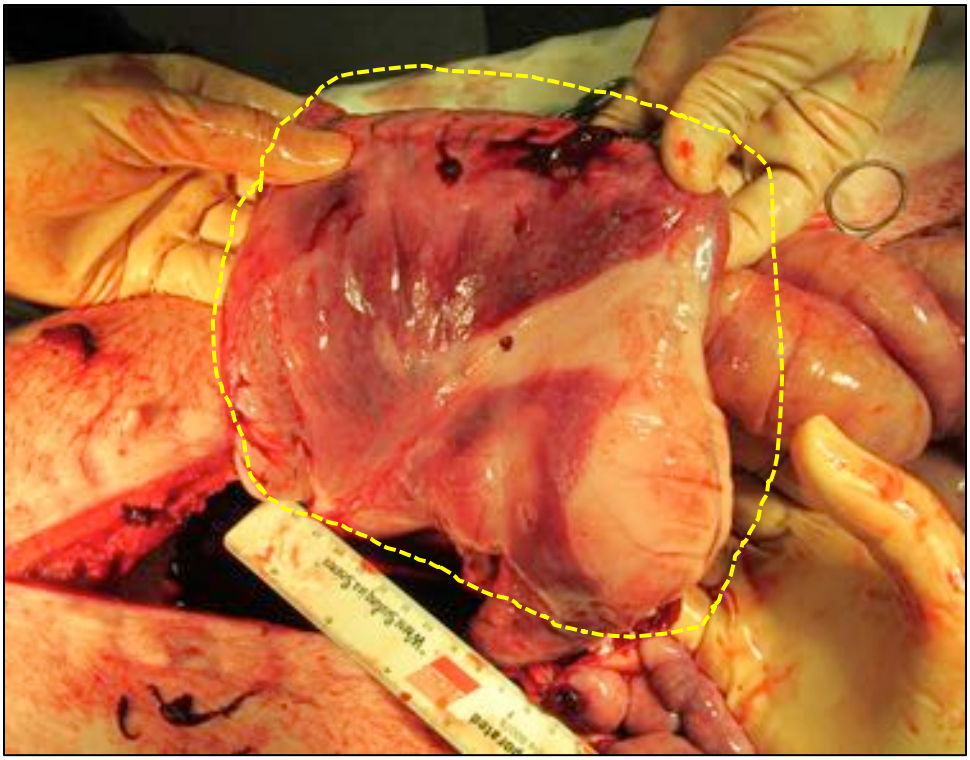

Figure 6, swine 262. Overhead view of abdomen immediately after euthanasia (IVC transection), showing surface changes to anterior stomach (encircled).

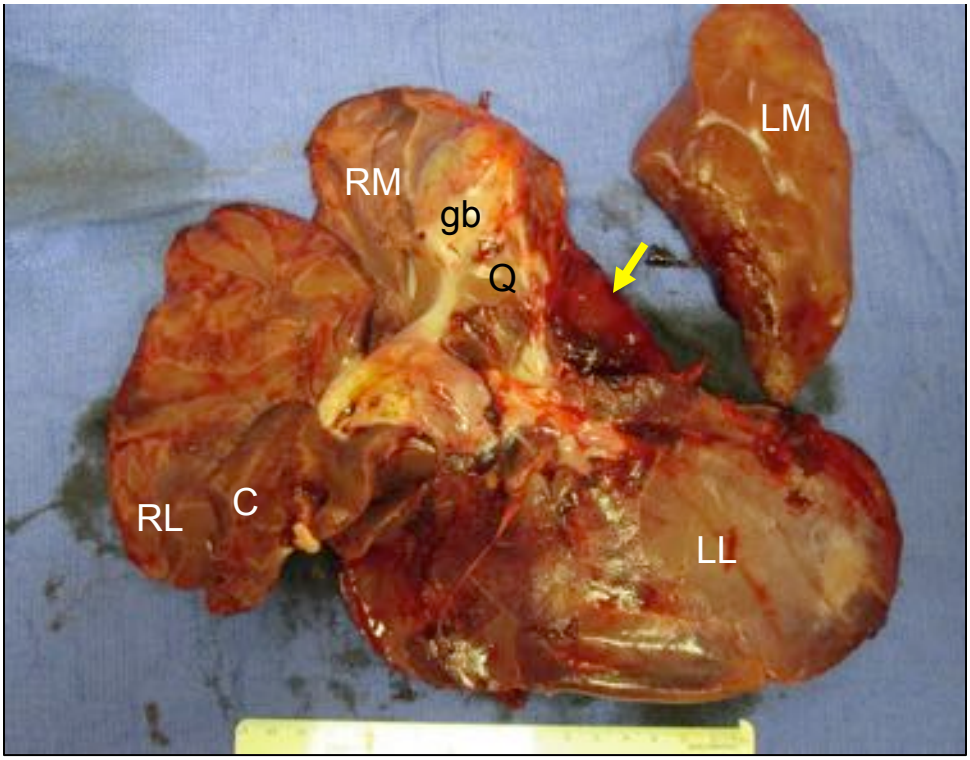

Figure 7, swine 262. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Arrow = large clot overlying surface of injury. Anterior is at top of image. Note diffuse surface changes to liver; contrast with previous subjects, whose livers are uniformly reddish.

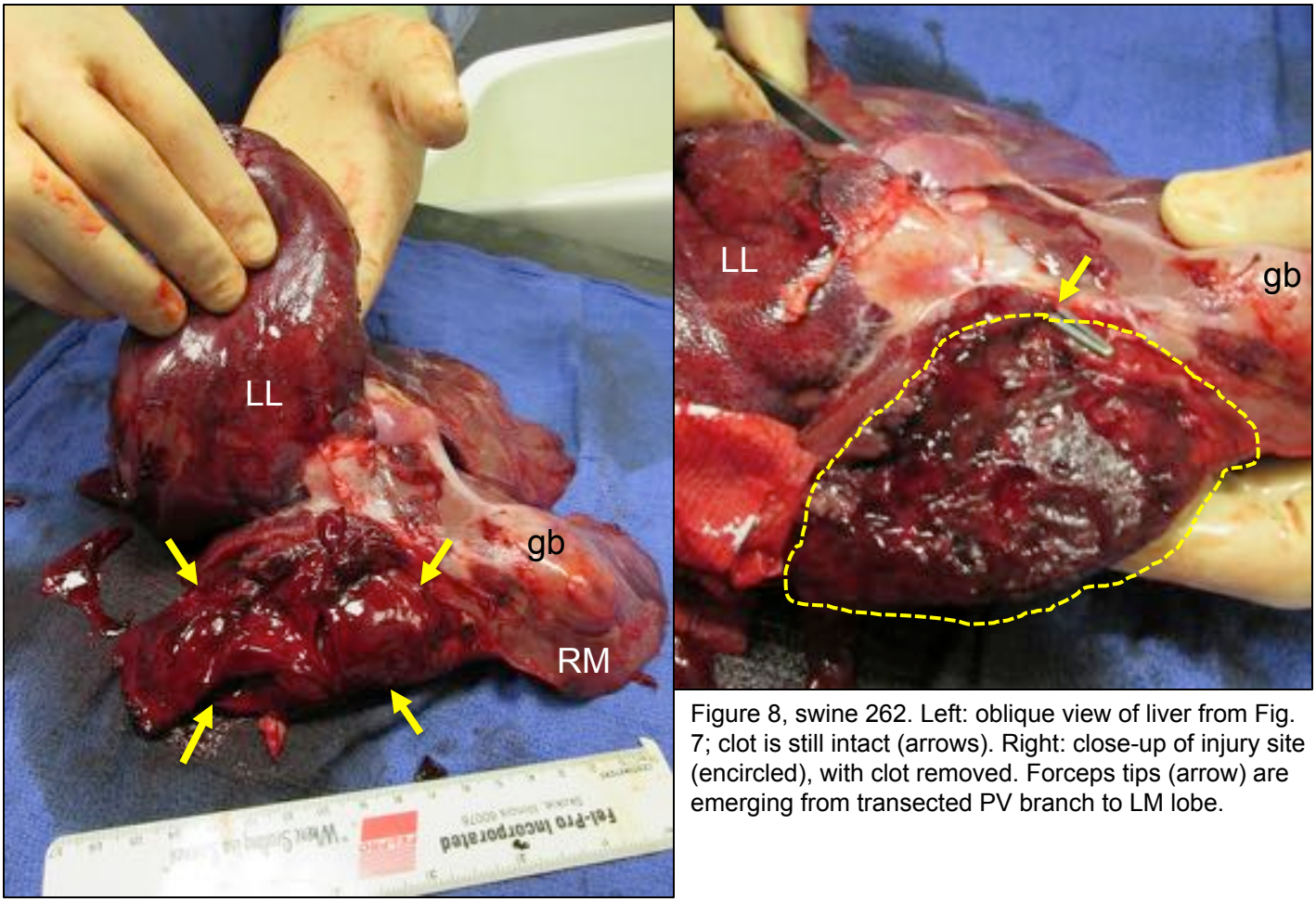

Figure 8, swine 262. Left: oblique view of liver from Fig. 7; clot is still intact (arrows). Right: close-up of injury site (encircled), with clot removed. Forceps tips (arrow) are emerging from transected PV branch to LM lobe.

I. OVERVIEW
Date: March 13, 2014
Swine no: 263
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (pdFI, FII, FXIII)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian.

II. PRE-INJURY PHASE

Start time: 07:55 AM
Swine sex: male
Date swine received from UNL Mead: 03/06/2015
Pre-procedure wt: 37.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 136
- MAP: 108
- Temp: 38.0
- EtCO2: 42

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:45 AM
Spleen wt: 429.3 gm
LR (22°C) infused after splenectomy: 1820 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 429.3 + 20 + 60.2 = 509.5 mL
- LR (22°C) infused (spleen replacement + incidental): 1820 + 0 = 1820 mL

Pre-injury VS

- HR: 122
- MAP: 102
- Temp: 36.5

- EtCO2 : 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:05 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 84 mL/min of 1.14 M $CaCl_2$ (20 mL/min x 4 syringe injectors).

Clotting factors: 810 mg Fibrinogen, 32.4 mg Factor XIII, 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around the upper quadrants of the abdomen.

Total mass injected: 778 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.9 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in this hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:06AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:15 AM

10 min post-injury VS

- HR: 141
- MAP: 41
- Temp: 36.4
- EtCO2: 33
- IAP: 7

Blood draw no. 3: (30 min post-injury): 09:35 AM

30 min post-injury VS

- HR: 122
- MAP: 43
- Temp: 36.2

- EtCO2: 33
- IAP: 3

Blood draw no. 4: (60 min post-injury): 10:05 AM

60 min VS
- HR: 124
- MAP: 49
- Temp:35.9
- EtCO2: 34
- IAP: 1

Blood draw no. 5: (90 min post-injury): 10:35 AM

90 min post-injury VS
- HR: 129
- MAP: 47
- Temp: 35.1
- EtCO2: 33
- IAP: 3

Blood draw no. 6: (120 min post-injury): 11:05 AM

120 min post-injury VS
- HR: 127
- MAP: 47
- Temp: 34.8
- EtCO2: 33
- IAP: 5

Blood draw no. 7: (150 min post-injury): 11:35 AM

150 min post-injury VS
- HR: 125
- MAP: 45
- Temp: 34.6
- EtCO2: 33
- IAP: 9

Blood draw no. 8: (180 min post-injury): 12:05 PM

180 min post-injury VS
- HR: 122
- MAP: 59
- Temp: 34.5
- EtCO2: 31
- IAP: 11

Survival at 180 min? YES
Target MAP attained ? No.
Time of death: 12:05 PM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 799.8 mL (suction) + 247.5 mL (clot + lap pads) + 140 (phlebotomies) = 1047.3 mL
- IV fluid given: LR (37°C): 2200 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~ 11 mm Hg). Upon re-opening abdomen, Foam not mixed with blood for the most part and moderate amounts of unclotted blood and some clots are seen (see Figs). Few ischemic areas are noted over small intestine (see Figs). A large clot was covering the surface of the injury site (~62 gms, see Figs); no active bleeding.
Volume foam recovered: 812.3 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 92.0 (resected LM lobe) + 837.3 (remaining liver) = 929.8 g

Tissue harvested: None

VI. COMMENTS

N = 2 of NC model (LM resection) treated with Ca alginate + biologics; 3 h survivor with 1.0 L blood loss. Injury site covered with large clot. Less surface discoloration to the intestine than what was seen with no. 262, but still remarkable. Similar to no. 262, IAP remained around 10 mm Hg after initial injection period (went up into 20's briefly at time of injection.

VII. PLAN

Continue with this series, for N of 8-10 swine. Next procedures on Mar 17th and 20th. Begin collecting clot specimen, fix in formalin, and then double IHC for porcine & human FI.

We have N = 5 of no-treatment controls with this injury mechanism from the Fall of 2014; we are tabulating results from these no-treatment controls. We will likely need 5 more no-treatment controls.

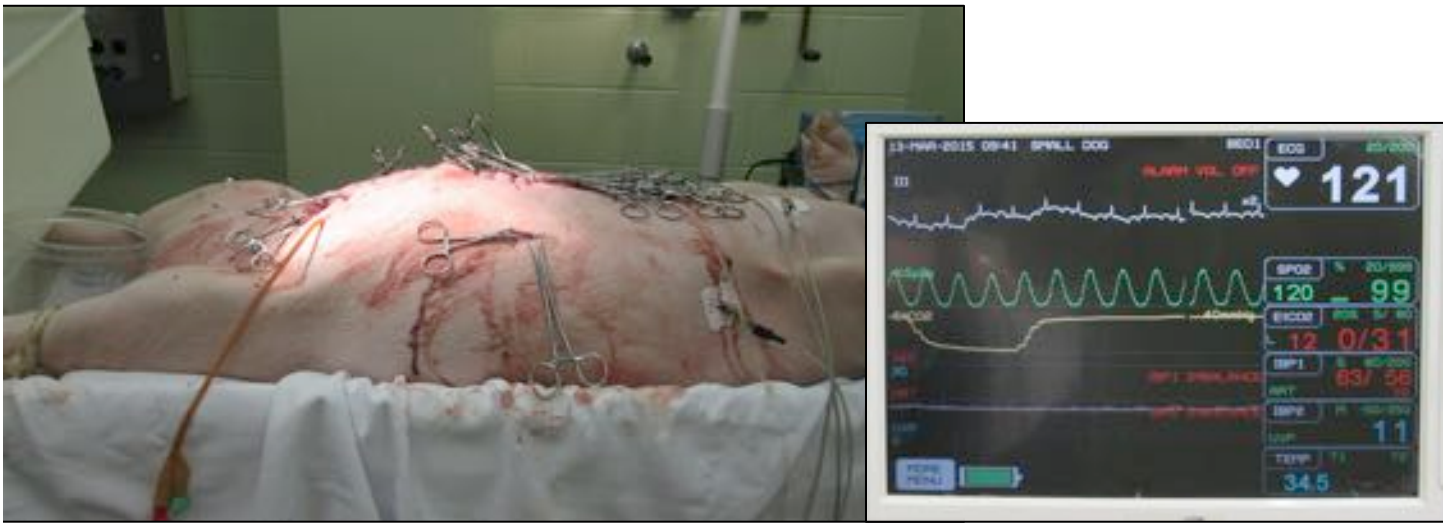

Figure 1, swine 263. Lateral view of swine after completion of 3 h observation period. Subject alive & well, with pulse = 121, MAP = 59 mm Hg, O2 sat = 99%, EtCO2 = 31 mm Hg, temp = 34.5˚C, IAP = 11 mm Hg (see monitor inset).

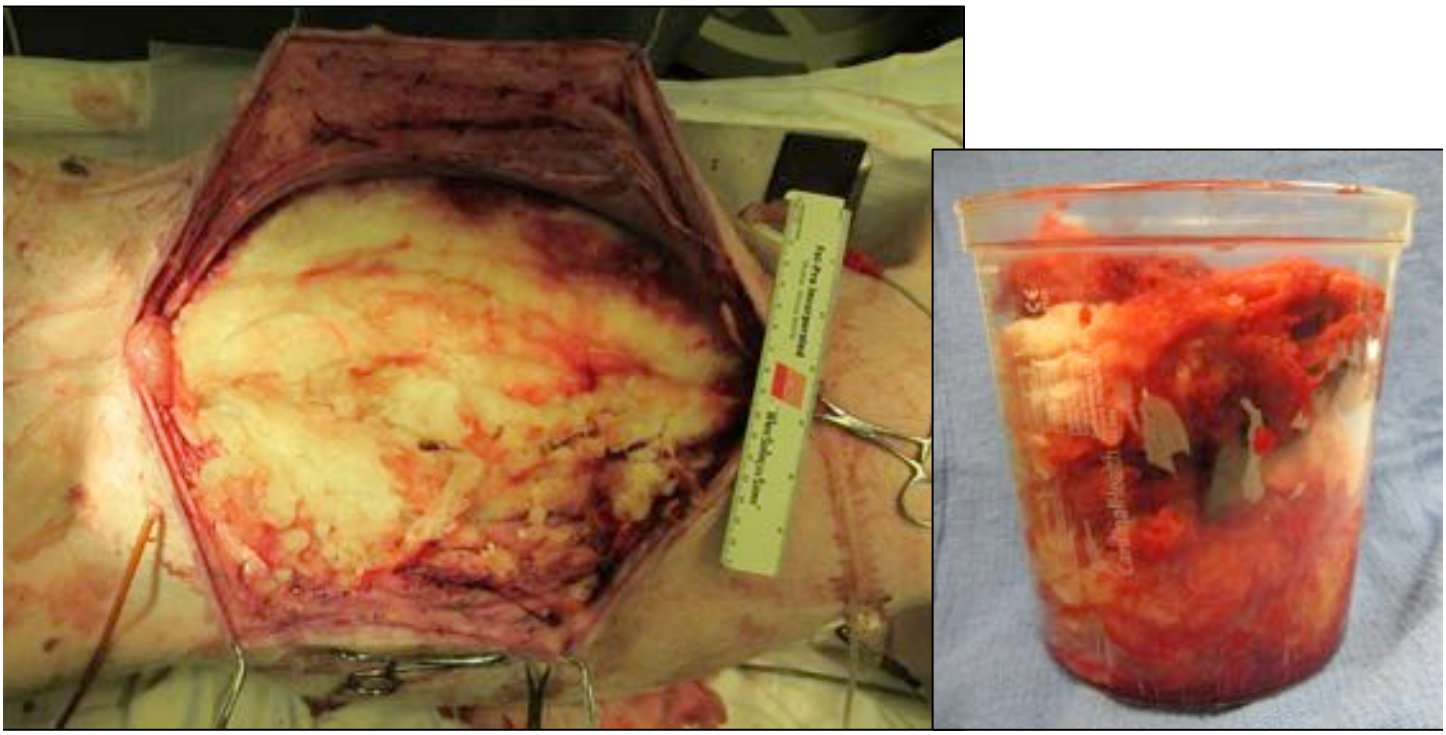

Figure 2, swine 263. Overhead view of re-opened abdomen immediately after Fig. 1 image. Subject alive & well. Cephalad is to right. Foam is overlying the intestines. No clot visible; small amount blood present at periphery. Inset: foam subsequently was removed, and placed into this 2 L canister.

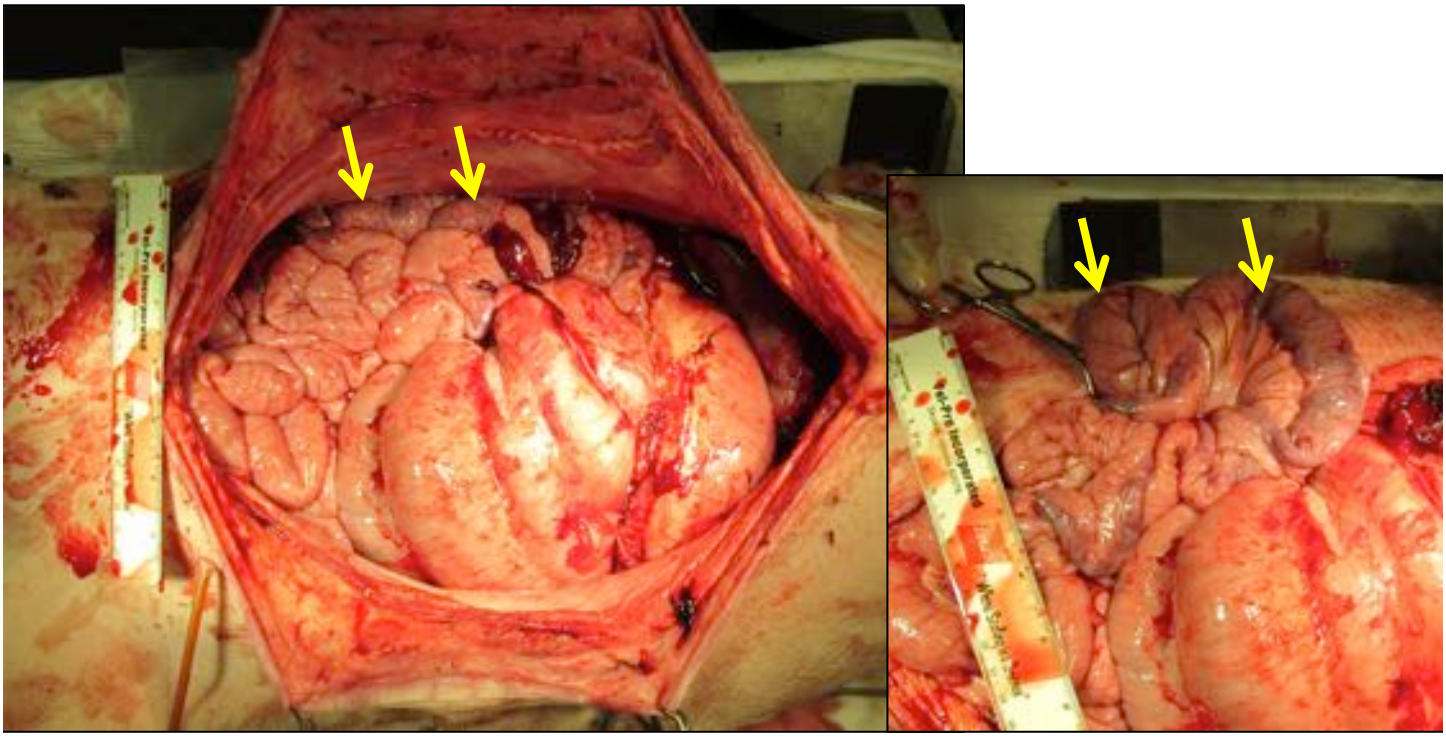

Figure 3, swine 263. Left: overhead view of re-opened abdomen immediately after Fig. 2 image; foam has been removed. Subject alive & well. Cephalad is to right. No clot or blood immediately obvious. Note ischemic-appearing loops of small bowel (arrows), which were only present in the right gutter. Right: discolored loops of intestine from left image now have been eviscerated for visualization.

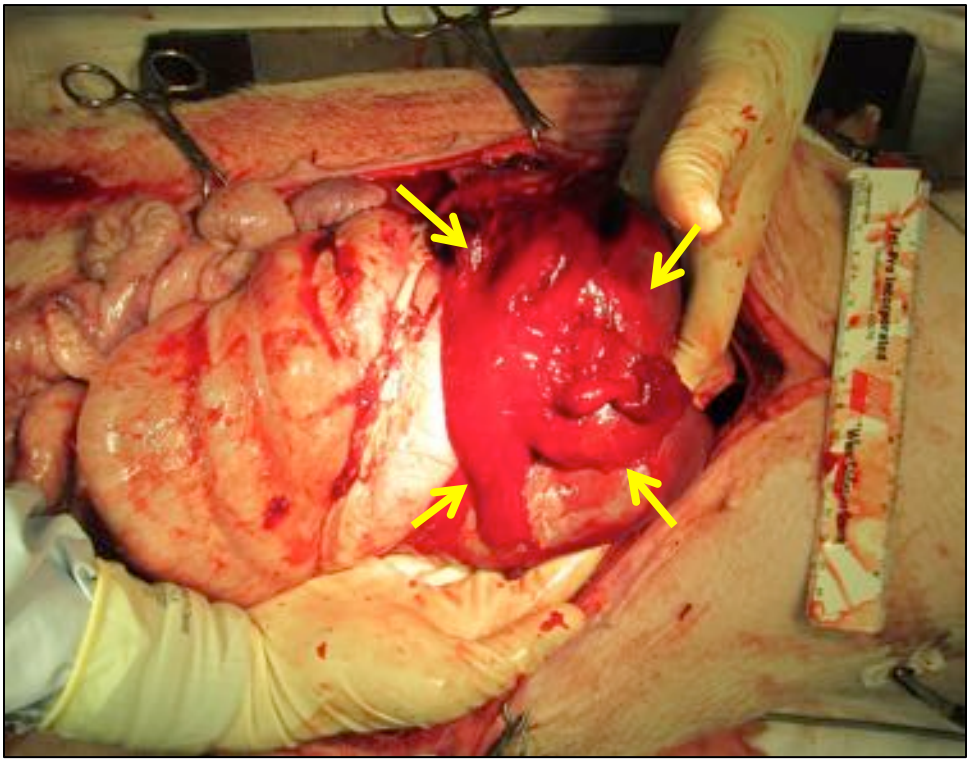

Figure 4, swine 263. View of superior abdomen immediately after Fig. 3 image; liver has been elevated into the wound. Subject alive & well. Cephalad is to right. There was a large clot overlying injury site (arrows) with no active bleeding.

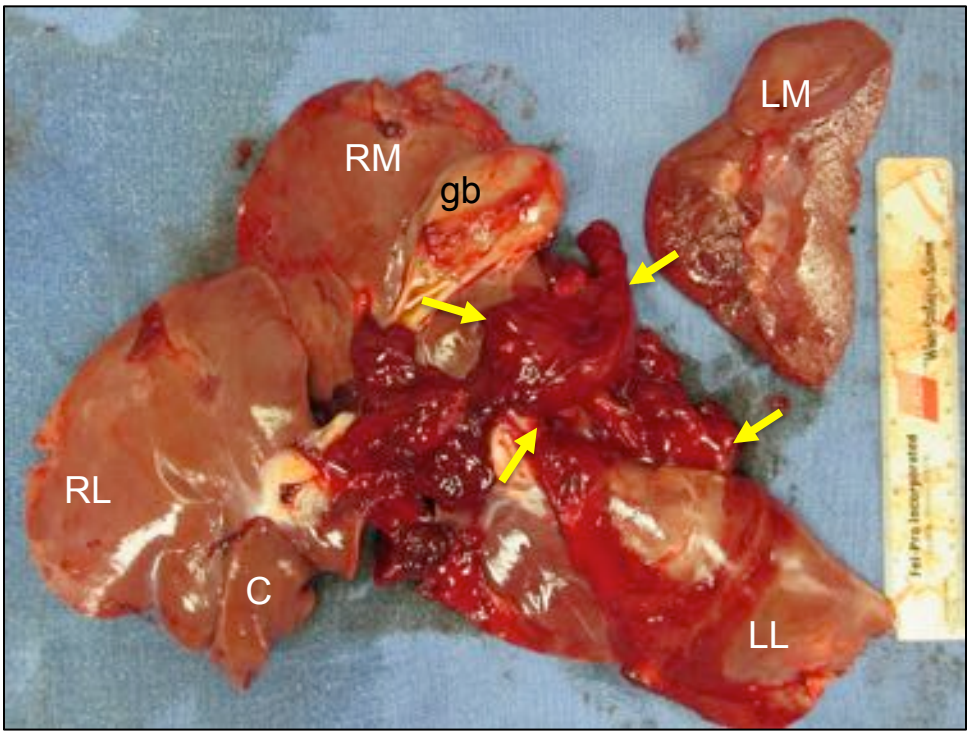

Figure 5, swine 263. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Arrows = large clot overlying surface of injury. Anterior is at top of image. Color of liver appears good (contrast #262).

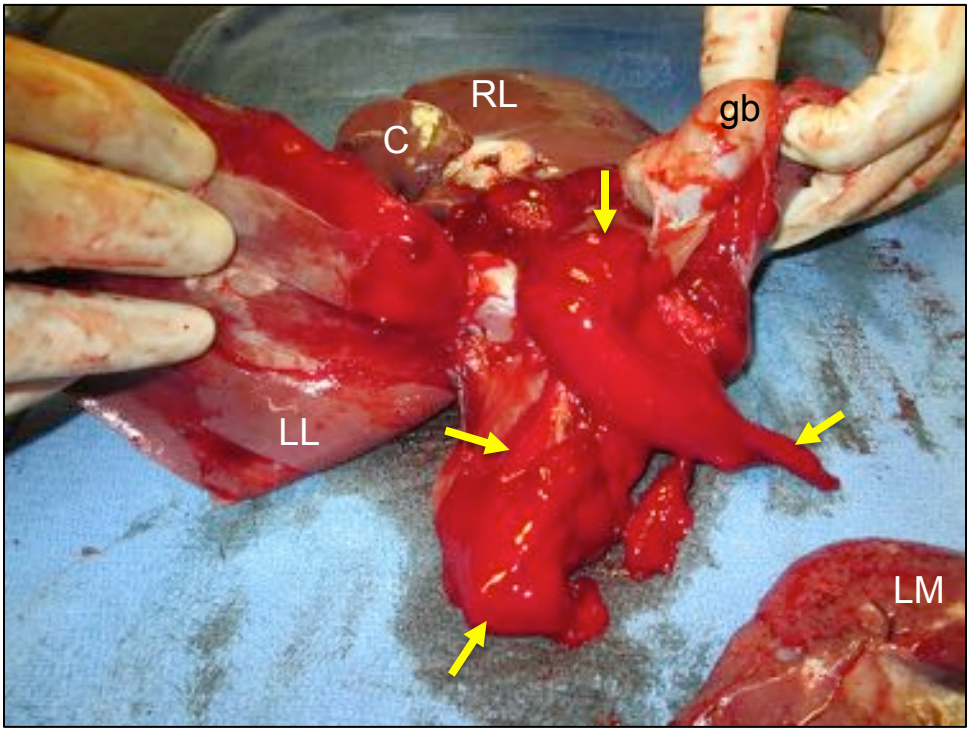

Figure 6, swine 263. Liver *ex vivo*, inferior left oblique view, showing large clot covering injury site; refer to Fig. 5.

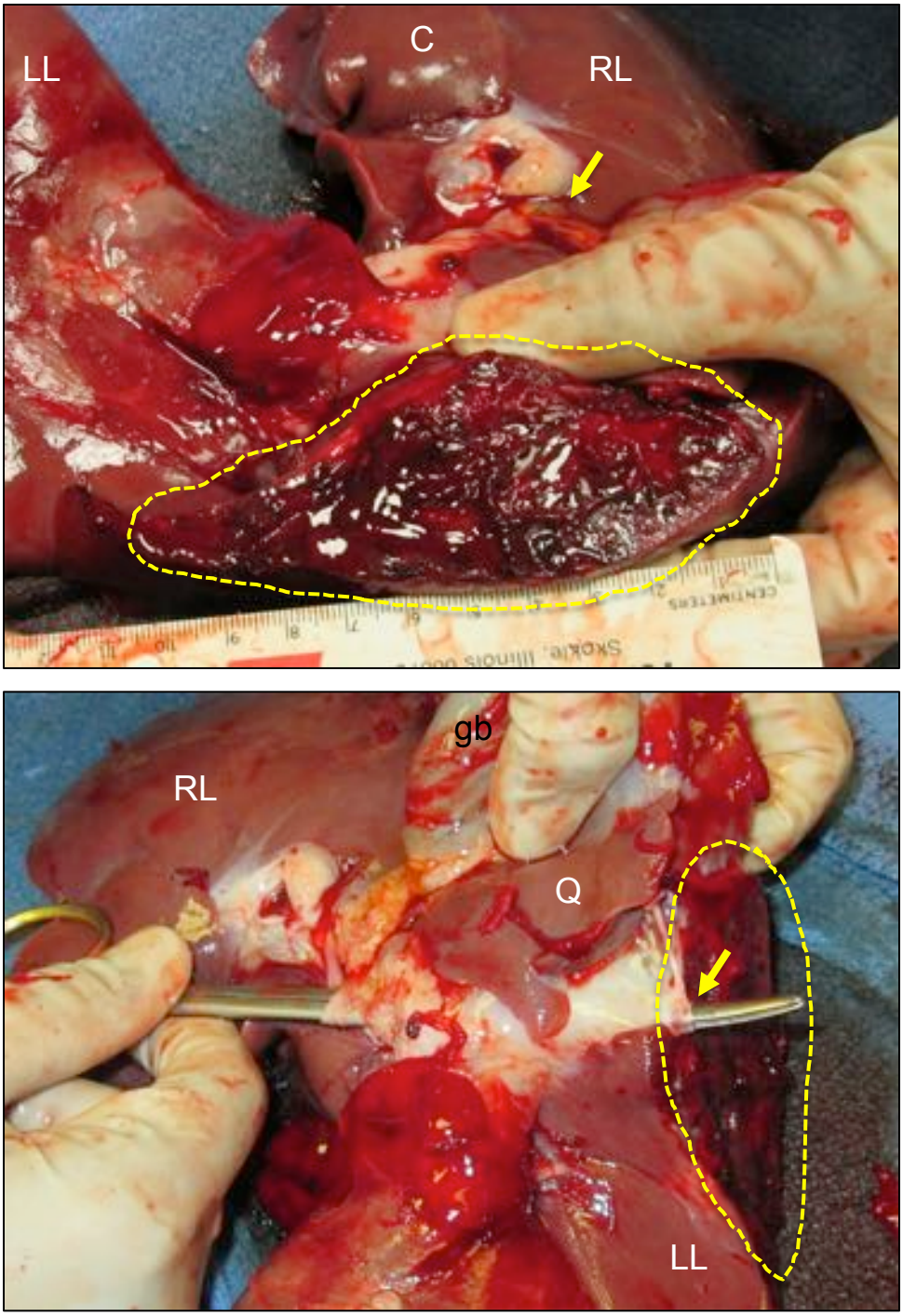

Figure 7, swine 263. Top: close-up of injury site after removal of clot. Bottom: tip of scissors shown emerging from transected end of PV branch to LM lobe. Dashed yellow line = cut surface of LM lobe (i.e., injury site).

I. OVERVIEW
Date: March 17, 2015
Swine no: 264
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (pdFI, FII, FXIII)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 03/06/2015
Pre-procedure wt: 38.5 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 88
- MAP: 98
- Temp: 38.1
- EtCO2: 40

Blood draw no. 1 (initial): 8:30 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:45 AM
Spleen wt: 280.9 gm
LR (22°C) infused after splenectomy: 850 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 280.9 + 20 + 4 = 304.9 mL
- LR (22°C) infused (spleen replacement + incidental): 850 + 0 = 850 mL

Pre-injury VS
- HR: 106
- MAP: 95
- Temp: 37.3

- EtCO2 : 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:03 AM

Injury type: hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 140 mL/min 1.14 M $CaCl_2$ (20 mL/min x 7 syringe injectors).

Clotting factors: 810 mg Fibrinogen, 32.4 mg Factor XIII, 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around the upper quadrants of the abdomen.

Total mass injected: 797.7 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 75

Resuscitation fluid: warm LR, 2.0 L preset maximum (50 mL/kg), given at constant rate of 10.7 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:04AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:13 AM

10 min post-injury VS

- HR: 142
- MAP: 21
- Temp: 36.9
- EtCO2: 11
- IAP: 4

Blood draw no. 3: (30 min post-injury): 09:30 AM

30 min post-injury VS

- HR: 122
- MAP: 17
- Temp: 36.7

- EtCO2: 9
- IAP: 7

Blood draw no. 4: (60 min post-injury): 10:00 AM

60 min VS
- HR: 114
- MAP: 19
- Temp:35.7
- EtCO2: 12
- IAP: 10

Blood draw no. 5: (90 min post-injury): 10:30 AM

90 min post-injury VS
- HR: 126
- MAP: 20
- Temp: 35.0
- EtCO2: 14
- IAP: 14

Blood draw no. 6: (120 min post-injury): 11:00 AM

120 min post-injury VS
- HR: 132
- MAP: 22
- Temp: 34.7
- EtCO2: 15
- IAP: 17

Blood draw no. 7: (150 min post-injury): 11:30 AM

150 min post-injury VS
- HR: 128
- MAP: 22
- Temp: 34.4
- EtCO2: 15
- IAP: 24

Blood draw no. 8: (180 min post-injury): 12:05 PM

180 min post-injury VS
- HR: 111
- MAP: 22
- Temp: 34.1
- EtCO2: 14
- IAP: 2

Survival at 180 min? Yes
Target MAP attained ? No
Time of death: 12:06 PM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 1467 mL (suction) + 329.7 mL (clot + lap pads) + 140 (phlebotomies) = 1936.7 mL
- IV fluid given: LR (37°C): 2300 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, mildly tense (IAP ~ 2 mm Hg). Upon re-opening abdomen, Foam not mixed with blood for the most part in the anterior region, and moderate amounts of unclotted blood and some clots are seen (see Figs). Some ischemic areas noted over small intestine, perhaps less than previously seen. A large clot mixed with foam was adherent to the surface of the injury site (~ 62 gms, see Figs).
Volume foam recovered: 728.2 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 133.6 (resected LM lobe) + 894.9 (remaining liver) = 1028.5 g

Tissue harvested: two specimens: (1) clot from the site of injury, and (2) intraabdominal clot remote from injury. For porcine-human fibrinogen double IHC.

VI. COMMENTS

N = 3 of LM resection/noncompressible, with alginate foam + FS Rx. Another survivor, though quite hypotensive at end of 3 h (MAP = 22), and 1.9 L blood loss. Less visceral surface changes today. We are getting good clot adherence with foam at the injury site.

We have N = 5 subjects with no treatment from last Fall. Results from those procedures are being tabulated.

VII. PLAN

Continue with same injury/Rx on 3/20. Obtain FI IHC.

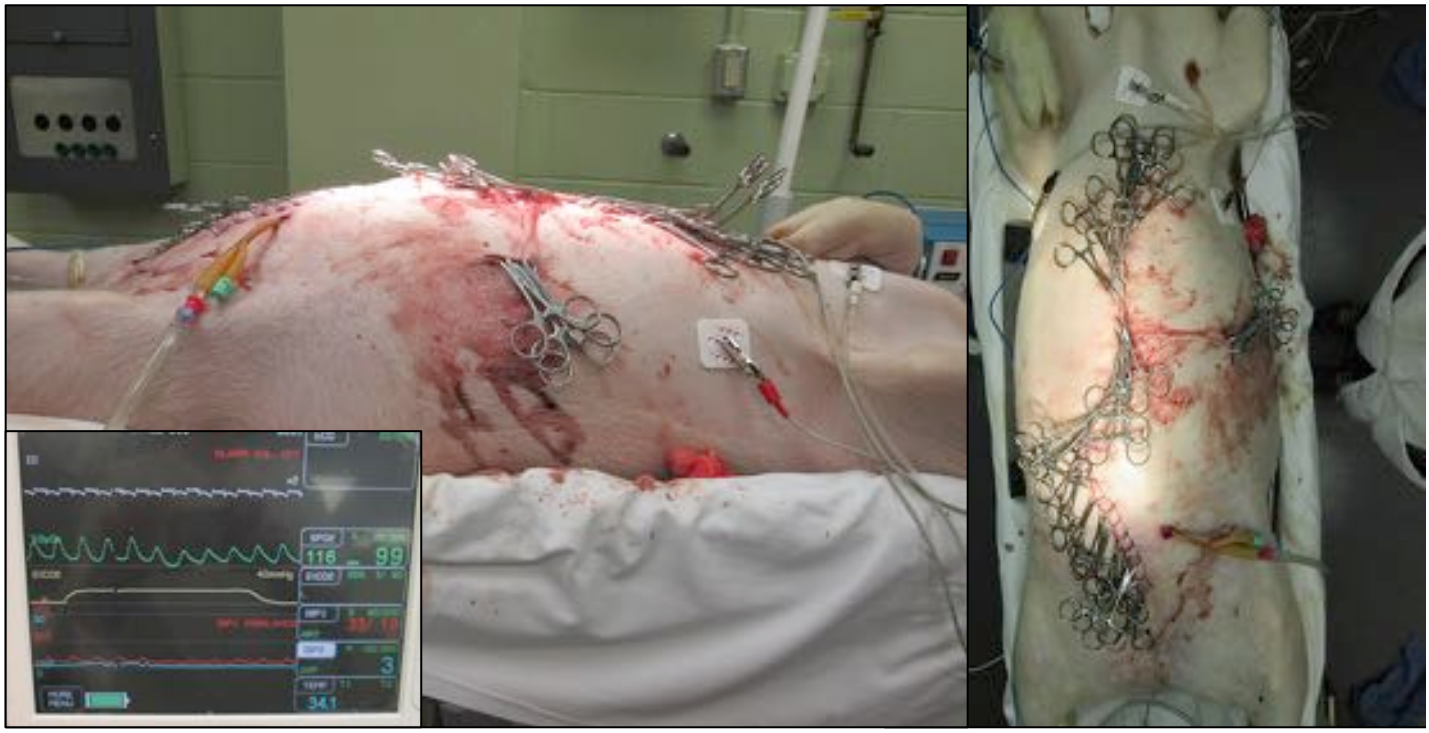


Figure 1, swine 264. Lateral & overhead views of swine after completion of 3 h observation period. Subject alive bu hypotensive, with pulse = 99, MAP = 26 mm Hg, O2 sat = 99%, EtCO2 = 14 mm Hg, temp = 34.1˚C, IAP = 3 mm Hg (see monitor inset).

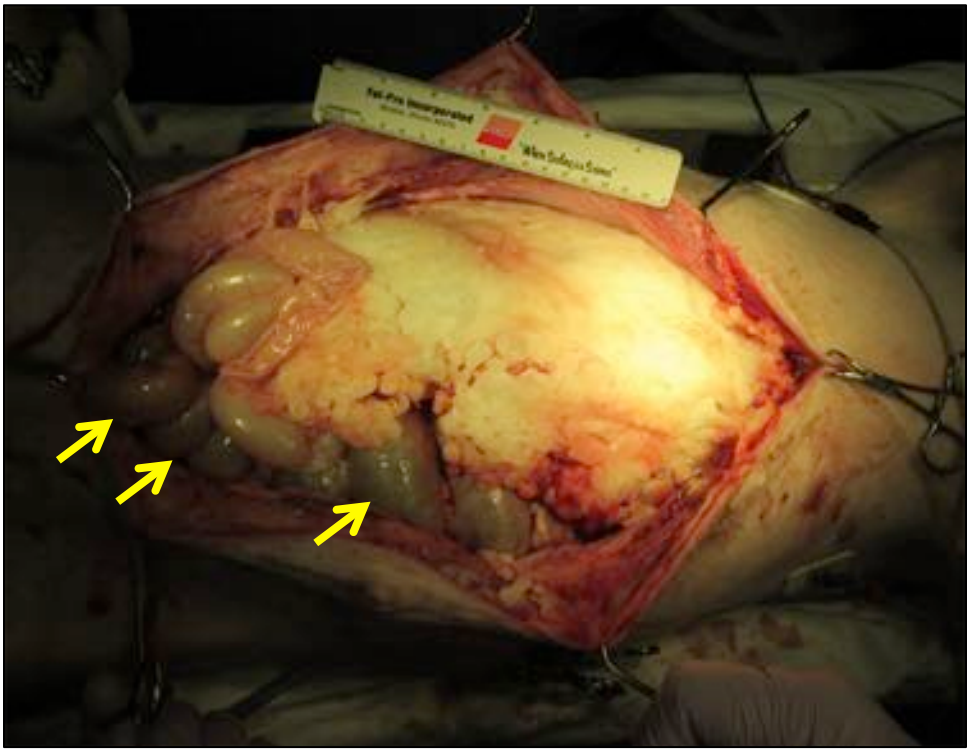


Figure 2, swine 264. Overhead view of re-opened abdomen immediately after Fig. 1 image. Subject alive but hypotensive. Cephalad is to right. Foam is overlying the intestines. No clot visible; small amount blood present at periphery. Surface changes to intestines indicated with arrows.

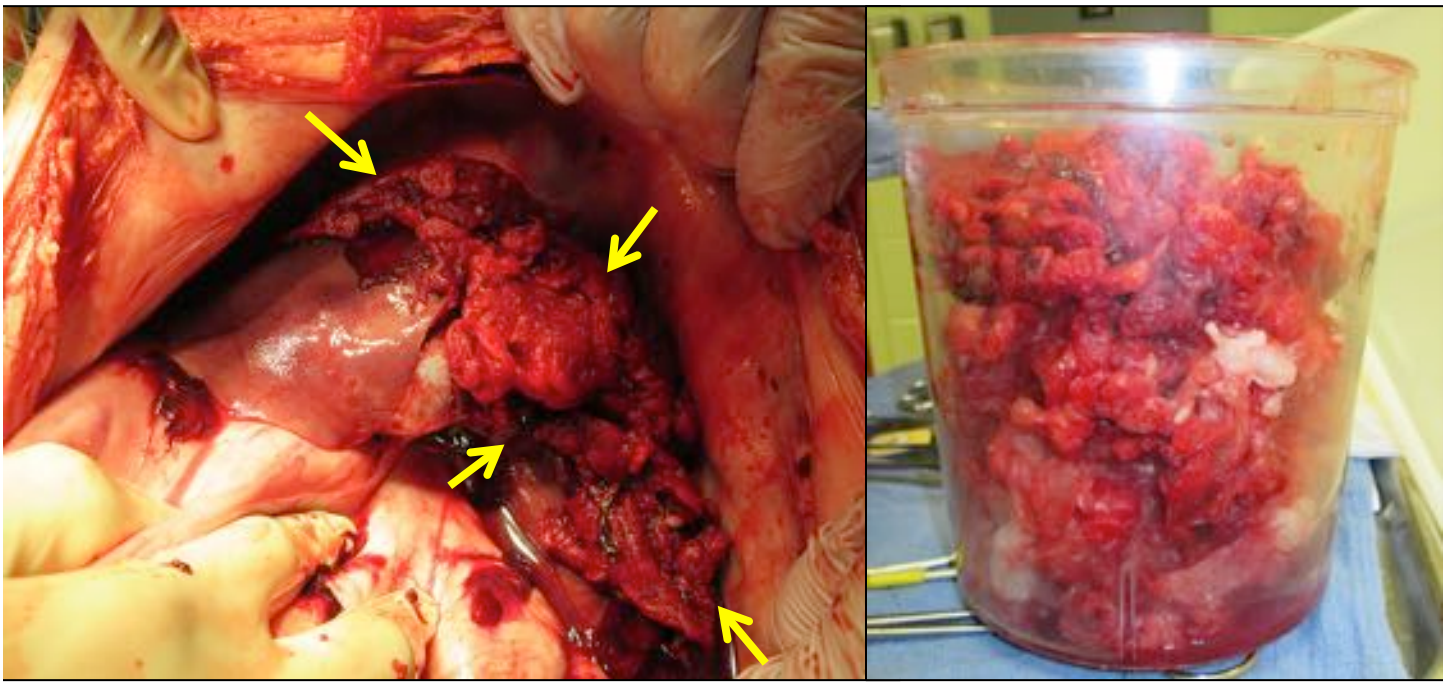


Figure 3, swine 264. Left: view of superior abdomen immediately after Fig. 3 image; foam has been removed and placed into 2 L canister (right image). Arrows indicated injury site covered with admixture of clot and foam. This clot/ foam mix is shown removed from the injury site in Fig. 4 below. Cephalad at top of image.

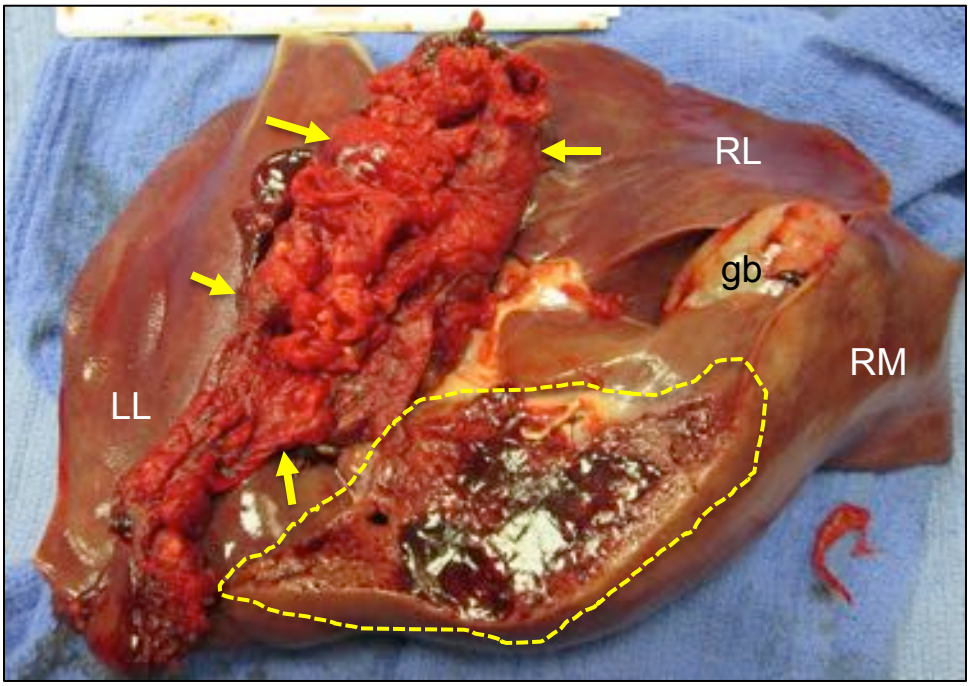
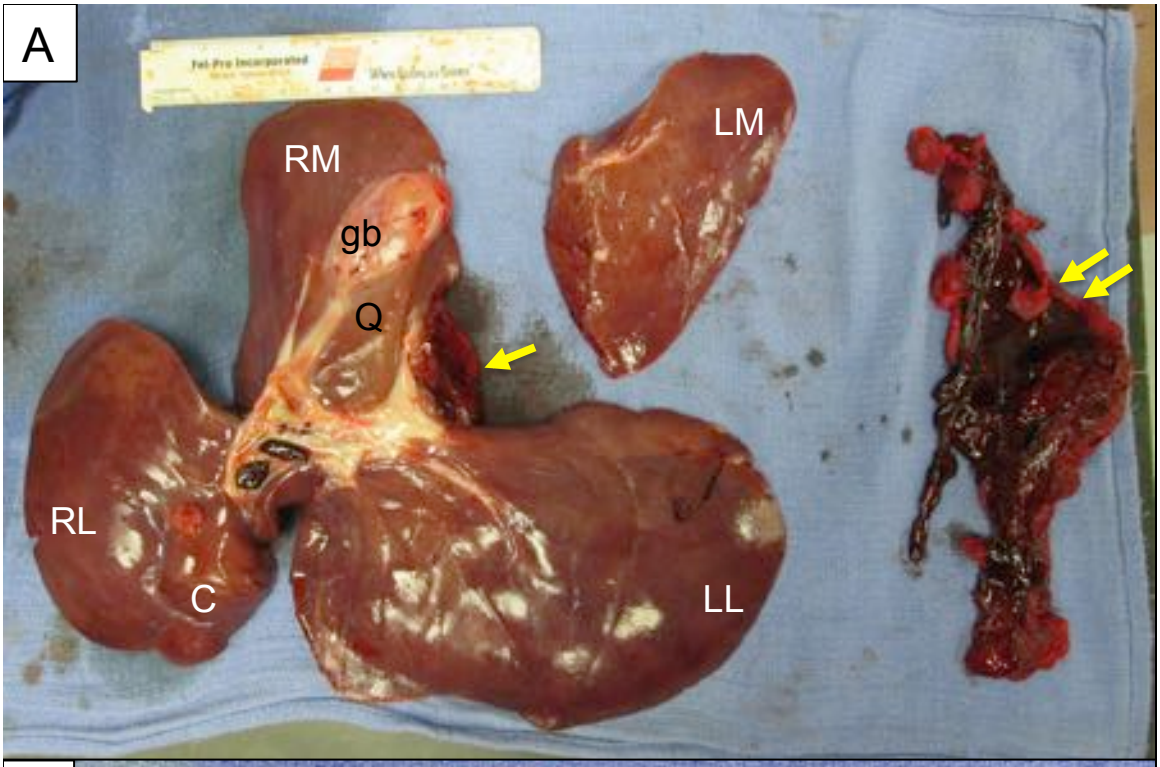
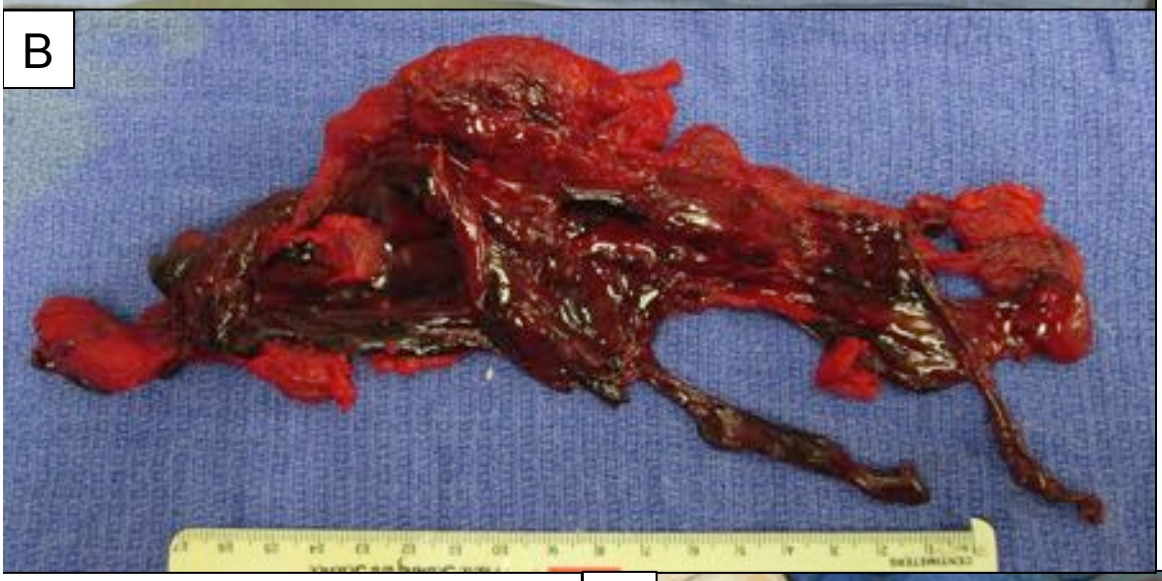


Figure 4, swine 264. Liver *ex vivo*, left inferior oblique view. Mass of clot and foam (arrows) has been swept away from the injury site (dashed line).





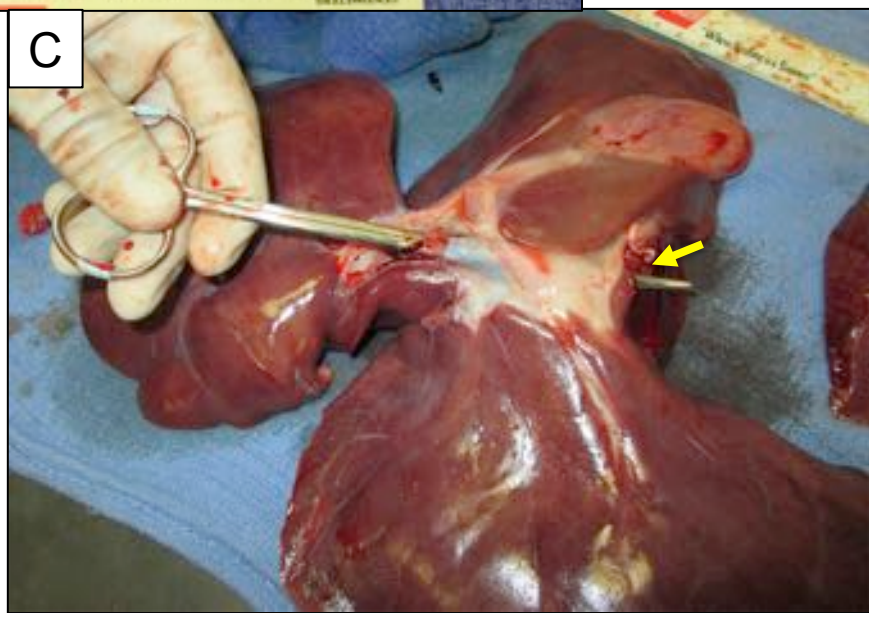


Figure 5, swine 263. (A) Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection site; double arrow = clot removed from resection site. Anterior is at top of image. (B) Close up of clot/foam mass removed from resection site. (C) Demonstration of PV injury. Scissors inserted through end of PV, with tips (arrow) emerging from transected PV branch to LM lobe.

I. OVERVIEW
Date: March 20, 2015
Swine no: 265
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (pdFI, FII, FXIII)
Personnel: Carlson, Yanala, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:40 AM
Swine sex: male
Date swine received from UNL Mead: 03/06/2015
Pre-procedure wt: 42.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 87
- MAP: 106
- Temp: 38.3
- EtCO2: 40

Blood draw no. 1 (initial): 8:50 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 09:05 AM
Spleen wt: 253.5 gm
LR (22°C) infused after splenectomy: 760.5 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 253.5 + 20 + 4 = 277.5 mL
- LR (22°C) infused (spleen replacement + incidental): 760.5 + 10 = 770.5 mL

Pre-injury VS
- HR: 104
- MAP: 112
- Temp: 37.5

- EtCO2 : 44
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 09:13 AM

Injury type: hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 240 mL/min 1.14 M $CaCl_2$ (20 mL/min x 4 syringe injectors).

Clotting factors: 810 mg Fibrinogen, 32.4 mg Factor XIII, 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around the upper quadrants of the abdomen.

Total mass injected: 767.7 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 2.1 L preset maximum (50 mL/kg), given at constant rate of 11.7 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:14AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:25 AM

10 min post-injury VS

- HR: 112
- MAP: 42
- Temp: 37.6
- EtCO2: 35
- IAP: 22

Blood draw no. 3: (30 min post-injury): 09:45 AM

30 min post-injury VS

- HR: 143
- MAP: 58
- Temp: 37.5

- EtCO2: 38
- IAP: 26

Blood draw no. 4: (60 min post-injury): 10:15 AM

60 min VS
- HR: 153
- MAP: 54
- Temp: 37.4
- EtCO2: 36
- IAP: 30

Blood draw no. 5: (90 min post-injury): 10:45 AM

90 min post-injury VS
- HR: 165
- MAP: 59
- Temp: 37.6
- EtCO2: 39
- IAP: 34

Blood draw no. 6: (120 min post-injury): 11:15 AM

120 min post-injury VS
- HR: 162
- MAP: 58
- Temp: 37.8
- EtCO2: 36
- IAP: 37

Blood draw no. 7: (150 min post-injury): 11:45 AM

150 min post-injury VS
- HR: 185
- MAP: 78
- Temp: 37.9
- EtCO2: 40
- IAP: 40

Blood draw no. 8: (180 min post-injury): 12:15 PM

180 min post-injury VS
- HR: 152
- MAP: 54
- Temp: 37.9
- EtCO2: 38
- IAP: 42

Survival at 180 min? Yes
Target MAP attained ? No
Time of death: 12:16 PM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 1449.4 mL (suction) + 111 mL (clot + lap pads) + 160 (phlebotomies) = 1720.4 mL
- IV fluid given: LR (37°C): 2200 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, mildly tense (IAP ~ 40 mm Hg, but monitor not working properly today; IAP really felt like ~5 mm of Hg). Upon re-opening abdomen, Foam not mixed with blood for the most part and moderate amounts of unclotted blood and some clots are seen (see Figs). Lots of surface changes noted over liver, stomach, & small intestine (see Figs). Clot mixed with foam was adherent to the surface of the injury site *in situ*.
Volume foam recovered: 531.9 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 147.0 (resected LM lobe) + 789.1 (remaining liver) = 936.1 g

Tissue harvested: two specimens: (1) clot from the site of injury, and (2) intraabdominal clot remote from injury. For porcine-human fibrinogen double IHC.

VI. COMMENTS

N = 4 of LM resection/noncompressible, with alginate foam + FS Rx. Another survivor, with reasonable MAP (54) at end of 3 h observation period, and 1.7 L blood loss. Lots of visceral surface changes today (liver, intestine, stomach, indicating substantial toxicity. We still are getting good clot adherence with foam at the injury site. So good efficacy, but toxicity still appears to be major issue.

VII. PLAN

Next several subjects will be protocol 827 (NEDED; hep resection with PCL bandage + FS). Further subjects for this protocol in a week or so, pending announcement.

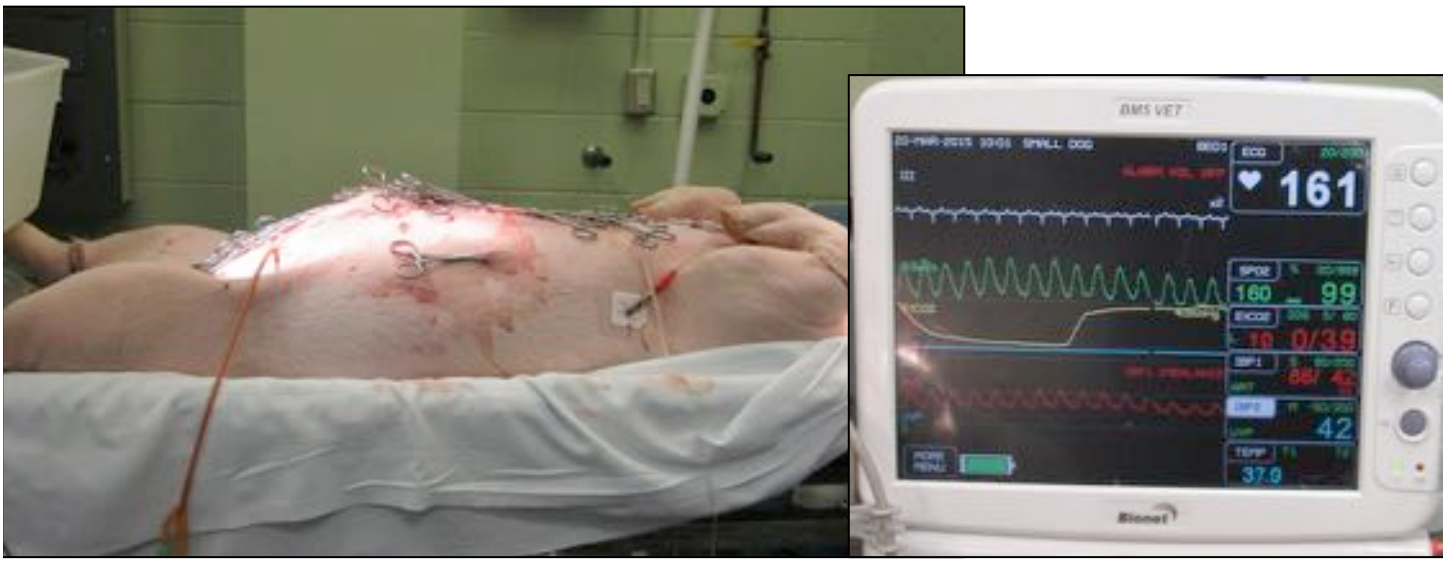

Figure 1, swine 265. Lateral view of swine after completion of 3 h observation period. Subject alive and stable, with pulse = 161, MAP = 55 mm Hg, O2 sat = 99%, EtCO2 = 39 mm Hg, temp = 37.0˚C (see monitor inset). IAP misreading, but generally was 2-3 mm Hg in this subject at end of procedure. Cephalad is to the right.

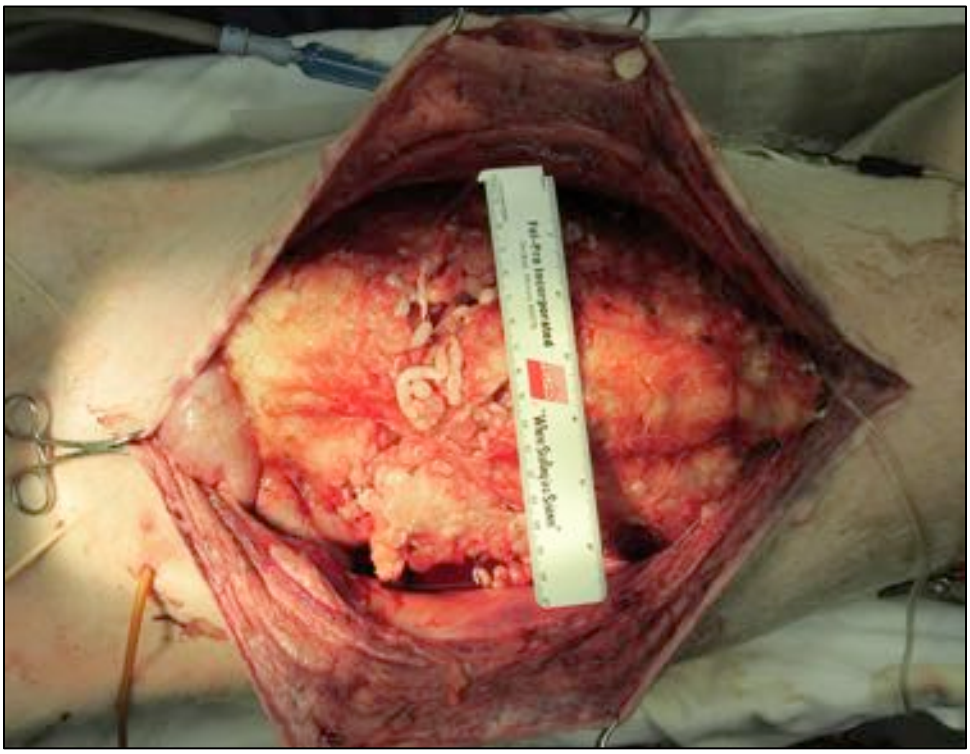

Figure 2, swine 265. Overhead view of re-opened abdomen immediately after Fig. 1 image. Subject alive. Cephalad is to right. Foam is overlying the intestines. No clot visible; small amount blood present at periphery.

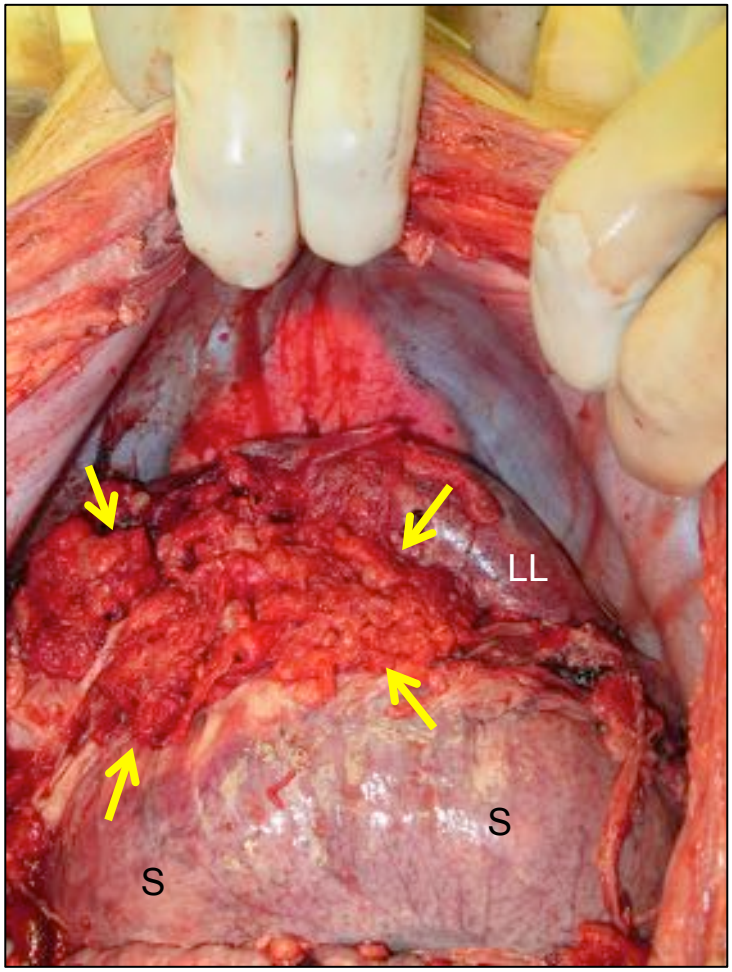

Figure 3, swine 264. Overhead view of abdomen immediately after Fig. 3 image; foam has been removed. Arrows indicated injury site covered with admixture of clot and foam. Cephalad at top of image. S = stomach, with diffuse surface changes. LL = left lateral lobe of liver.

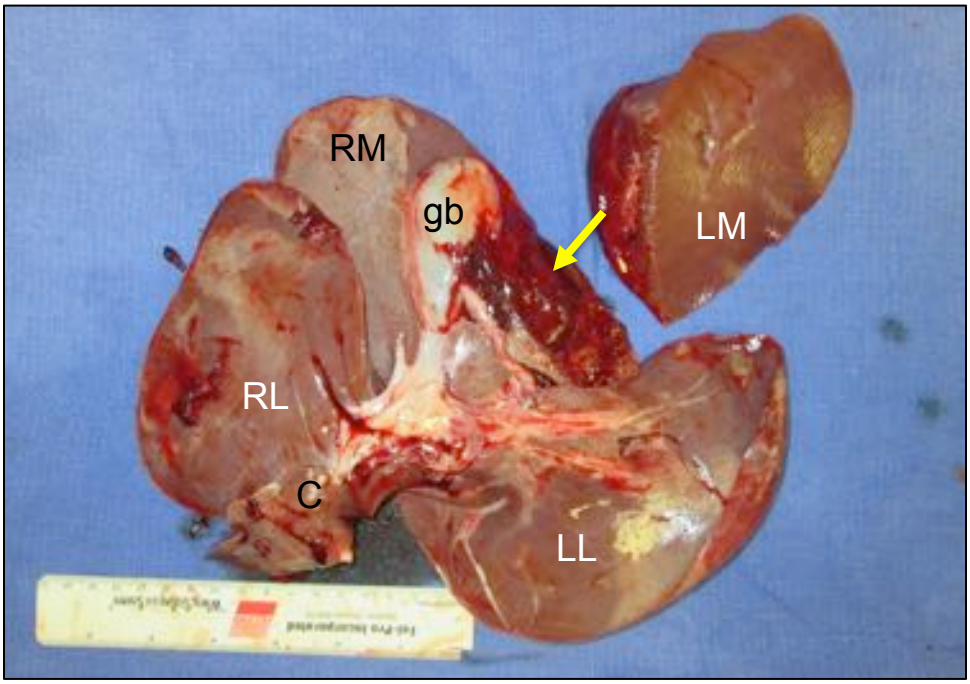

Figure 4, swine 265. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Arrow = resection site; still covered with some clot (foam/clot mix in Fig. 3 has been removed). Anterior is at top of image.

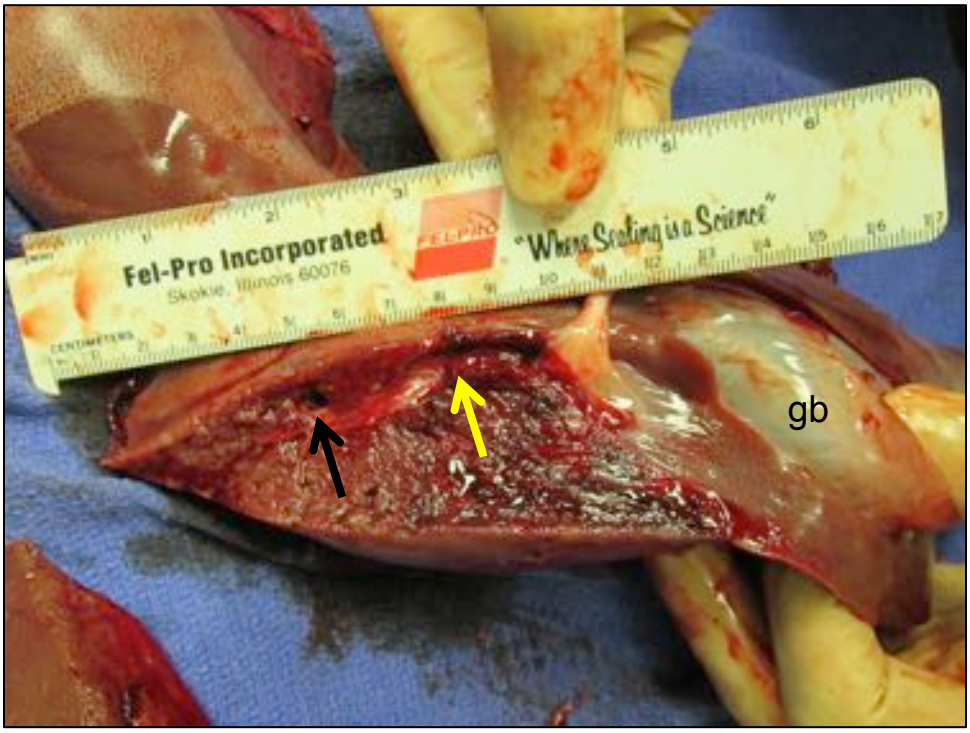

Figure 5, swine 265. Close-up of injury site (LM lobe resection). All clot and foam have been swept away. Black arrow: transected hepatic vein branch. Yellow arrow: site of transected portal vein branches.

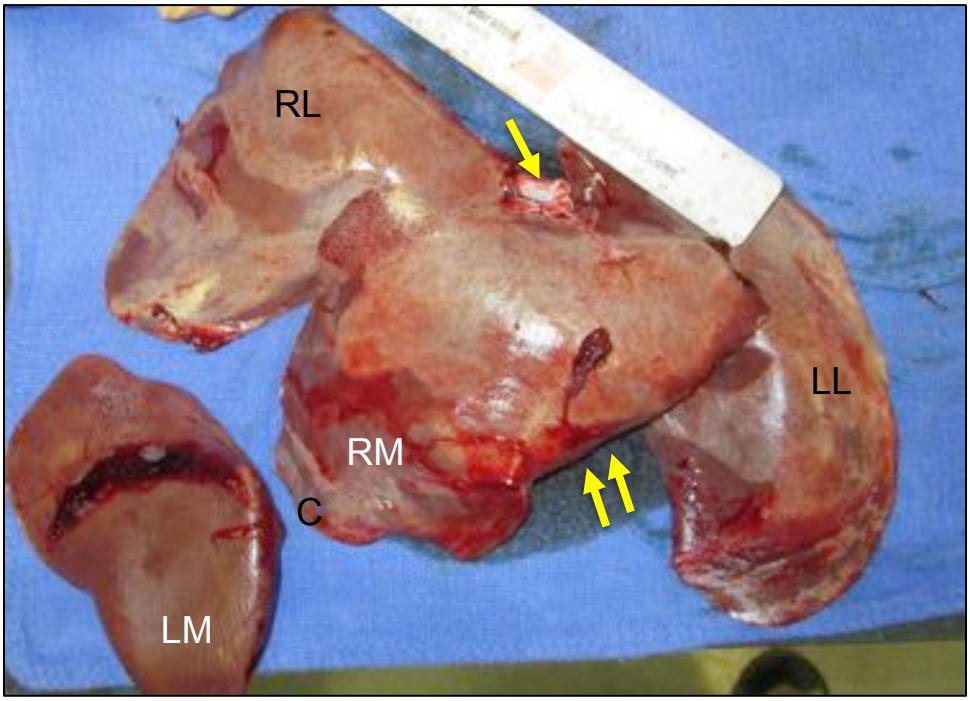

Figure 6, swine 265. Liver *ex vivo*, superior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen); LL = left lateral. Arrow = IVC; double arrow: location of LM resection site. Note diffuse surface changes to liver; contrast this with normal appearing liver of LM resection specimen, which did not come into contact with treatment. Anterior is at bottom of image.

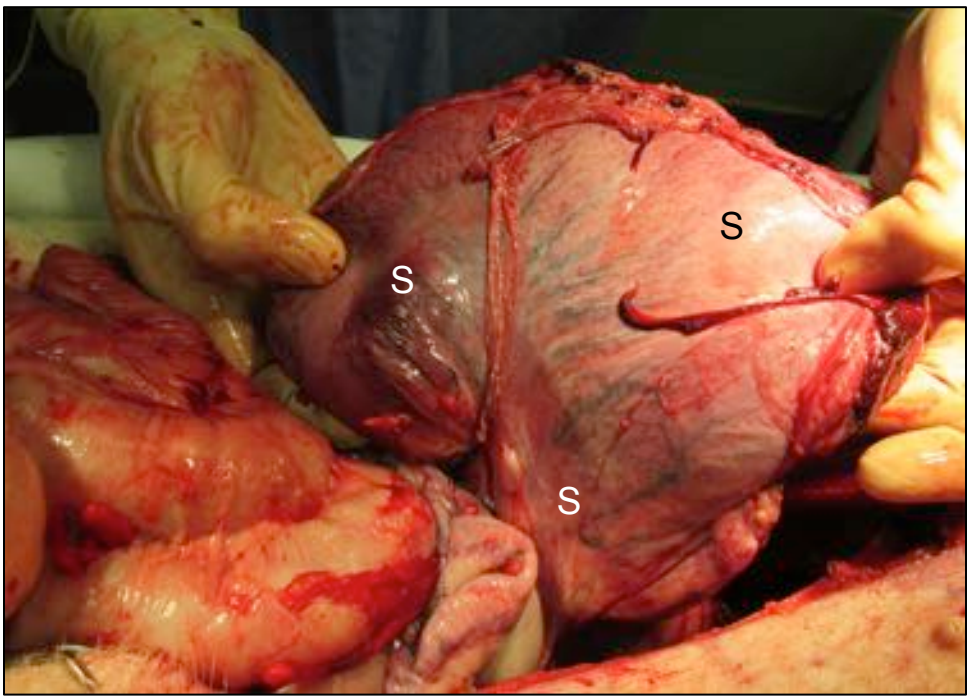

Figure 7, swine 265. Posterior wall of stomach (S), retracted up out of the wound, showing diffuse surface changes. Postmortem image. Cephalad is to the right.

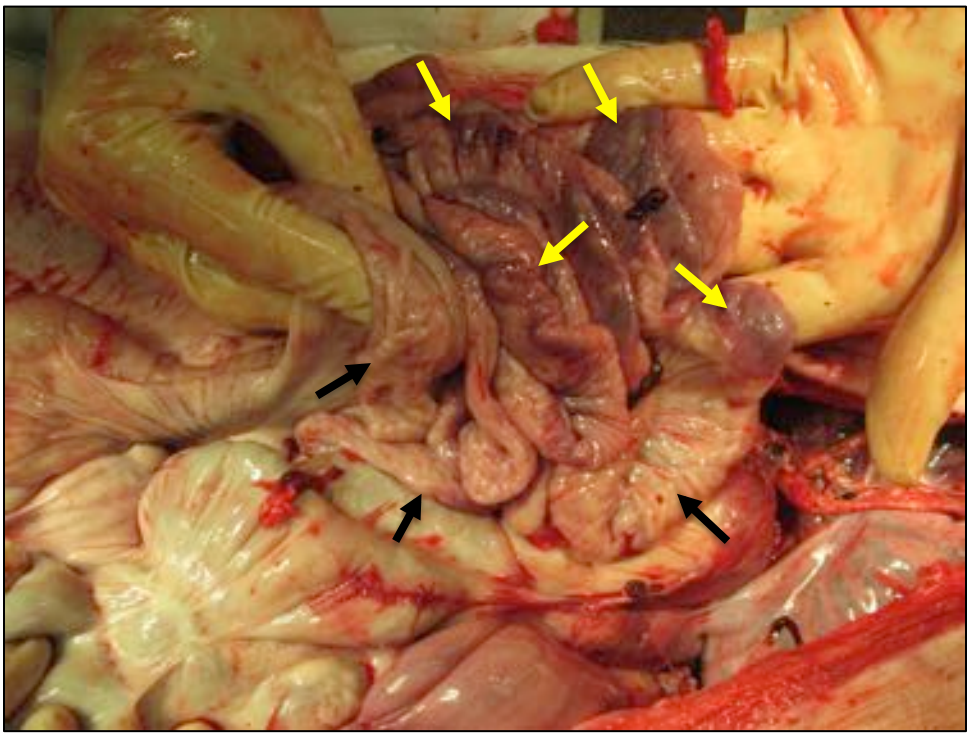

Figure 8, swine 265. Small intestine, postmortem. Contrast normal-appearing loops of bowel (black arrows) with loops having surface changes (yellow arrows). Cephalad is to the right.

I. OVERVIEW
Date: April 10, 2015
Swine no: 271
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: alginate foam + clotting factors (FII, FXIII; no fibrinogen)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: male
Date swine received from UNL Mead: 04/03/2015
Pre-procedure wt: 36.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 128
- MAP: 94
- Temp: 38.5
- EtCO2: 44

Blood draw no. 1 (initial): 7:50 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:05 AM
Spleen wt: 304.3 gm
LR (22°C) infused after splenectomy: 1000 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 304.3 + 20 + 5 = 329.3 mL
- LR (22°C) infused (spleen replacement + incidental): 850 + 0 = 1000 mL

Pre-injury VS

- HR: 102
- MAP: 99
- Temp: 36.1

- EtCO2 : 30
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:33 AM

Injury type: hepatic left medial lobectomy, non anatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through a separate stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %); no xanthan gum; Tween 20 = 0.6%; 140 mL/min 1.14 M $CaCl_2$ (20 mL/min x 7 syringe injectors).

Clotting factors: 0 mg Fibrinogen, 32.4 mg Factor XIII, 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Note: this subject had very vigorous bleeding from the injury. The blood shot upward like a geyser, and covered one of us (MAC) with blood. We have not seen such brisk hemorrhage from this injury type before. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 30 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 792.1 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:34AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:45 AM

10 min post-injury VS

- HR: 97
- MAP: 18
- Temp: 34.0
- EtCO2: 9
- IAP: 8

Blood draw no. 3: (30 min post-injury): 09:05 AM

30 min post-injury VS

- HR: 152

- MAP: 23
- Temp: 34.1
- EtCO2: 8
- IAP: 10

Survival at 180 min? NO
Target MAP attained ? No.
Time of death: 09:10 AM
Cause of death: Exsanguination from injury
Interval from injury to death: 37 min

Post-treatment fluid data:
- Blood loss: 1672.9 mL (suction) + 182.1 mL (clot + lap pads) + 40 (phlebotomies) = 1895 mL
- IV fluid given: LR (37°C): 350 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended, tense (IAP ~ 10 mm Hg). Upon re-opening abdomen, Foam mixed with blood in upper regionis, and large amounts of unclotted blood (~2 L) underneath foam, in gutters; very few clots are seen (see Figs). No ischemic areas are noted. Injury site was entirely exposed to foam with only a very thin film of clot on the injury; no adherent clots or foam clumps as previously seen.
Volume foam recovered: 848.8 g (see Figs)
Heart: right ventricle and right atrium opened after liver removed. Empty; no clots/ foam components.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe, plus multiple small branches (see Figs)
Other: none
Ex vivo liver wt: 115.9 (resected LM lobe) + 838.4 (remaining liver) = 954.3 g
Tissue harvested: None

VI. COMMENTS

Alginate + FII + FXIII (no fibrinogen) treatment of noncompressible injury. Unexpectedly, this subject died early (37 min) after the injury. Some interesting observations: (1) bleeding was stronger/faster than in any subject we've seen thus far; (2) compared to previous subjects, there was very little clotted blood within the abdomen (both the no-treatment controls and the alginate + FS subjects had lots of clot); (3) very little clot/foam was stuck to the injury site (contrast with previous alginate + FS subjects); (4) there were no surface changes to the viscera (contrast with previous alginate + FS subjects)—formulation issue? The initial bleeding was so brisk that my immediate reaction was that the pig was not going to do well. And the subsequent lack of clotting was so profound, it was as if the injected material (alginate + FII + FXIII) had an *anti-thrombotic* effect, reminiscent of what we used to see when we used Barbasol as a carrier foam. So I'm really not sure what was going on today…

This only is an N of 1, and given the bizarre results, I'm not sure how strong I would rely on today's data.

VII. PLAN

Repeat this procedure, same treatment, on Wed Apr 15th.

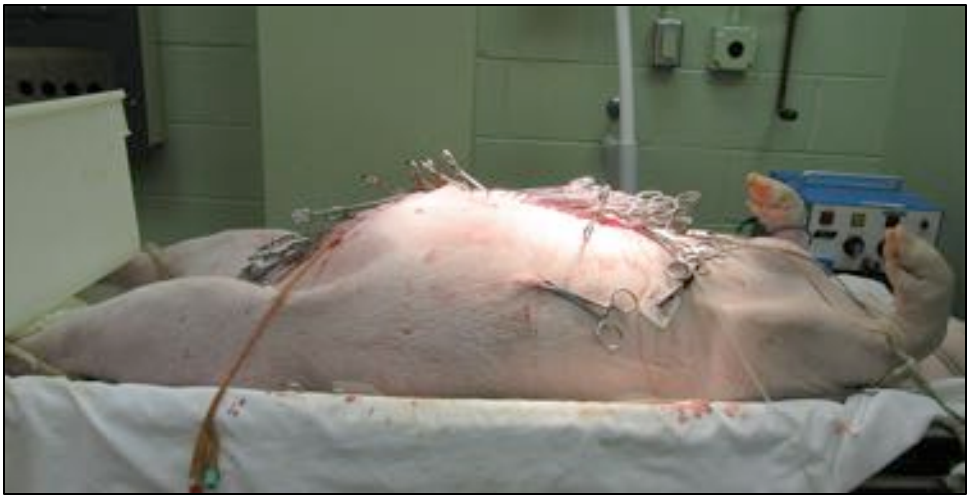

Figure 1, swine 271. Lateral view of swine at time of expiration, 37 min after injury. Cause of death = exsanguination. IAP = 8 at time of death. Cephalad is to the right.

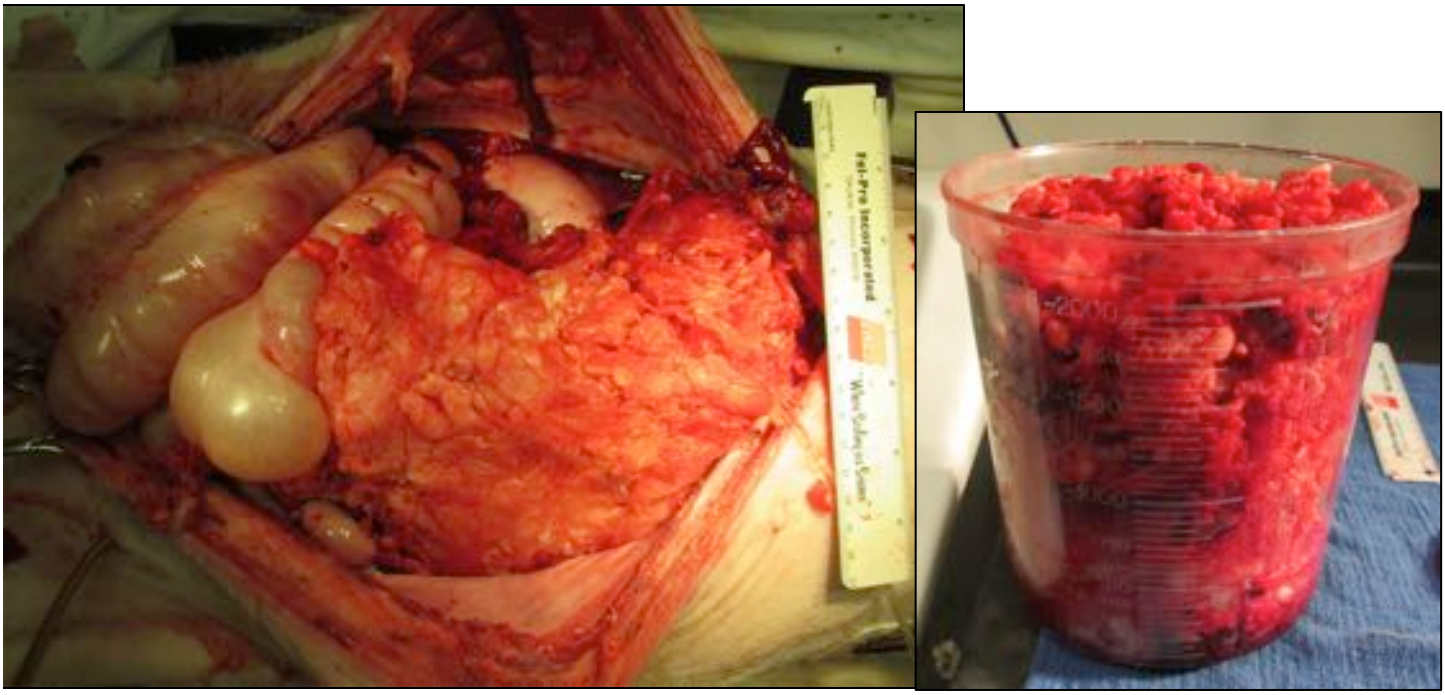

Figure 2, swine 271. Left: overhead view of re-opened abdomen at necropsy, immediately after Fig. 1 image. Cephalad is to right. Foam is overlying the intestines. About 2 L of unclotted blood was present at necropsy, underneath & around the foam; most shed blood had been suctoned out prior to this image. Right: >2 L foam recovered at necropsy. Very little clot associated with this foam.

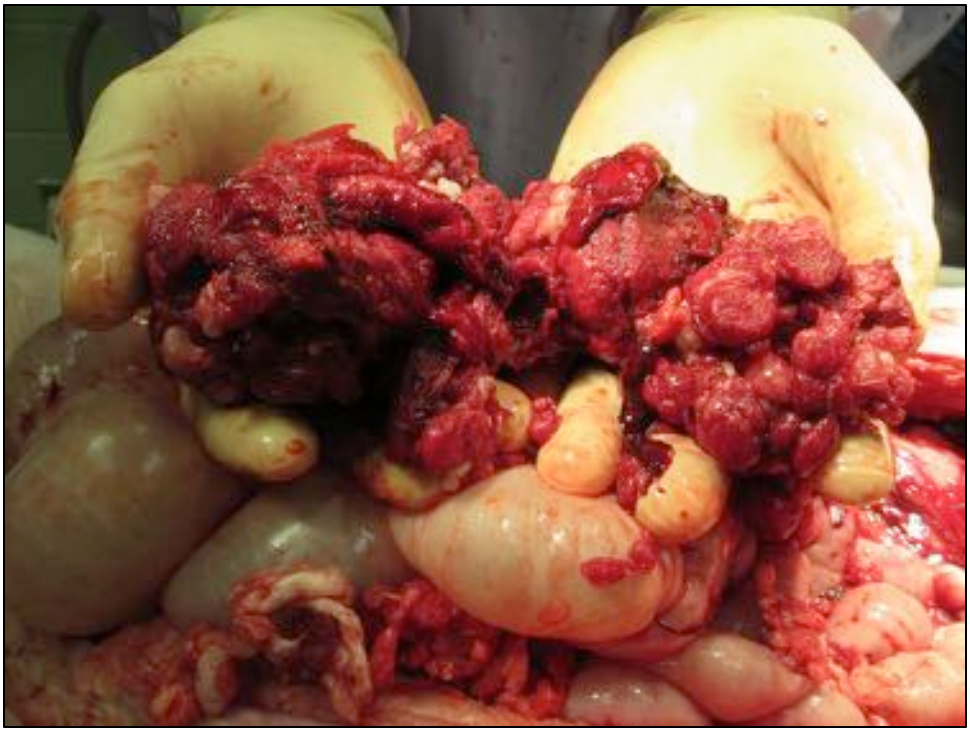

Figure 3, swine 271. Example of foam removed at necropsy. Foam was wet with blood, but very little clot associated with foam.

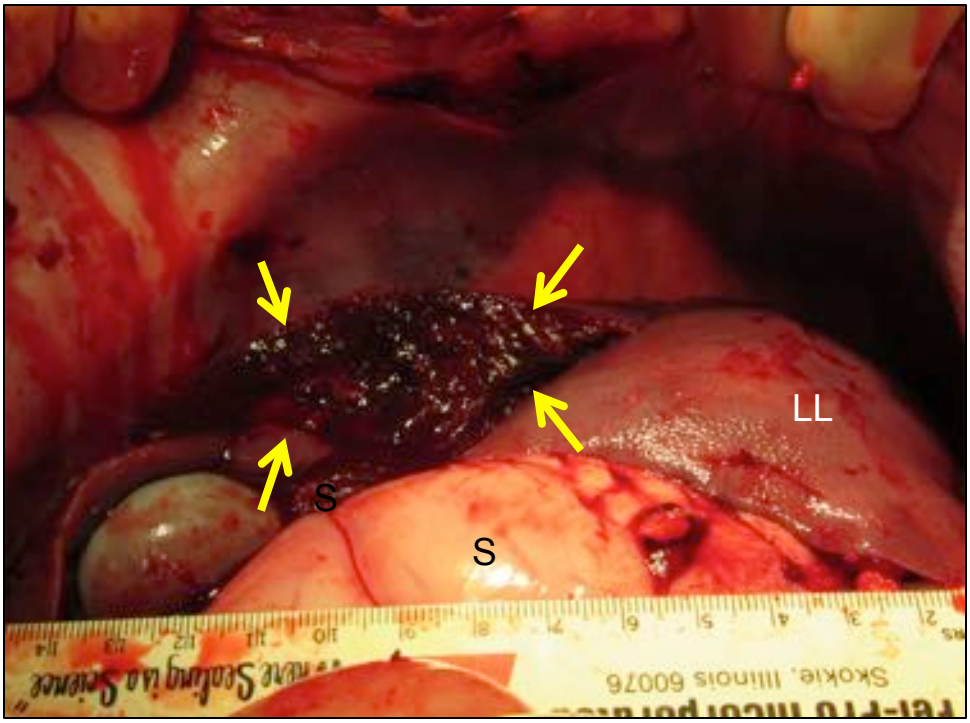

Figure 4, swine 271. Overhead view of abdomen at necropsy; foam + shed blood have been removed. Arrows indicated injury site, which was not covered with clot nor foam. Cephalad at top of image. S = stomach; LL = left lateral lobe of liver.

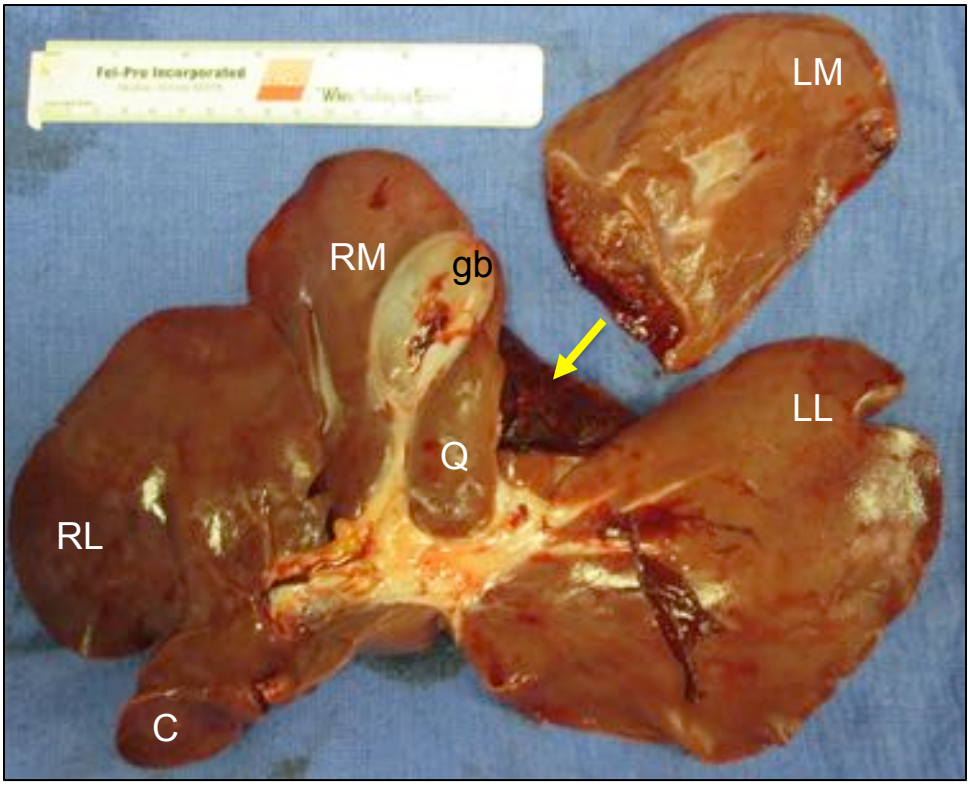

Figure 5, swine 271. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection site. Anterior is at top of image.

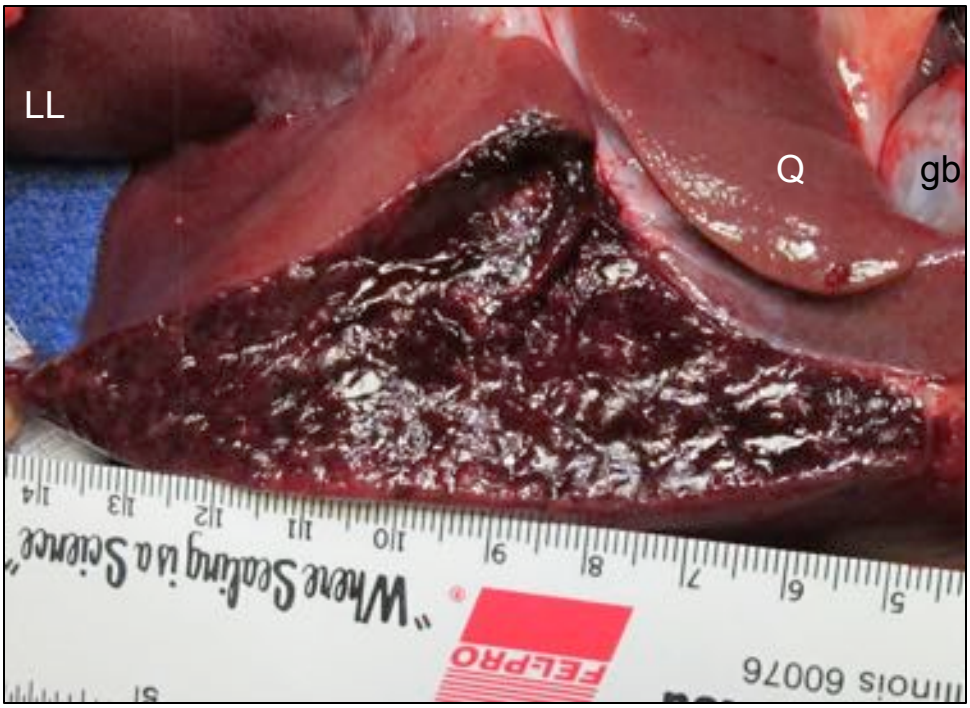

Figure 6, swine 271. Close-up of resection site (base of LM lobe). Site was actually covered with a thin film of fibrin.

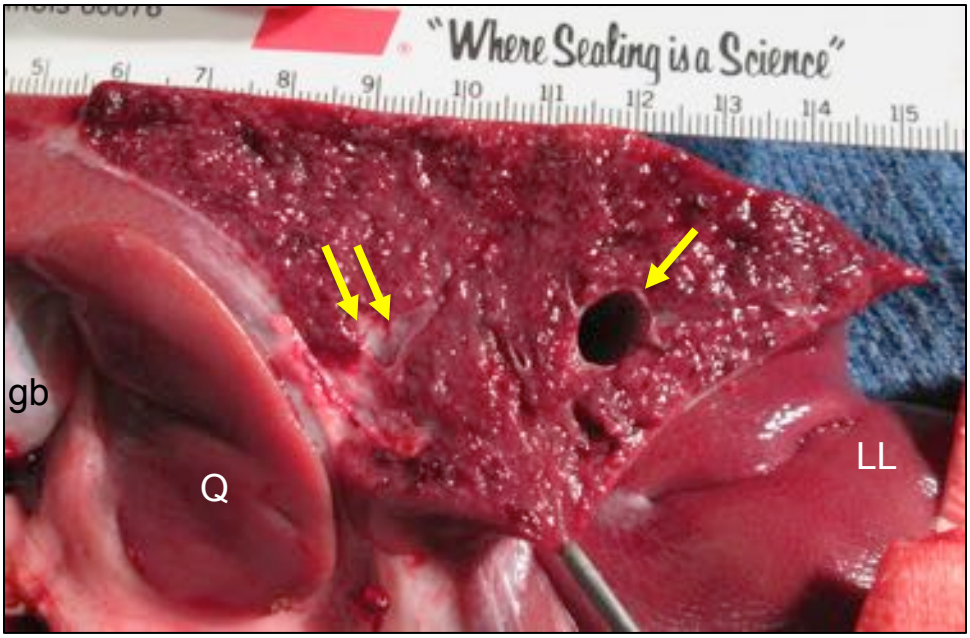

Figure 7, swine 271. Close-up of resection site (base of LM lobe), all fibrin has been swiped away. Single arrow = hepatic vein to LM lobe. Double arrow: transected branches of PV. Superior is at top of image.

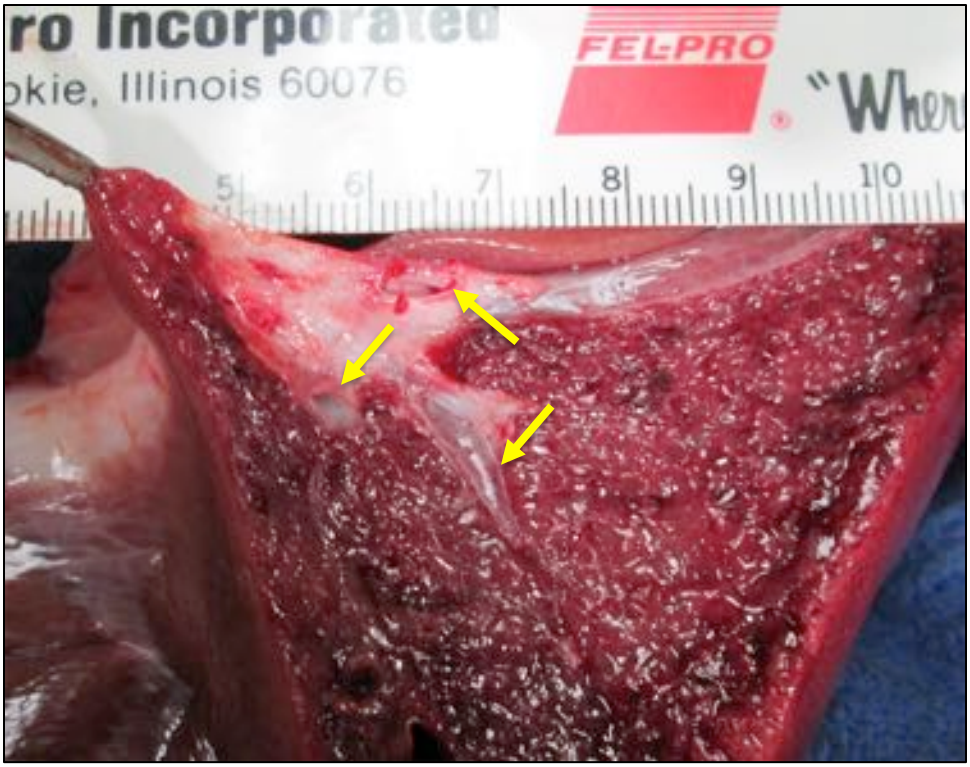

Figure 8, swine 271. Another view of resection site (base of LM lobe), showing multiple cut branches of PV to LM lobe. Superior is at lower right of image.

I. OVERVIEW
Date: April 15, 2015
Swine no: 273
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (FII + FXIII, no FI)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: male
Date swine received from UNL Mead: 04/03/2015
Pre-procedure wt: 35.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 102
- MAP: 106
- Temp: 37.9
- EtCO2: 36

Blood draw no. 1 (initial): 7:55 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:10 AM
Spleen wt: 311.7 gm
LR (22°C) infused after splenectomy: 950 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 311.7 + 30 + 0 = 341.7 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 25 = 975 mL

Pre-injury VS

- HR: 93
- MAP: 128
- Temp: 36.7

- EtCO2 : 33
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:32 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 100 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: 0 mg Fibrinogen, 32.4 mg Factor XIII, and 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 664.0 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR, 1.75 L preset maximum (50 mL/kg), given at constant rate of 9.7 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:33AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:40 AM

10 min post-injury VS
- HR: 87
- MAP: 47
- Temp: 36.6
- EtCO2: 27
- IAP: 10

Blood draw no. 3: (30 min post-injury): 09:02 AM

30 min post-injury VS
- HR: 116
- MAP: 54
- Temp: 36.4
- EtCO2: 26

- IAP: 5

Blood draw no. 4: (60 min post-injury): 09:32 AM

60 min VS
- HR: 107
- MAP: 78
- Temp: 34.1
- EtCO2: 31
- IAP: 3

Blood draw no. 5: (90 min post-injury): 10:00 AM

90 min post-injury VS
- HR: 108
- MAP: 71
- Temp: 34.7
- EtCO2: 30
- IAP: 3

Blood draw no. 6: (120 min post-injury): 10:30 AM

120 min post-injury VS
- HR: 105
- MAP: 78
- Temp: 33.9
- EtCO2: 30
- IAP: 2

Blood draw no. 7: (150 min post-injury): 11:00 AM

150 min post-injury VS
- HR: 103
- MAP: 84
- Temp: 34.0
- EtCO2: 29
- IAP: 2

Blood draw no. 8: (180 min post-injury): 11:30 AM

180 min post-injury VS
- HR: 100
- MAP: 80
- Temp: 33.9
- EtCO2: 28
- IAP: 2

Survival at 180 min? YES
Target MAP attained ? No.

Time of death: 11:30 AM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 633.8 mL (suction) + 160.4 mL (clot + lap pads) + 210 (phlebotomies) = 1004.2 mL
- IV fluid given: LR (37°C): 1850 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; mildly tense (IAP ~ 2 mm Hg). Upon re-opening abdomen, Foam not mixed with blood (see Figs) for the most part except for the surface in contact with viscera; moderate amounts of unclotted blood and few clots are seen (see Figs). There was a large mass of foam mixed with clot overlying and adherent to the injury site. Upon removal of this mass, no active bleeding from the site was seen. There was a film of clot covering the injury (see Figs). A few ischemic areas over small intestine are noted.
Volume foam recovered: 658.6 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 130.7 (resected LM lobe) + 824.5 (remaining liver) = 955.2 g

Tissue harvested: Clots from the site of injury and away from injury; portion of resected LM lobe

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VI. COMMENTS

Noncompressible injury treated with alginate foam + thrombin/FXIII, no fibrinogen (N = 2 for this Rx). Survived full 3 h easily with 1.0 L blood loss. Lump of clot and foam covering injury site with no active bleeding at 3 h.

VII. PLAN

Repeat this experiment again on 4/17/15.

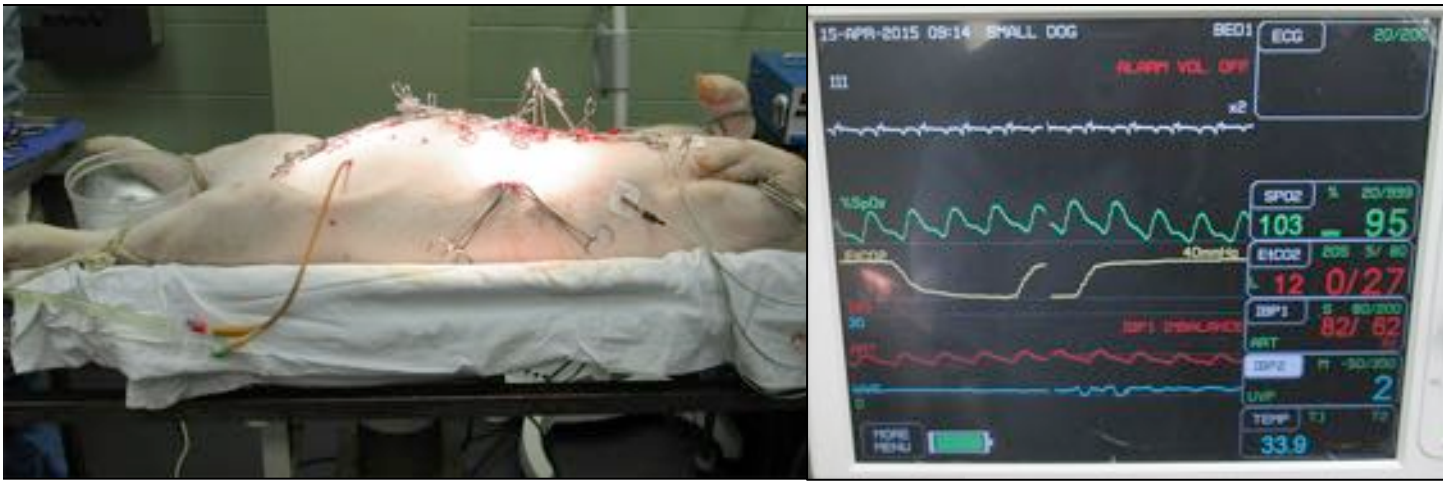

Figure 1, swine 273. Lateral view of swine at time of expiration, 180 min after injury. Subject alive and stable; HR = 103; MAP = 72 mm Hg; O2 Sat = 95%; ETCO2 = 27 mm Hg; IAP = 2 mm Hg; T = 33.9˚C. Cephalad is to the right.

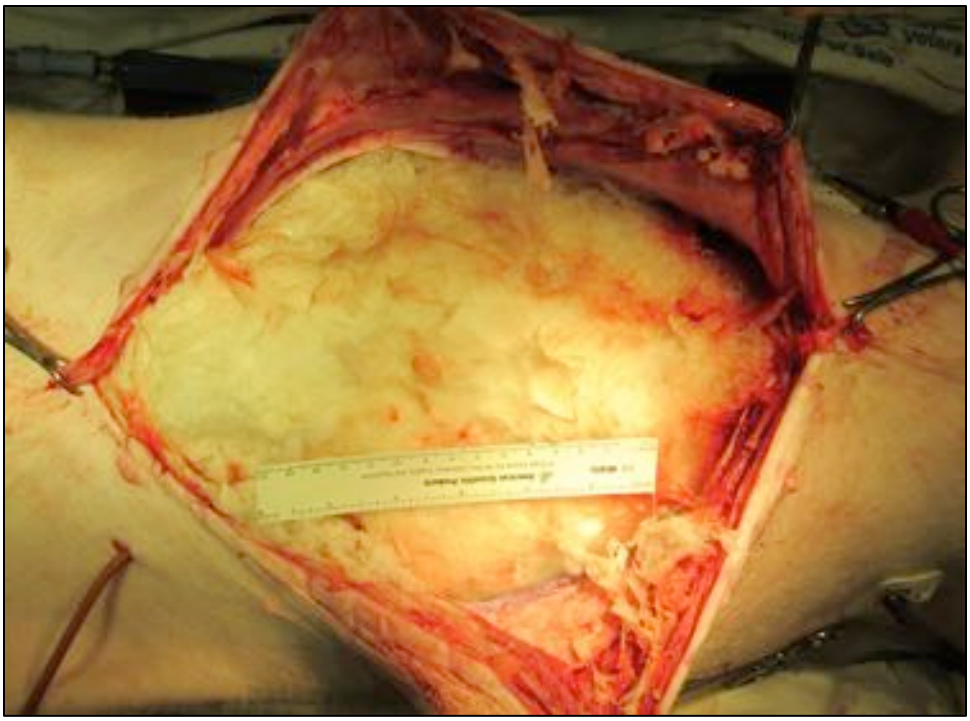

Figure 2, swine 273. Overhead view of re-opened abdomen after 180 min, immediately after Fig. 1 image. Cephalad is to right. Foam is overlying the intestines. Very little blood visible upon re-opening incision. No gas pockets.

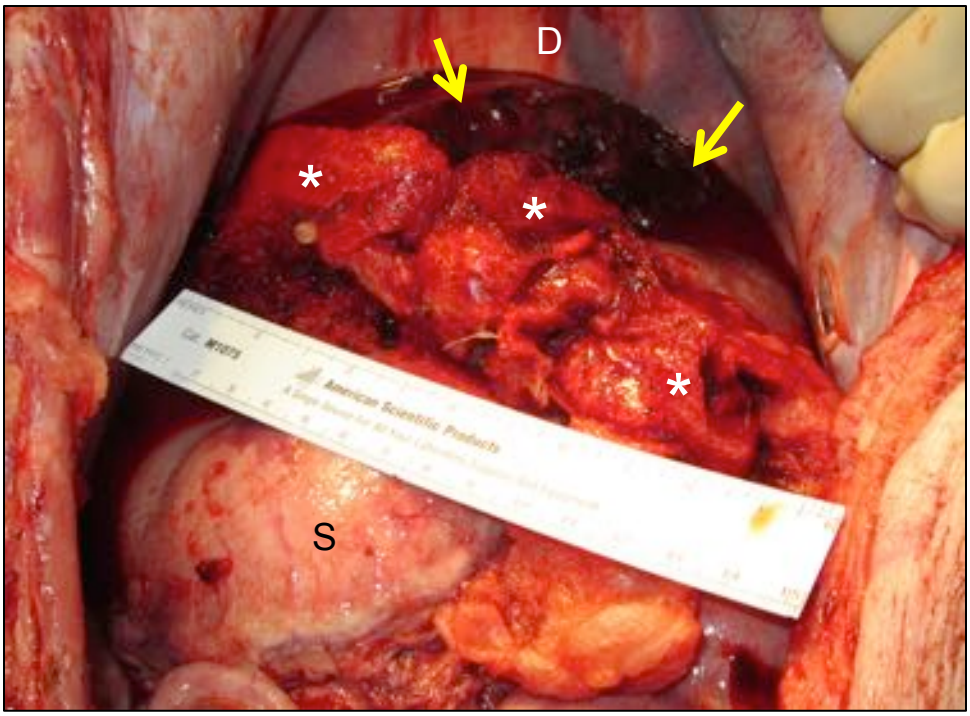

Figure 3, swine 273. Overhead view of upper abdomen after 180 min; subject still alive in this image. Nonclotted blood around liver has been suctioned away. Large mass of foam + clot was covering/adherent to injury site (*); this mass was partially pulled away in this image, exposing the top part of the injury site (arrows). S = stomach; D = diaphragm.

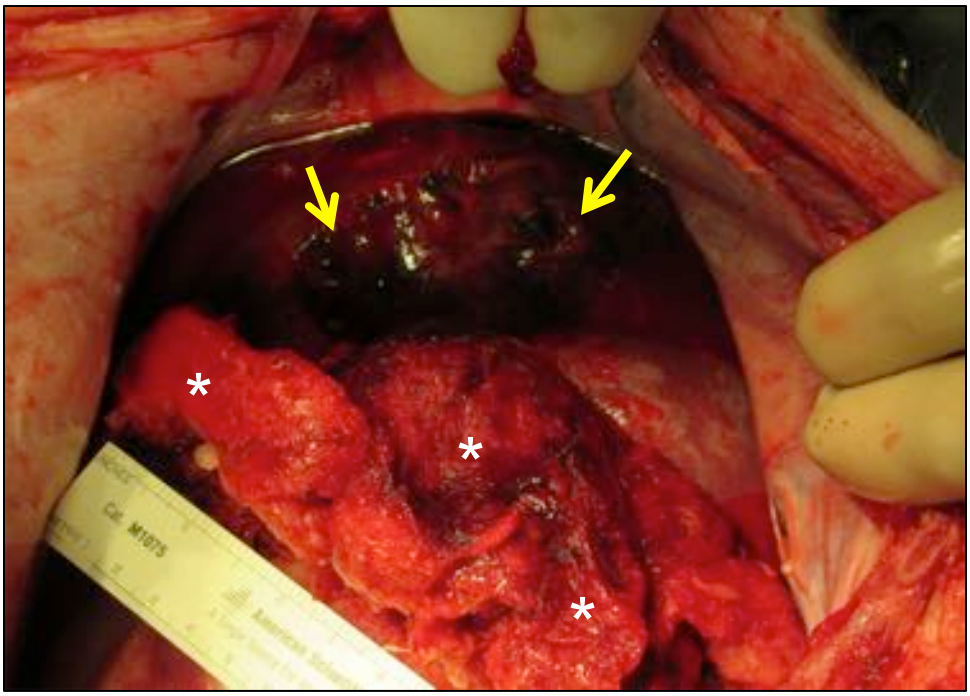

Figure 4, swine 273. Same view as Fig. 3, except that mass of clot + foam (*) has been pulled completely away from injury site (arrows).

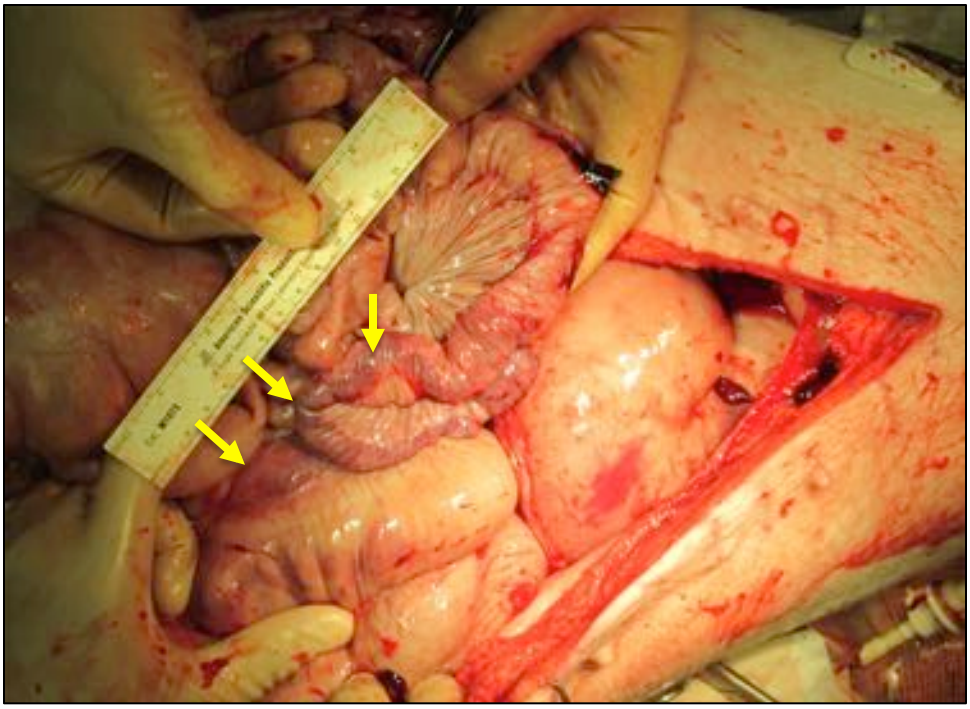

Figure 5, swine 273. Surface changes in the small bowel (arrows). Image taken after euthanasia and removal of liver.

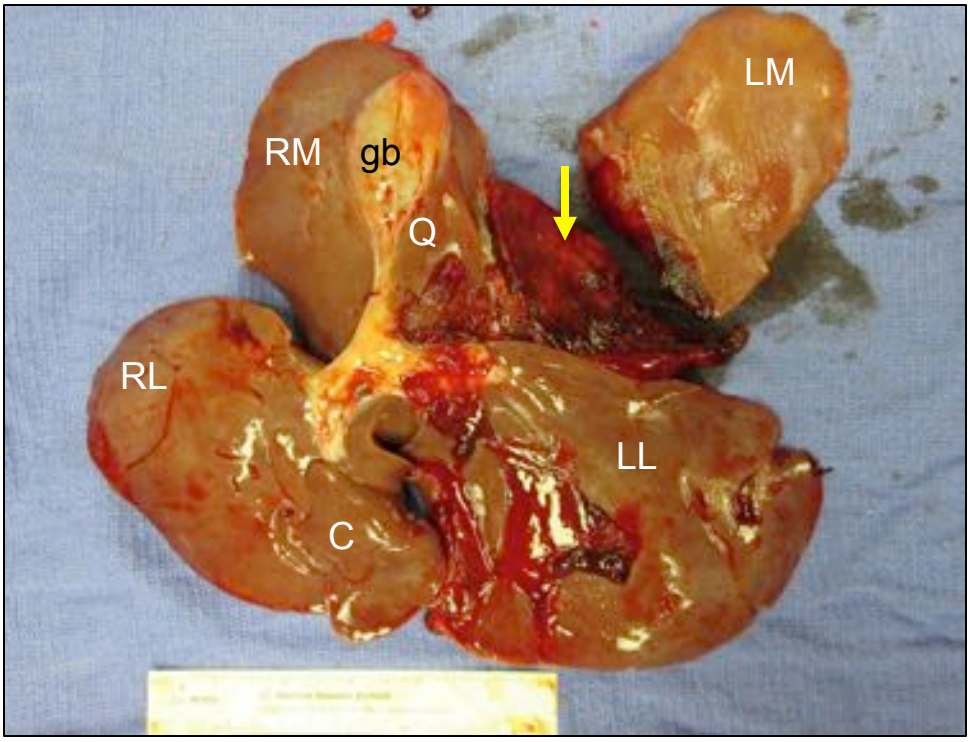

Figure 6, swine 273. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection site, covered with adherent clot. Anterior is at top of image.

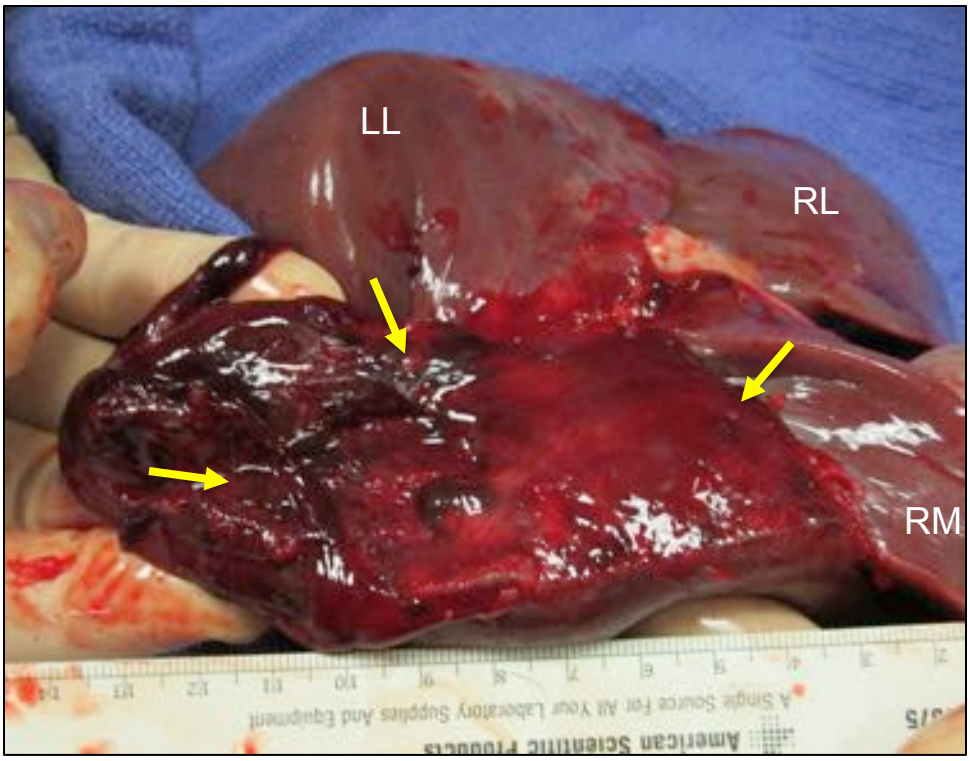

Figure 7, swine 273. Close-up of resection site (base of LM lobe), completely covered with adherent clot (arrows). Superior is at bottom of image.

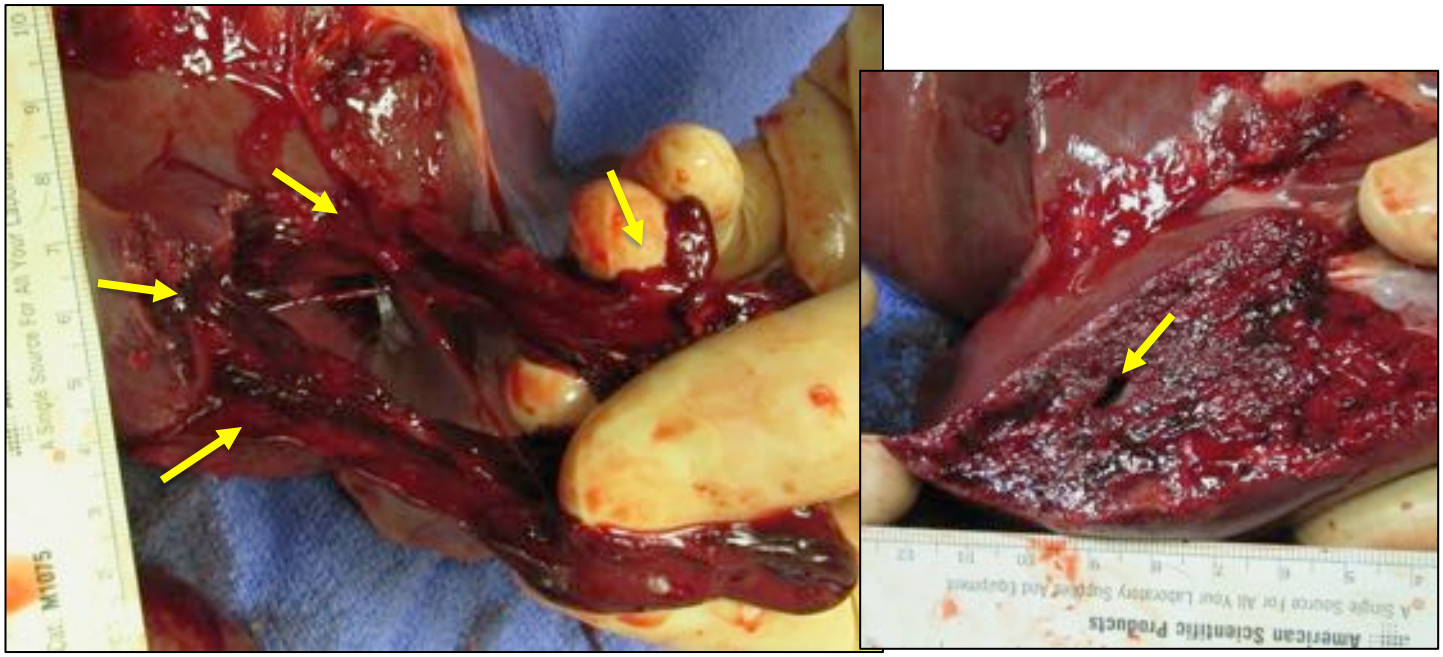

Figure 8, swine 273. Left: removal of adherent clot from injury site (arrows), showing tension as clot is lifted. Right: injury site with most clot wiped away; similar orientation to Fig. 7. Arrow = lumen of transected hepatic vein to LM lobe.

I. OVERVIEW
Date: April 17, 2015
Swine no: 274
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (FII + FXIII, no FI)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: male
Date swine received from UNL Mead: 04/03/2015
Pre-procedure wt: 32.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 128
- MAP: 104
- Temp: 38.1
- EtCO2: 39

Blood draw no. 1 (initial): 8:10 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:20 AM
Spleen wt: 298.6 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 298.6 + 30 + 8.2 = 336.8 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 250 = 1200 mL
- Note: subject was hypotensive (MAP <30 mm Hg) after splenectomy, though very little blood was lost. Subject responded with LR splenic replacement and 0.3 mg epinephrine.

Pre-injury VS

- HR: 115

- MAP: 98
- Temp: 36.4
- EtCO2 : 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:57 AM

Injury type: Hepatic left medial lobectomy, non anatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 92 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: 0 mg Fibrinogen; 32.4 mg Factor XIII, and 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 471.0 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.63 L preset maximum (50 mL/kg), given at constant rate of 9.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:58AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:10 AM

10 min post-injury VS

- HR: 98
- MAP: 39
- Temp: 36.3
- EtCO2: 37
- IAP: 10

Blood draw no. 3: (30 min post-injury): 09:30 AM

30 min post-injury VS

- HR: 109
- MAP: 26

- Temp: 36.0
- EtCO2: 30
- IAP: 4

Blood draw no. 4: (60 min post-injury): 10:00 AM

60 min VS
- HR: 70
- MAP: 34
- Temp: 35.8
- EtCO2: 34
- IAP: 3

Blood draw no. 5: (90 min post-injury): 10:30 AM

90 min post-injury VS
- HR: 67
- MAP: 37
- Temp: 35.5
- EtCO2: 33
- IAP: 2

Blood draw no. 6: (120 min post-injury): 11:00 AM

120 min post-injury VS
- HR: 69
- MAP: 48
- Temp: 35.2
- EtCO2: 33
- IAP: 1

Blood draw no. 7: (150 min post-injury): 11:30 AM

150 min post-injury VS
- HR: 71
- MAP: 41
- Temp: 35.3
- EtCO2: 32
- IAP: 1

Blood draw no. 8: (180 min post-injury): 12:00 PM

180 min post-injury VS
- HR: 72
- MAP: 45
- Temp: 35.2
- EtCO2: 30
- IAP: 1

Survival at 180 min? YES
Target MAP attained ? No.
Time of death: 12:00 PM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 820.4 mL (suction) + 197.6 mL (clot + lap pads) + 210 (phlebotomies) = 1228.0 mL
- IV fluid given: LR (37°C): 1950 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen minimally distended; tense (IAP ~1 mm Hg). Upon re-opening abdomen, Foam not mixed with blood for the most part in the superior abdomen except for the surface in contact with viscera and moderate amounts of unclotted blood and few clots are seen (see Figs). Similar to our previous subject, there was a large mass of foam mixed with clot overlying and adherent to the injury site (see Figs). A few ischemic areas over small intestine are noted.
Volume foam recovered: 469.2 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 128.4 (resected LM lobe) + 907.3 (remaining liver) = 1035.7 g

Tissue harvested: Clots and liver specimen from the site of injury.

VI. COMMENTS

Noncompressible injury treated with alginate foam + thrombin/FXIII, no fibrinogen (N = 3 for this Rx). Survived full 3 h easily with 1.2 L blood loss. Lump of clot and foam covering and adherent to injury site, with no active bleeding at 3 h.

Note: this particular subject had a hypotensive episode after spleen was removed during the prep phase. Not related to blood loss. This responded to fluid resuscitation and 0.3 mg of epinephrine given IV. Subject then stabilized and was injured as planned. Not sure what the cause of the pre-injury hypotension was…

Also note that initially during injection, IAP climbs into the 20-30 mm Hg range, but by end of 180 min, almost all subjects have low IAP (<5 mm Hg). So persistent hemostatic effect does not appear to require persistently elevated IAP, at least as measured by our improvised IAP measuring device (a 50 mL IV bag filled with saline and transduced to the monitor).

VII. PLAN

No procedures week of Apr 20th, MAC will be out of town. Next subjects for this protocol will be on Wed Apr 29th and Fri May 1st.

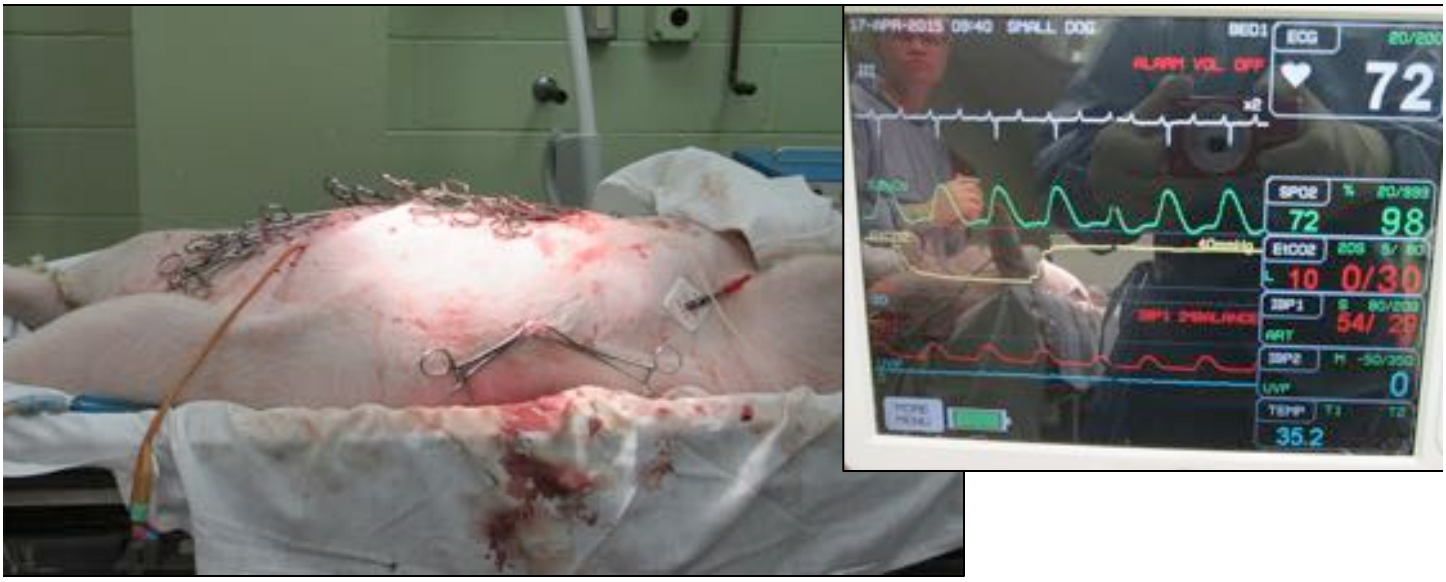

Figure 1, swine 274. Lateral view of swine at end of 180 min observation. Subject alive and stable; HR = 72; MAP = 37mm Hg; O2 Sat = 98%; ETCO2 = 30 mm Hg; IAP = 0 mm Hg; T = 35.2˚C. Cephalad is to the right.

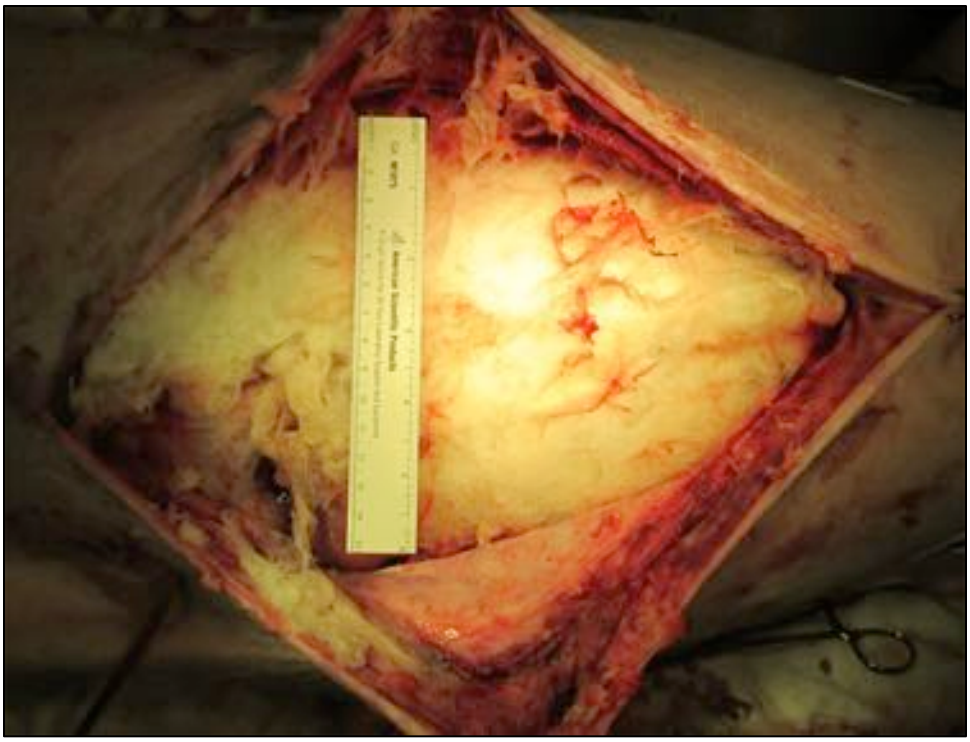

Figure 2, swine 274. Overhead view of re-opened abdomen after 180 min, immediately after Fig. 1 image. Cephalad is to right. Foam is overlying the intestines. Very little blood visible upon re-opening incision. No gas pockets.

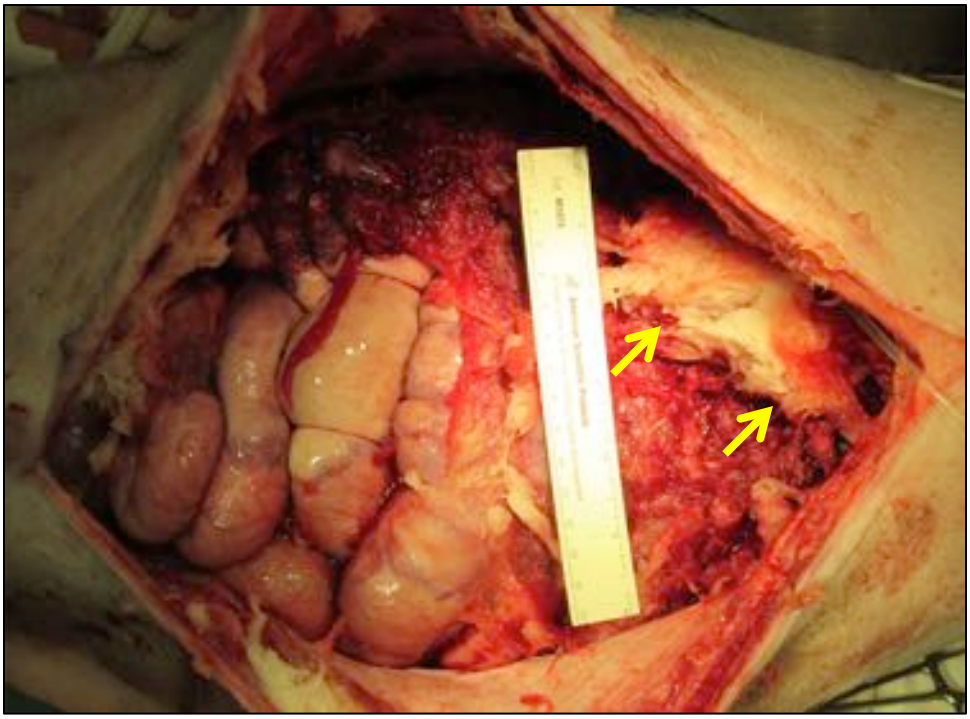

Figure 3, swine 274. Overhead view of upper abdomen after 180 min; subject still alive in this image. Nonclotted blood around liver has been suctioned away. Large mass of foam + clot was covering/adherent to injury site (arrows). Cephalad to left of image.

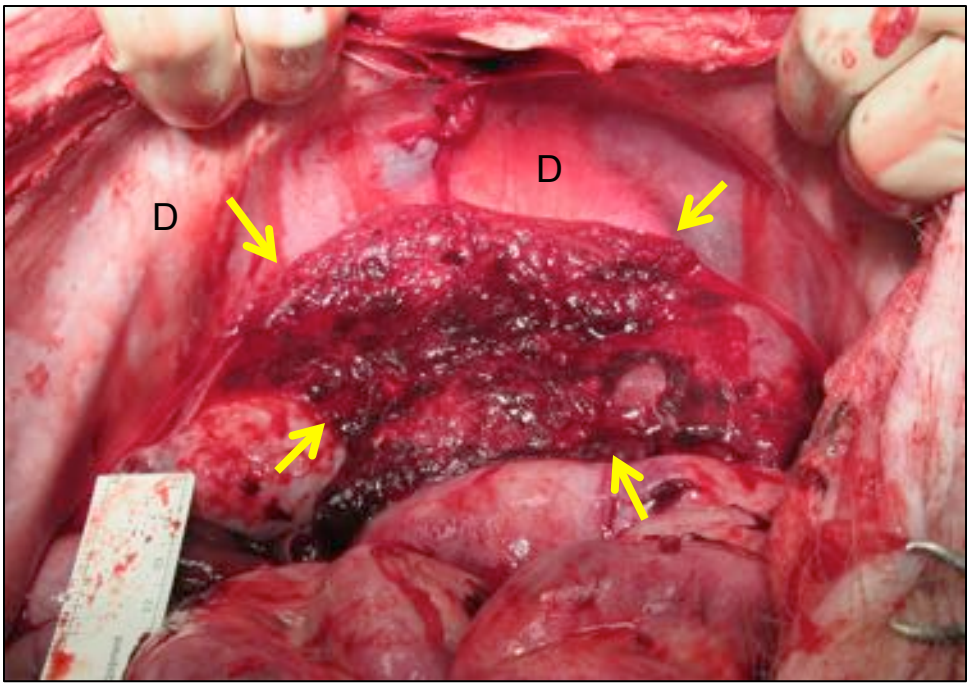

Figure 4, swine 274. View of injury site (arrows) after most of foam has been removed. Cephalad toward top of image.

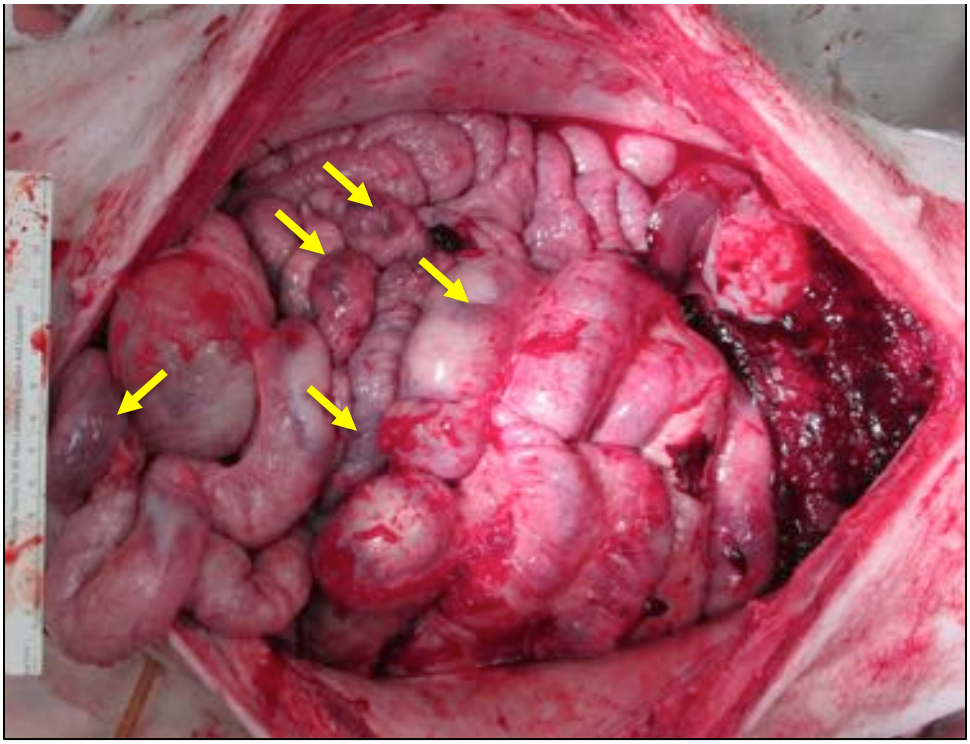

Figure 5, swine 274. Surface changes in the small bowel (arrows). Image taken prior to euthanasia. Cephalad to left of image.

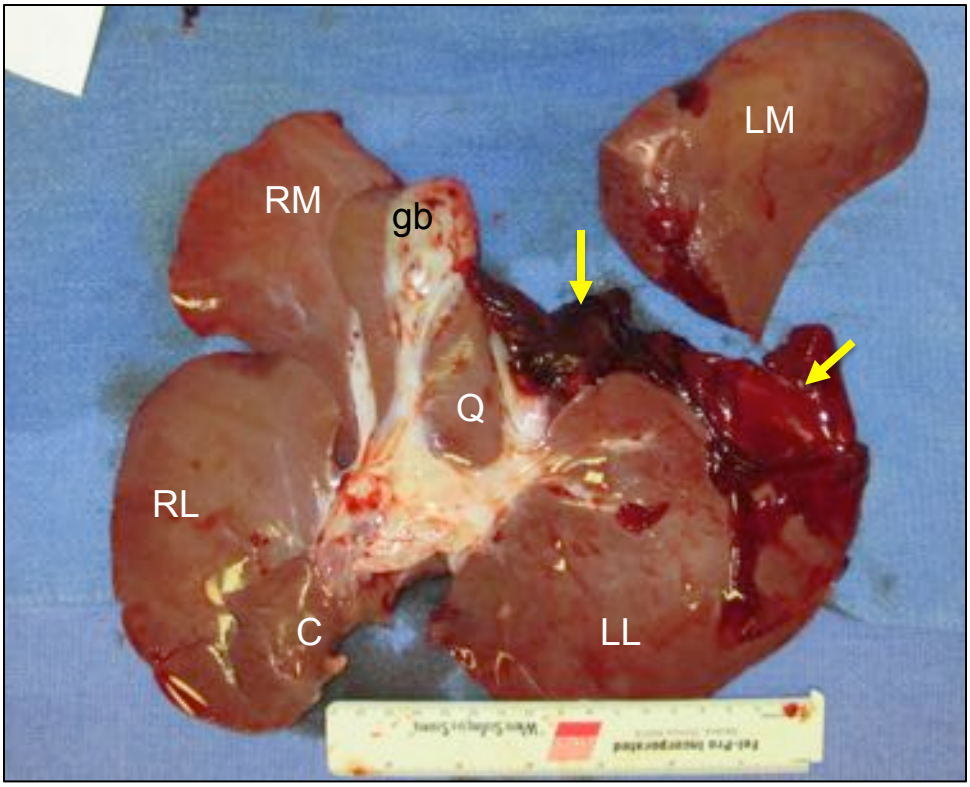

Figure 6, swine 274. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection site, covered with large adherent clot. Anterior is at top of image.

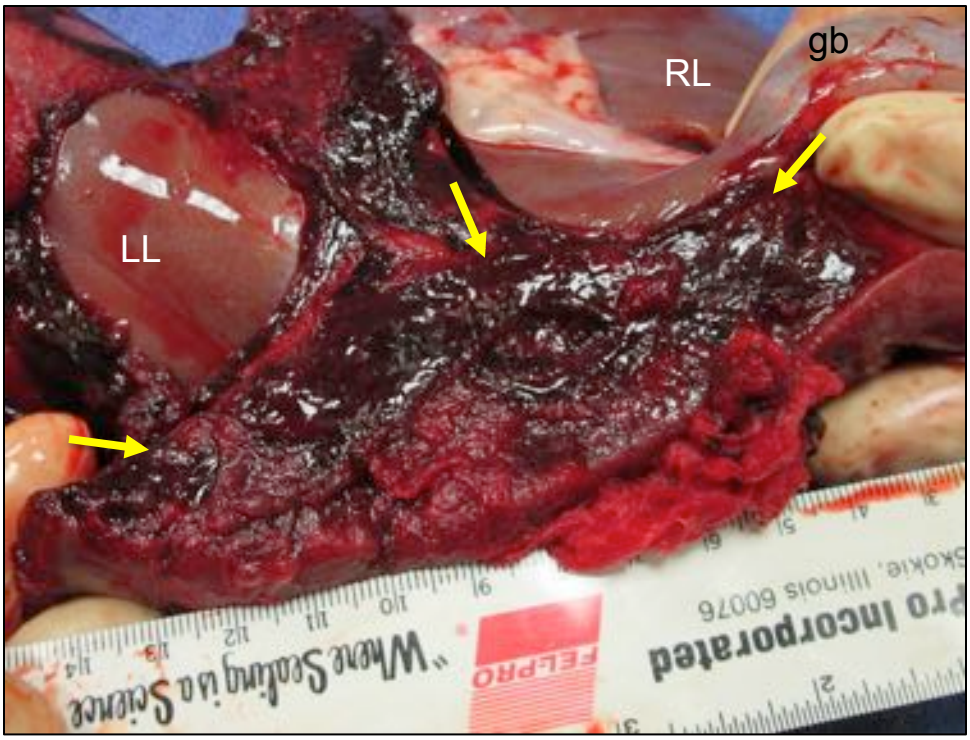

Figure 7, swine 274. Close-up of resection site (base of LM lobe), completely covered with adherent clot + foam (arrows). Superior is at bottom of image.

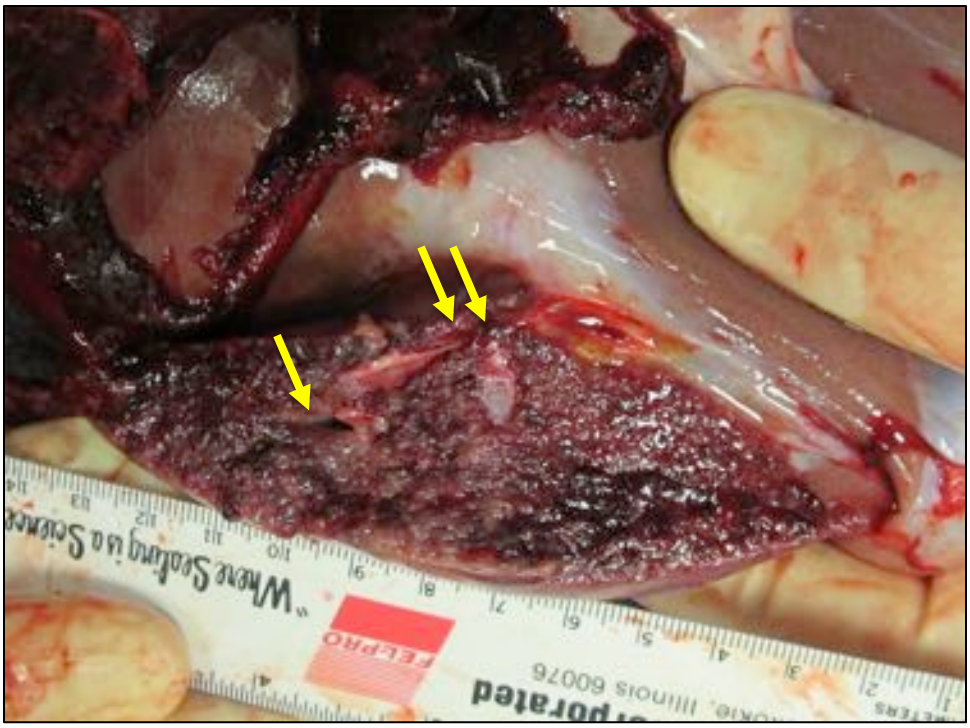

Figure 8, swine 274. Injury site with most clot wiped away; similar orientation to Fig. 7. Single arrow = lumen of transected hepatic vein to LM lobe; double arrow = transected PV branches.

I. OVERVIEW
Date: April 29, 2015
Swine no: 277
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (FII, FXIII, **no FI**)
Personnel: Carlson, Yanala, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: male
Date swine received from UNL Mead: 04/16/2015
Pre-procedure wt: 36.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 89
- MAP: 105
- Temp: 37.1
- EtCO2: 35

Blood draw no. 1 (initial): 8:10 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:25 AM
Spleen wt: 323.3 gm
LR (22°C) infused after splenectomy: 1000 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 323.3 + 30 + 15.5 = 368.8 mL
- LR (22°C) infused (spleen replacement + incidental): 1000 + 200 = 1200 mL

Pre-injury VS

- HR: 90
- MAP: 119
- Temp: 36.1

- EtCO2 : 38
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:45 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 32.4 mg Factor XIII, and 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 997.2 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 100

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.0 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:45AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:55 AM

10 min post-injury VS
- HR: 110
- MAP: 38
- Temp: 35.6
- EtCO2: 27
- IAP: 14

Blood draw no. 3: (30 min post-injury): 09:15 AM

30 min post-injury VS
- HR: 107
- MAP: 50
- Temp: 35.2
- EtCO2: 37

- IAP: 11

Blood draw no. 4: (60 min post-injury): 09:45 AM

60 min VS
- HR: 97
- MAP: 59
- Temp: 35.0
- EtCO2: 37
- IAP: 9

Blood draw no. 5: (90 min post-injury): 10:15 AM

90 min post-injury VS
- HR: 103
- MAP: 57
- Temp: 35.0
- EtCO2: 36
- IAP: 7

Blood draw no. 6: (120 min post-injury): 10:45 AM

120 min post-injury VS
- HR: 95
- MAP: 66
- Temp: 34.7
- EtCO2: 33
- IAP: 6

Blood draw no. 7: (150 min post-injury): 11:15 AM

150 min post-injury VS
- HR: 94
- MAP: 65
- Temp: 34.4
- EtCO2: 33
- IAP: 5

Blood draw no. 8: (180 min post-injury): 11:45 AM

180 min post-injury VS
- HR: 94
- MAP: 63
- Temp: 34.0
- EtCO2: 31
- IAP: 5

Survival at 180 min? YES
Target MAP attained ? No

Time of death: 11:45 AM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 818.0 mL (suction) + 109.7 mL (clot + lap pads) + 220 (phlebotomies) = 1147.7 mL
- IV fluid given: LR (37°C): 1900 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~ 5 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Some ischemic areas over small intestine were noted, along with generalized discoloration of colon. Injury site was covered with a large mass of foam + clot (see Figs). When this mass peeled away, hemorrhage resumed.
Volume foam recovered: 838.0 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 137.3 (resected LM lobe) + 726.0 (remaining liver) = 863.3 g

Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam + thrombin/FXIII, no fibrinogen (N = 4 for this Rx). Survived full 3 h easily with 1.1 L blood loss. Lump of clot and foam covering and adherent to injury site, with no active bleeding at 3 h.

VII. PLAN

Continue with this series on Fri 5/1 (alginate + FII/FXIII, no FI).

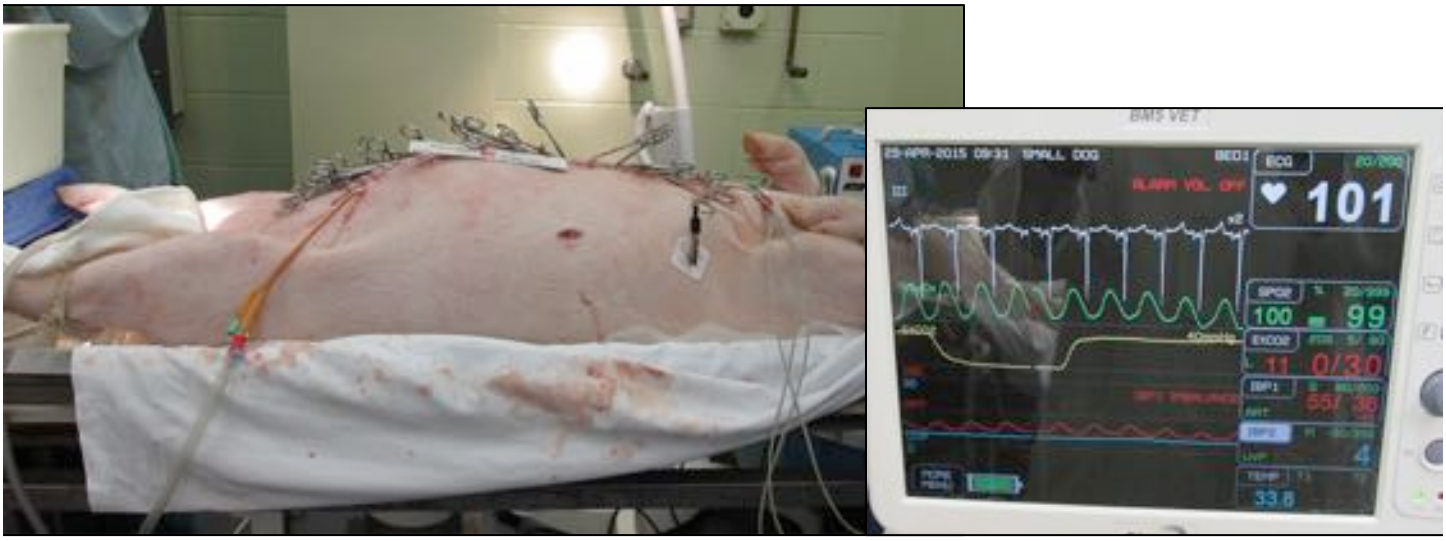

Figure 1, swine 277. Lateral view of swine at end of 180 min observation. Subject alive and stable; HR = 101; MAP = 45 mm Hg; O2 Sat = 99%; ETCO2 = 30 mm Hg; IAP = 4 mm Hg; T = 33.8˚C. Cephalad is to the right.

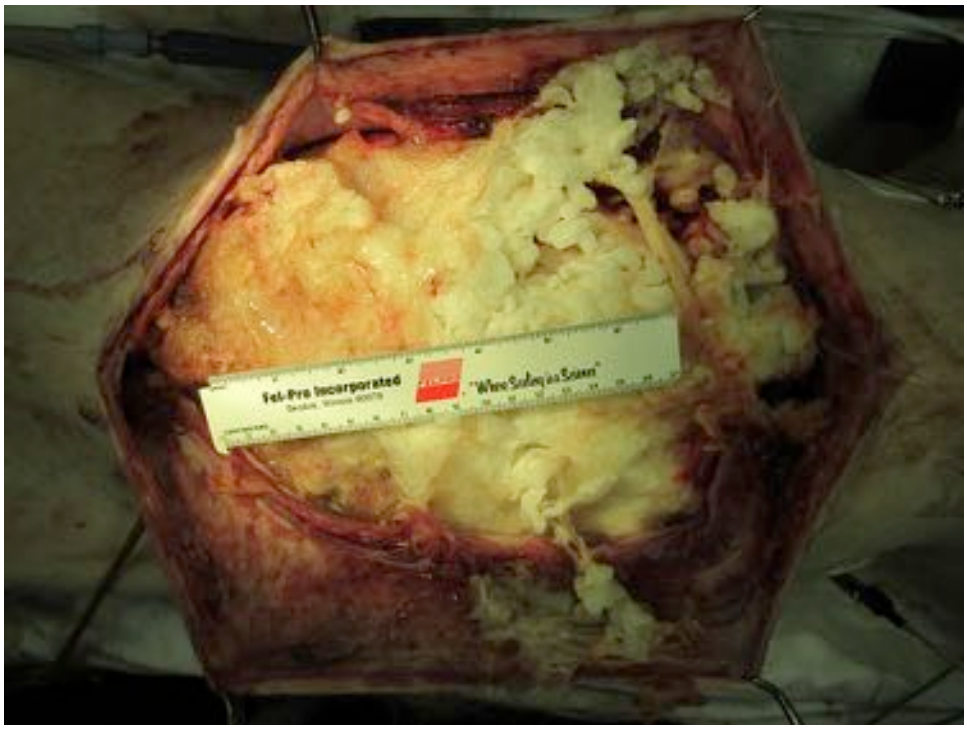

Figure 2, swine 277. Overhead view of re-opened abdomen after 180 min, immediately after Fig. 1 image. Cephalad is to right. Foam is overlying the intestines. Very little blood visible upon re-opening incision. No gas pockets.

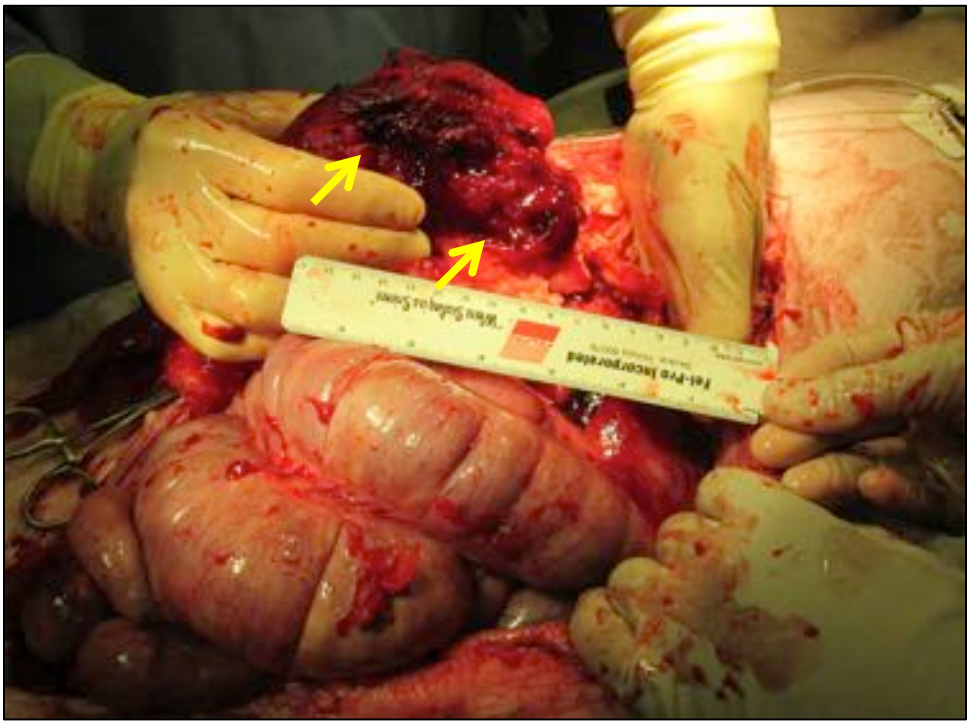

Figure 3, swine 277. View of upper abdomen after 180 min; subject still alive in this image. Large mass of foam + clot was covering/adherent to injury site, and has been peeled away from injury in this image. Arrows indicate surface of foam/clot mass that was adherent to injury. Cephalad at upper left of image.

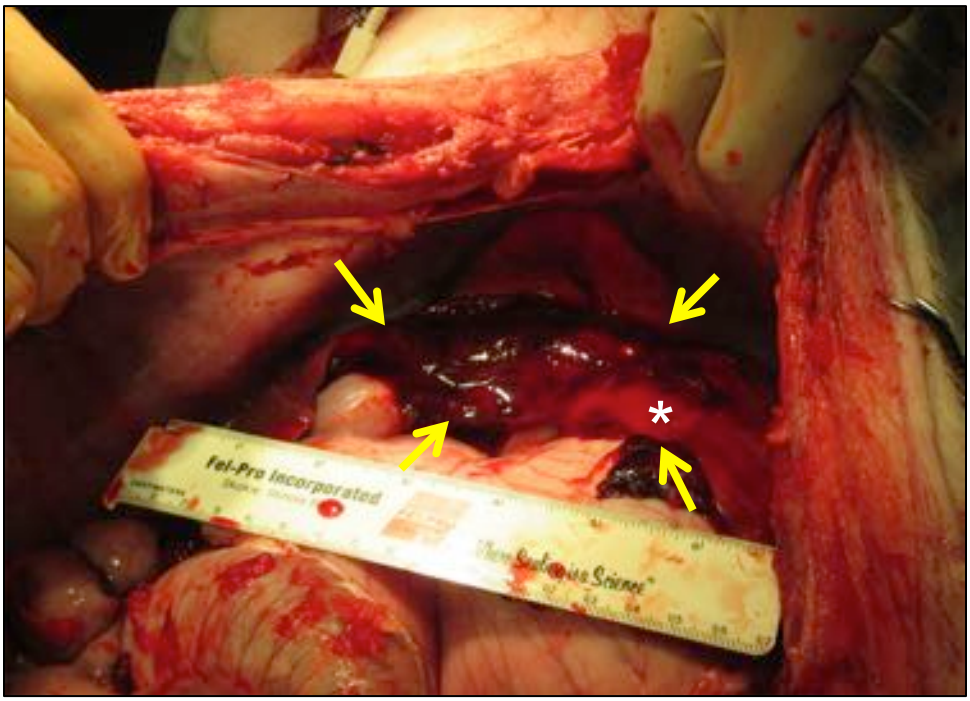

Figure 4, swine 277. View of injury site (arrows) within upper abdomen after most of foam has been removed. Subject still alive in this image. Cephalad toward top of image. Some active hemorrhage (*) occurred after clot/foam mass in Fig. 3 was peeled away.

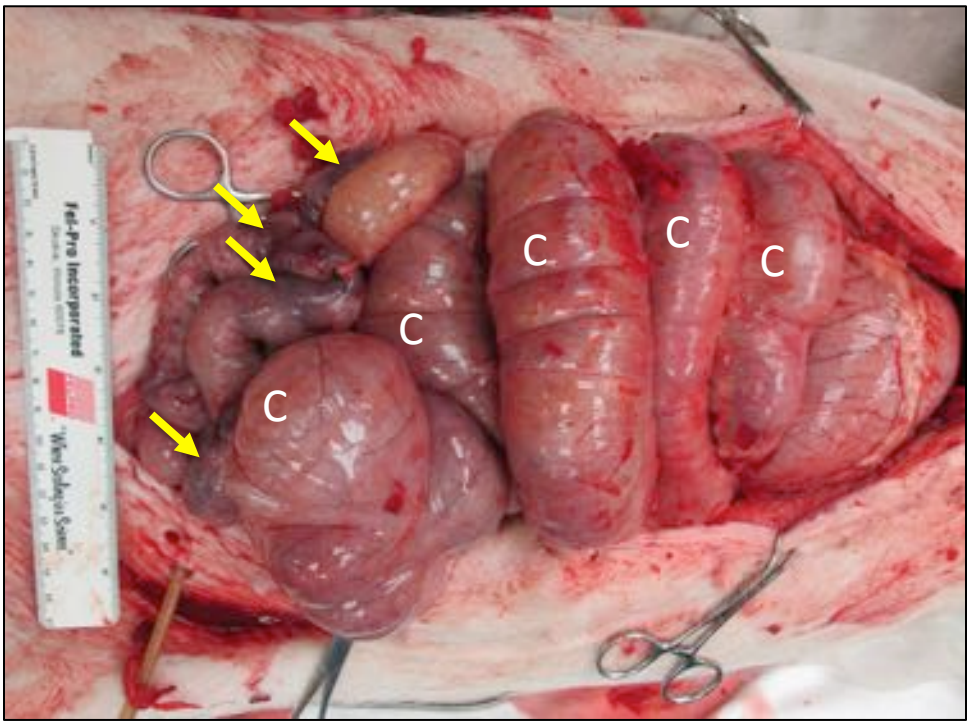

Figure 5, swine 277. Surface changes in the small bowel (arrows). Image taken prior to euthanasia. Cephalad to left of image. Colon (C) in general is darkish in hue.

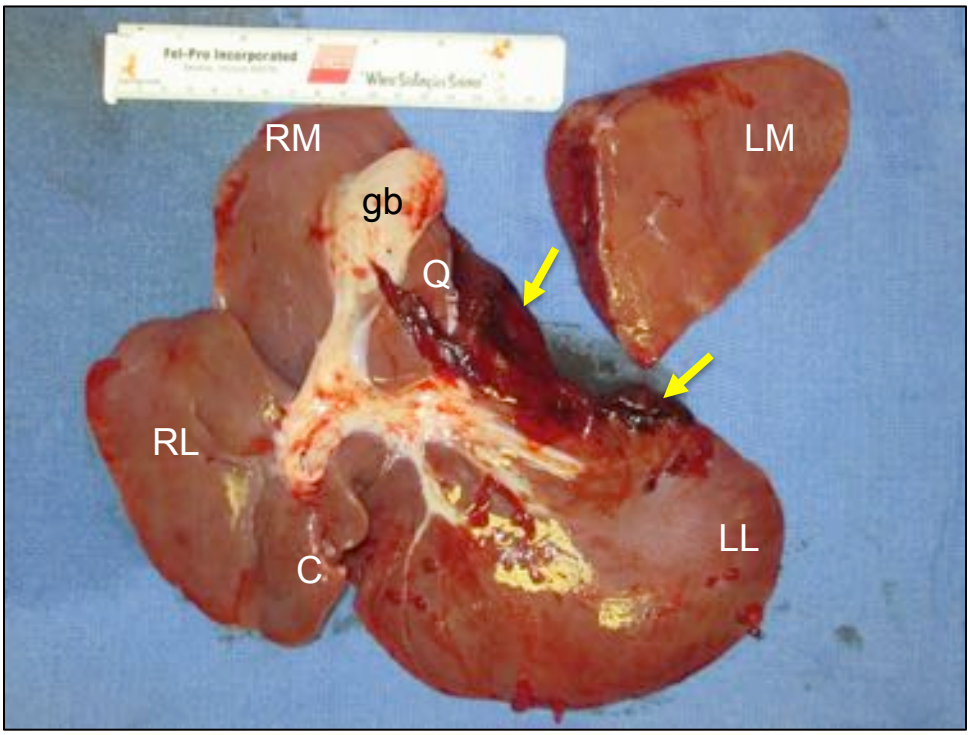

Figure 6, swine 277. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with large adherent clot. Anterior is at top of image.

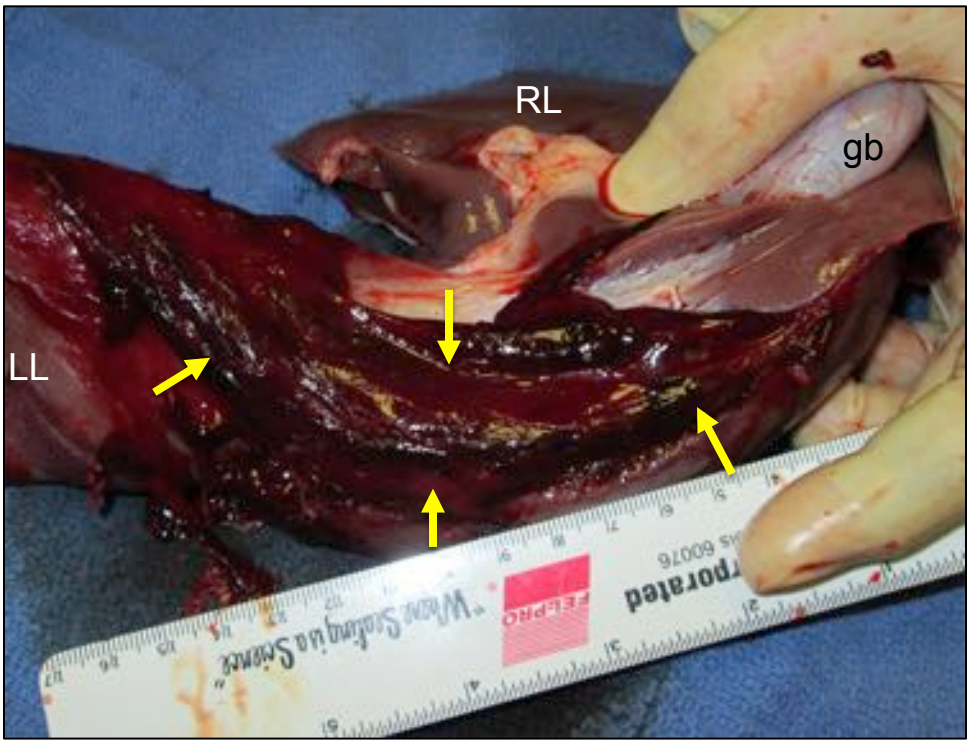

Figure 7, swine 277. Close-up of resection site (base of LM lobe), completely covered with adherent clot (arrows). Superior is at bottom of image.

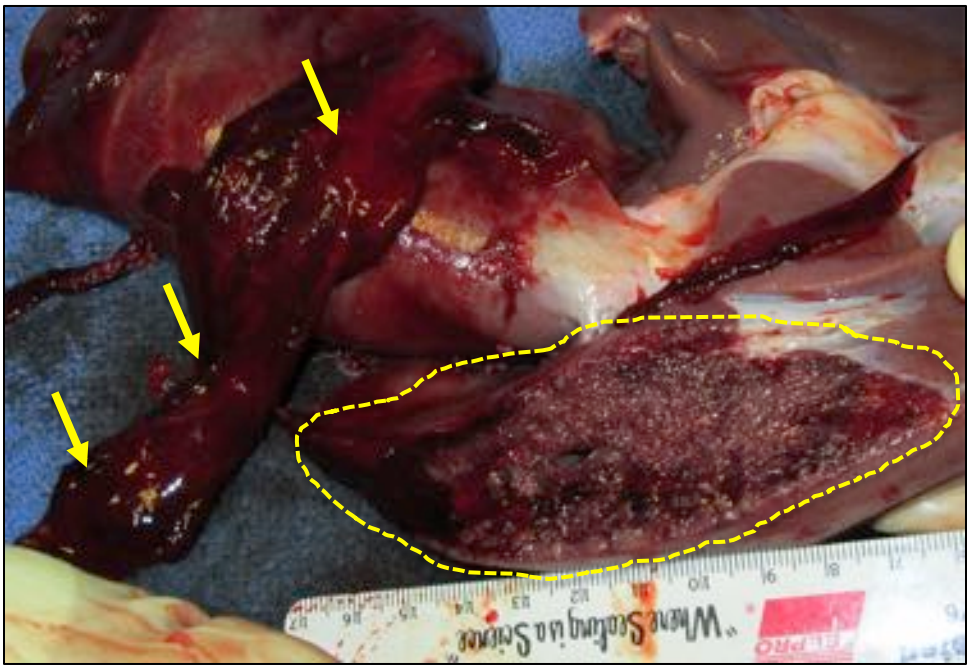

Figure 8, swine 277. Injury site (encircled) from Fig. 7; clot has been wiped away to the left (arrows).

I. OVERVIEW
Date: May 01, 2015
Swine no: 278
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam + clotting factors (FII, FXIII, **no FI**)
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 04/16/2015
Pre-procedure wt: 38.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 73
- MAP: 125
- Temp: 37.9
- EtCO2: 42

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:25 AM
Spleen wt: 249.8 gm
LR (22°C) infused after splenectomy: 750 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 249.5+ 30 + 15.5 = 295.00 mL
- LR (22°C) infused (spleen replacement + incidental): 750 + 300 = 1050 mL

Pre-injury VS
- HR: 93
- MAP: 128
- Temp: 37.2

- EtCO2 : 43
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:36 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 32.4 mg Factor XIII, and 9004 units thrombin.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 781.5 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.7 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:39AM (within 3 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:45 AM

10 min post-injury VS

- HR: 139
- MAP: 35
- Temp: 37.1
- EtCO2: 30
- IAP: 5

Blood draw no. 3: (30 min post-injury): 09:05 AM

30 min post-injury VS

- HR: 119
- MAP: 41
- Temp: 36.8
- EtCO2: 34

- IAP: 5

Blood draw no. 4: (60 min post-injury): 09:35 AM

60 min VS
- HR: 110
- MAP: 40
- Temp: 36.2
- EtCO2: 40
- IAP: 3

Blood draw no. 5: (90 min post-injury): 10:05 AM

90 min post-injury VS
- HR: 105
- MAP: 54
- Temp: 35.7
- EtCO2: 39
- IAP: 3

Blood draw no. 6: (120 min post-injury): 10:35 AM

120 min post-injury VS
- HR: 100
- MAP: 32
- Temp: 35.3
- EtCO2: 39
- IAP: 1

Blood draw no. 7: (150 min post-injury): 11:05 AM

150 min post-injury VS
- HR: 96
- MAP: 52
- Temp: 35.0
- EtCO2: 39
- IAP: 0

Blood draw no. 8: (180 min post-injury): 11:35 AM

180 min post-injury VS
- HR: 90
- MAP: 52
- Temp: 34.8
- EtCO2: 38
- IAP: 0

Survival at 180 min? YES
Target MAP attained ? No

Time of death: 11:35 AM
Cause of death: Exsanguination from euthanasia
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 929.4 mL (suction) + 238.2 mL (clot + lap pads) + 220 (phlebotomies) = 1387.6 mL
- IV fluid given: LR (37°C): 1950 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense, but IAP reading was 0 mm Hg. Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Some ischemic areas over colon were noted, see Figs. Injury site was covered with a large mass of foam + clot (see Figs). When this mass peeled away, minor hemorrhage resumed.
Volume foam recovered: 623.9.0 gms (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 90.7 (resected LM lobe) + 933.9 (remaining liver) = 1024.6 g

Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam + thrombin/FXIII, no fibrinogen (N = 5 for this Rx). Survived full 3 h easily with 1.4 L blood loss. Lump of clot and foam covering and adherent to injury site, with no active bleeding at 3 h while foam remained in place.

VII. PLAN

Begin series of alginate only controls on Wed 5/6.

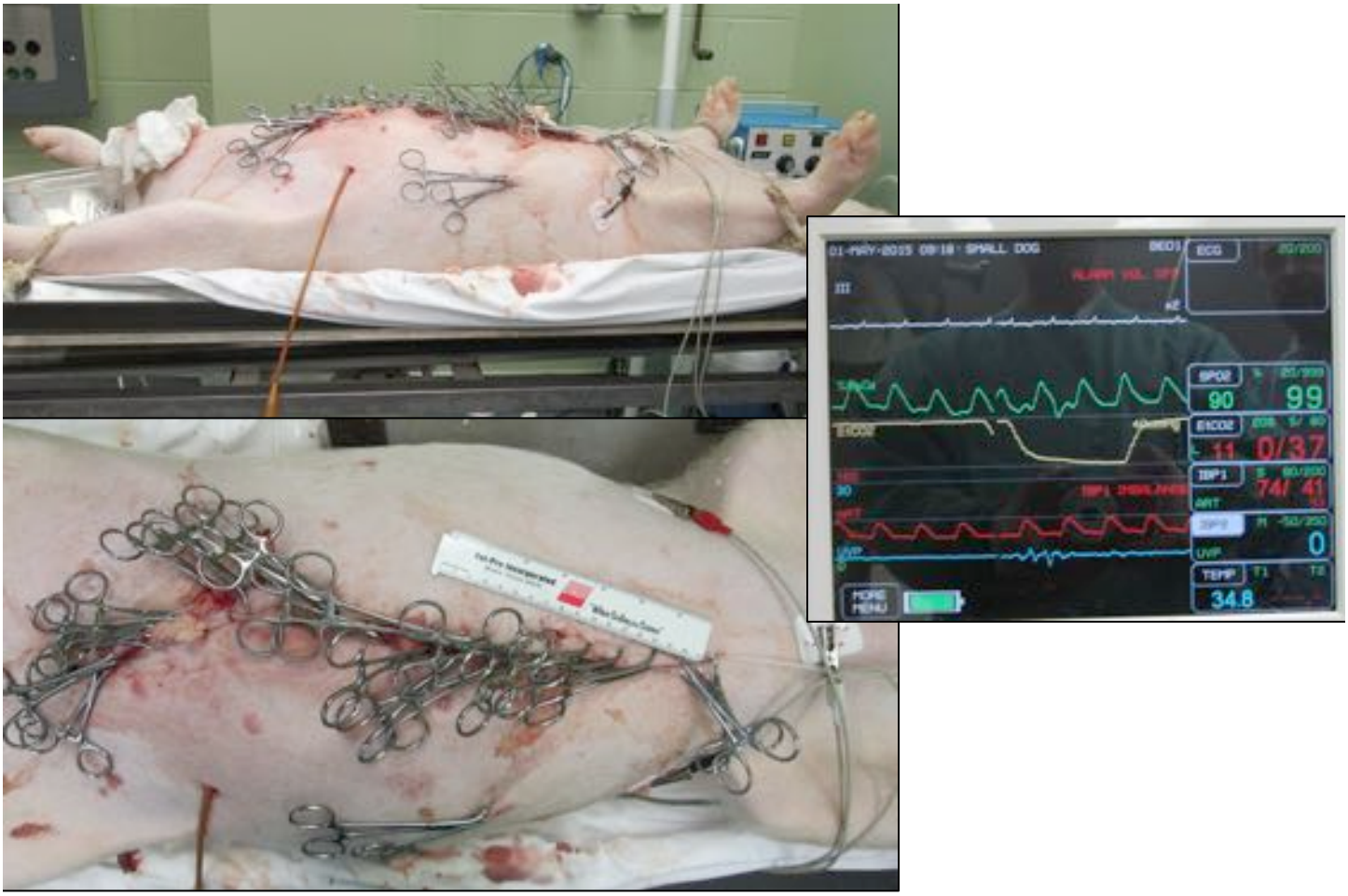

Figure 1, swine 278. Lateral and overhead views of swine at end of 180 min observation. Subject alive and stable; HR = 90; MAP = 53 mm Hg; O2 Sat = 99%; ETCO2 = 37 mm Hg; IAP = 0 mm Hg; T = 34.8˚C. Cephalad is to the right.

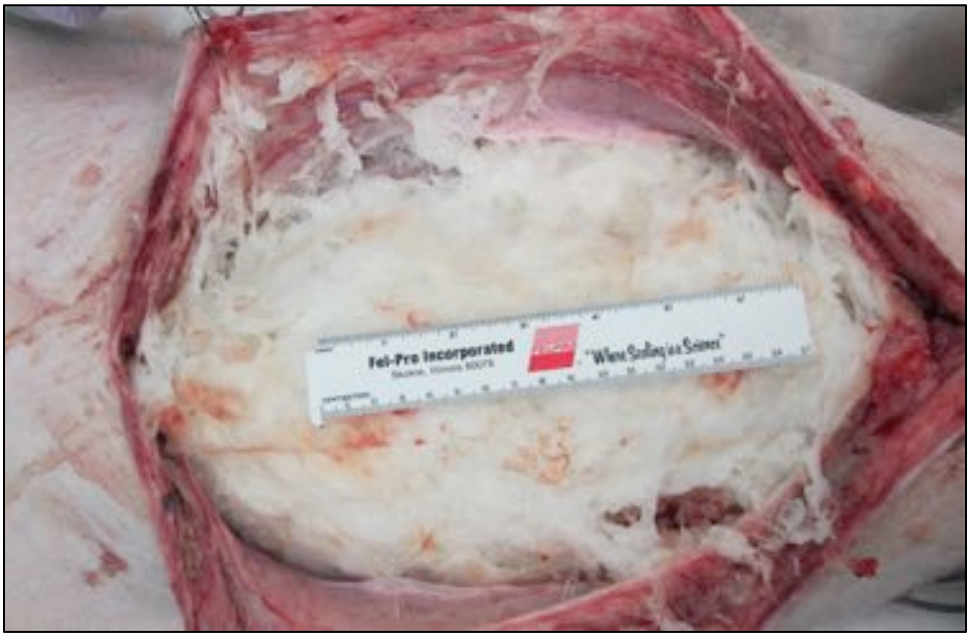

Figure 2, swine 278. Overhead view of re-opened abdomen after 180 min, immediately after Fig. 1 image; animal still alive, but MAP falling after incision re-opened. Cephalad is to right. Foam is overlying the intestines. No blood visible upon re-opening incision. No gas pockets.

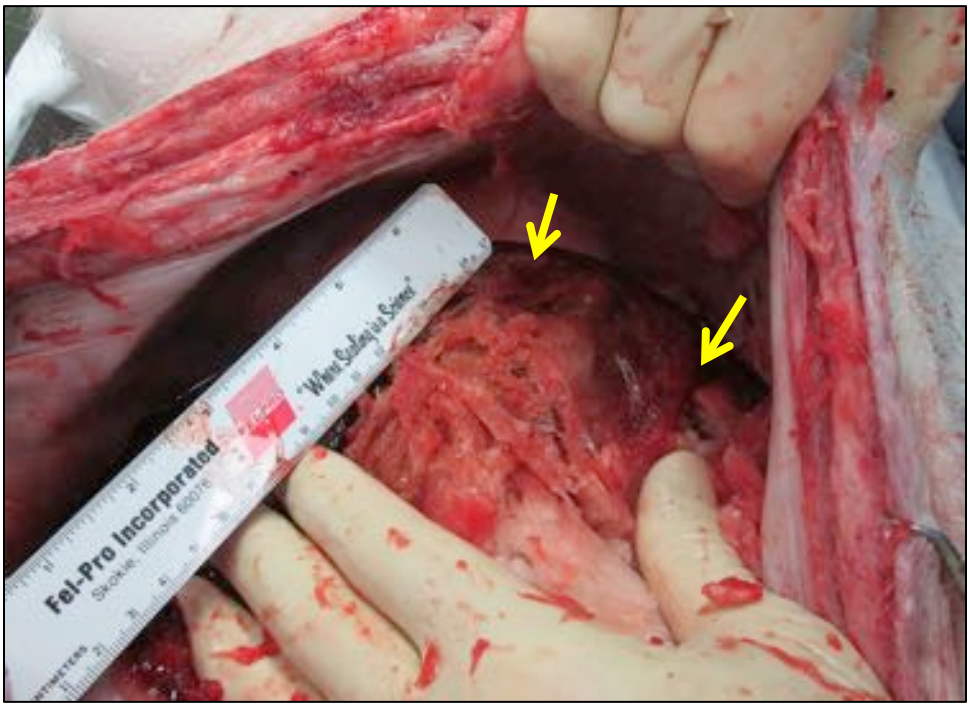

Figure 3, swine 278. View of upper abdomen after 180 min; subject still alive in this image, but MAP falling. Large mass of foam + clot (arrows) was covering & adherent to injury site. Cephalad at top of image.

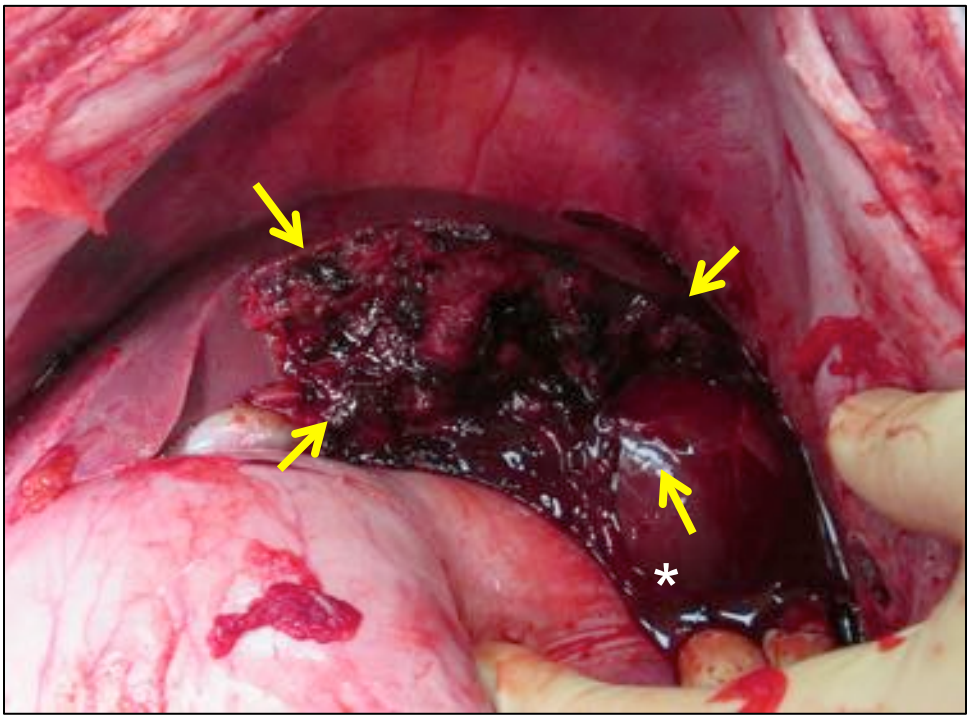

Figure 4, swine 278. View of injury site (arrows) within upper abdomen after most of foam has been removed. Subject still alive in this image. Cephalad toward top of image. Some active hemorrhage (*) occurred after clot/foam mass in Fig. 3 was peeled away.

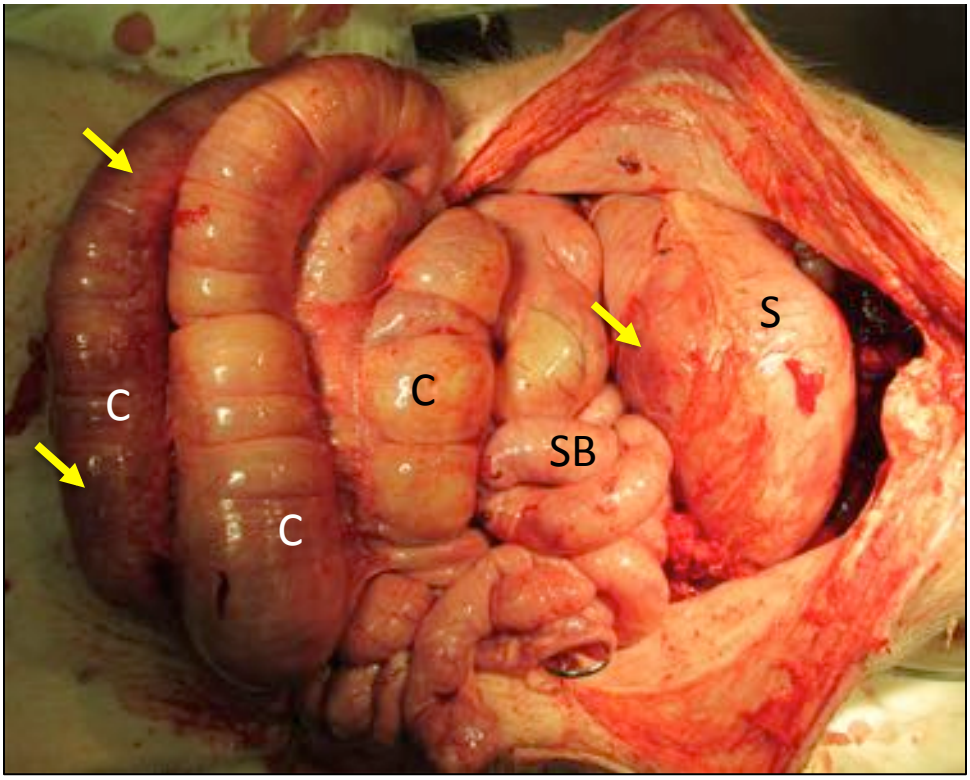

Figure 5, swine 278. Surface changes in the viscera (arrows). Image taken prior to euthanasia. Cephalad to left of image. Colon (C), small bowel (SB), and stomach (S) indicated.

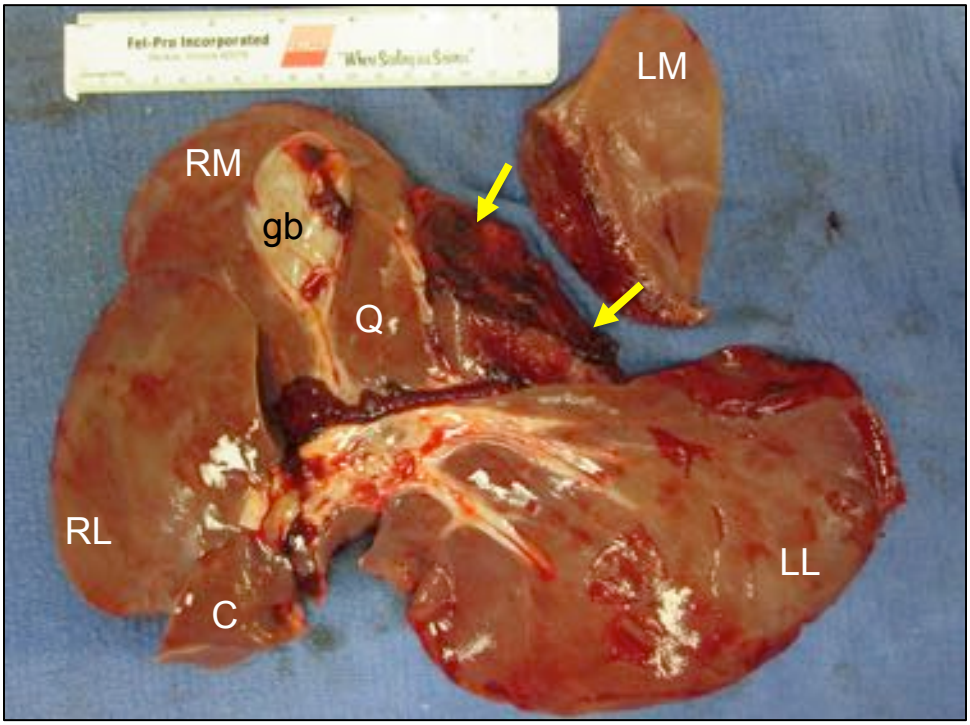

Figure 6, swine 278. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with adherent clot/foam. Anterior is at top of image.

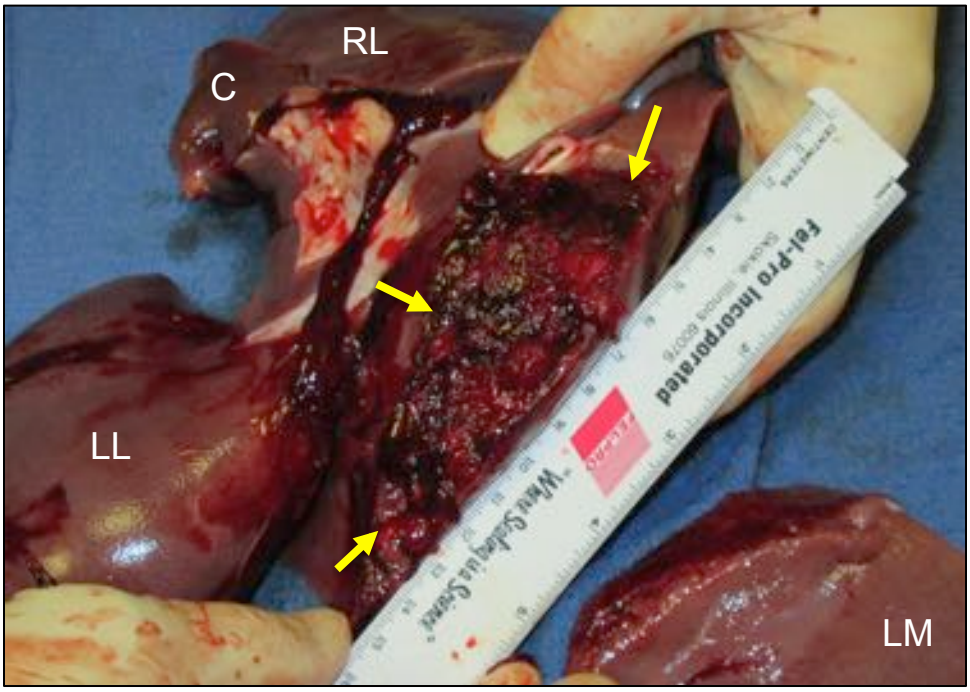

Figure 7, swine 278. Close-up of resection site (base of LM lobe), covered with adherent clot & faom (arrows). Superior is at bottom of image.

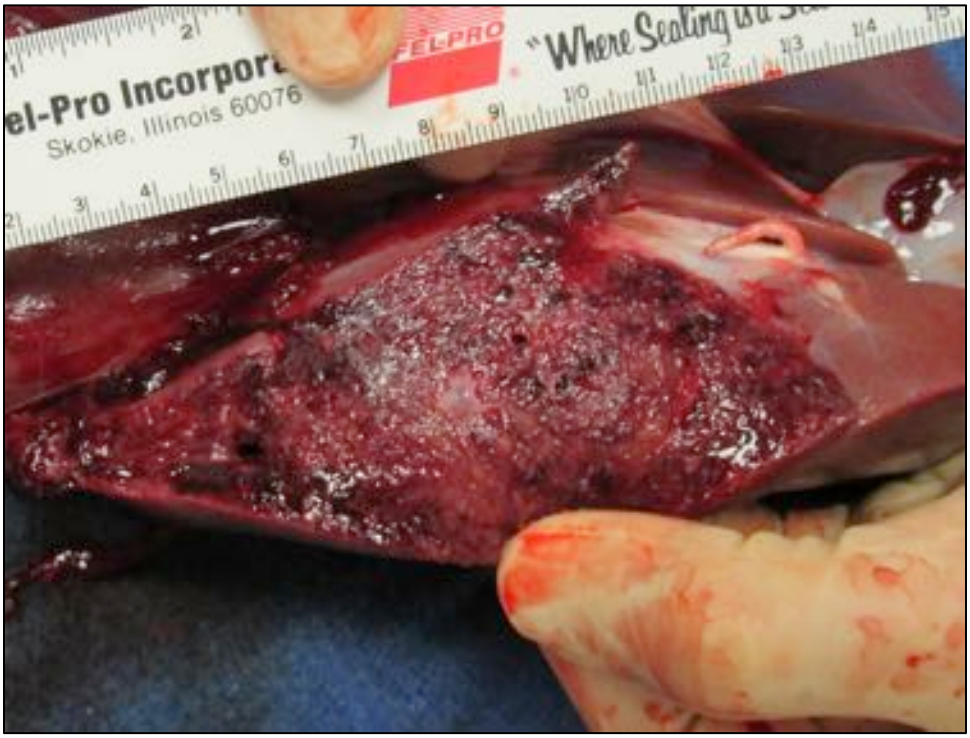

Figure 8, swine 278. Similar view as Fig. 7, but clot over injury site has been wiped away.

I. OVERVIEW
Date: May 06, 2015
Swine no: 280
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam only, **no biologics**
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Ragusa

II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: male
Date swine received from UNL Mead: 05/04/2015
Pre-procedure wt: 33.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 89
- MAP: 116
- Temp: 38.0
- EtCO2: 42

Blood draw no. 1 (initial): 8:35 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 227.9 gm
LR (22°C) infused after splenectomy: 800 mL at 150 mL/min (700 mL for spleen replacement + 100 mL for accidental blood loss from arterial line).

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 227.9 + 30 + 100 = 357.9 mL
- LR (22°C) infused (spleen replacement + incidental): 700 + 100 = 800 mL

Pre-injury VS

- HR: 107
- MAP: 91

- Temp: 38.0
- EtCO2 : 45
- IAP: 0

Note: this subject became hypotensive (MAP in the 40's) immediately upon infusion of LR for splenic replacement. We gave 0.2 mg epinephrine into auricular IV to resuscitate. Subject had rapid, appropriate, but unsustained response to epi Rx (MAP >120 mm Hg for several min). Prior to injury, however, MAP began drifting down again in the 80's and would not stabilize.

III. INJURY & TREATMENT PHASE

Time of injury: 09:05 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 0 mg Factor XIII, and 0 units thrombin (foam only; no biologics)

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 467.2 g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 70

Resuscitation fluid: warm LR, 1.7 L preset maximum (50 mL/kg), given at constant rate of 9.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 09:05AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 09:15 AM

10 min post-injury VS

- HR: 117
- MAP: 30
- Temp: 38.1
- EtCO2: 39
- IAP: 10

Blood draw no. 3: (30 min post-injury): 09:35 AM

30 min post-injury VS

- HR: 115
- MAP: 17
- Temp: 37.9
- EtCO2:7
- IAP: 7

Survival at 180 min? NO
Target MAP attained ? No
Time of death: 9:39 AM
Cause of death: not clear; combination of blood loss with depressed cardiac output, exacerbated by clot within right side of heart and major veins.
Interval from injury to death: 34 min

Post-treatment fluid data:

- Blood loss: mL 600 mL (suction) + 582.9 mL (clot + lap pads) + 90 mL (phlebotomies) = 1,272.9 mL
- IV fluid given: LR (37°C): 350 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~ 5 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a ++clots were seen (see Figs); more clot than typically present in other subjects. Only minor ischemic areas over intestine were noted. Injury site was covered with a mass of foam + clot (see Figs). No active hemorrhage.
Volume foam recovered: 416.7 gms (see Figs)
Heart: distended with blood. Large amount of clot in RV and RA, with clot extruding from SVC (see Figs). No obvious foam embolism.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: large clot extruded form transected orifice of hepatic vein to LM lobe (see Figs).
Ex vivo liver wt: 88.1 (resected LM lobe) + 987.1 (remaining liver) = 1075.2 g
Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam only, no biologics; first subject treated with foam only. Subject expired at 34 minutes, with 1.3 L blood loss. Lump of clot and foam covering and adherent to injury site, with no active bleeding at necropsy, though expired subject would only have leftover central venous pressure to drive hemorrhage.

Today's subject behaved strangely. Profound hypotension ensued with infusion of LR splenic replacement. It was as if the LR contained something that induced a systemic activation of inflammation/sepsis. We have seen this phenomenon several times in the past few months; never has been a problem before. This pig never behaved normally after this crystalloid-associated hypotensive episode. He got better temporarily with epinephrine injection, but then MAP starting steadily declining again and would not stabilize prior to injury. And at necropsy, he had diffuse intravascular clotting, even in SVC. This was not embolism, because clot extended all

the way up the SVC into the neck region. We never seen such extensive intravascular clotting at necropsy in these animals. And the amount of blood loss (1.3 L) was not enough by itself (based on previous pigs) to cause death. So I am not really sure what was happening with this pig. Only thing we can do is repeat the experiment. Ultimately we may have to throw out the data of this pig if the behavior persists as aberrant compared to other pigs.

VII. PLAN

Continue with the alginate-only series on Fri 5/8; we will test a different lot of LR to see if the crystalloid is causing the hypotension.

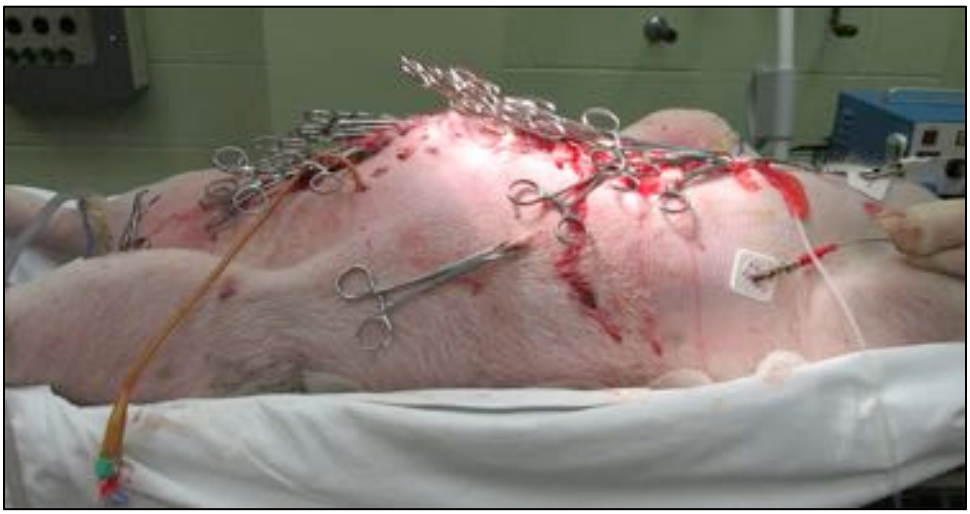

Figure 1, swine 280. Lateral and overhead views of swine just after expiration (~34 min after injury). Cephalad is to the right.

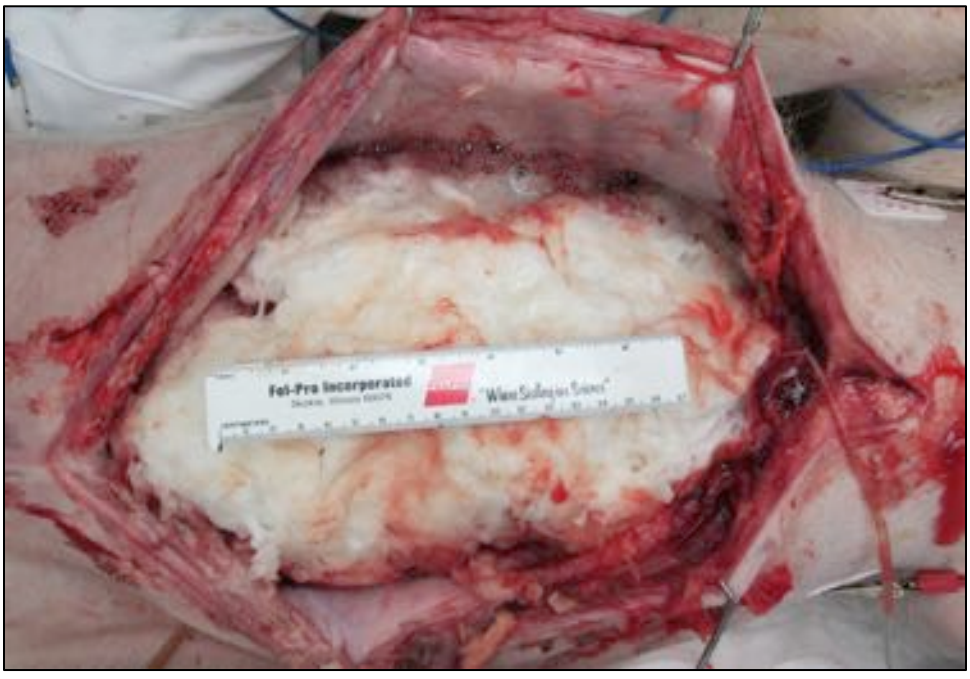

Figure 2, swine 280. Overhead view of re-opened abdomen after expiration, immediately after Fig. 1 image. Cephalad is to right. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision. No gas pockets.

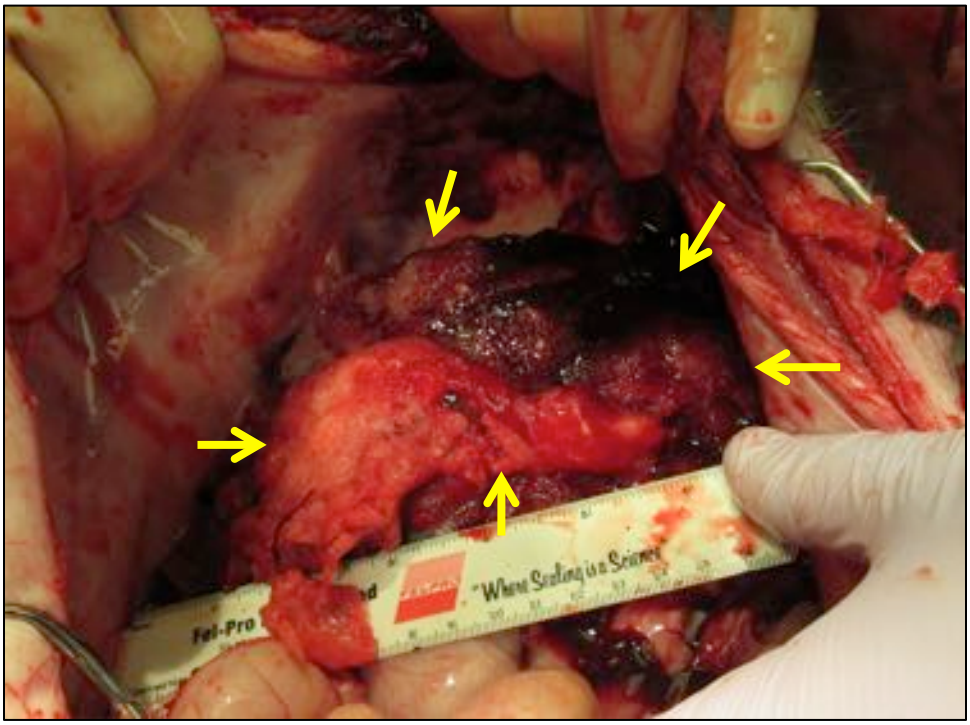

Figure 3, swine 280. View of upper abdomen after most of foam mass removed. Mass of foam + clot (arrows) was covering & adherent to injury site. Cephalad at top of image.

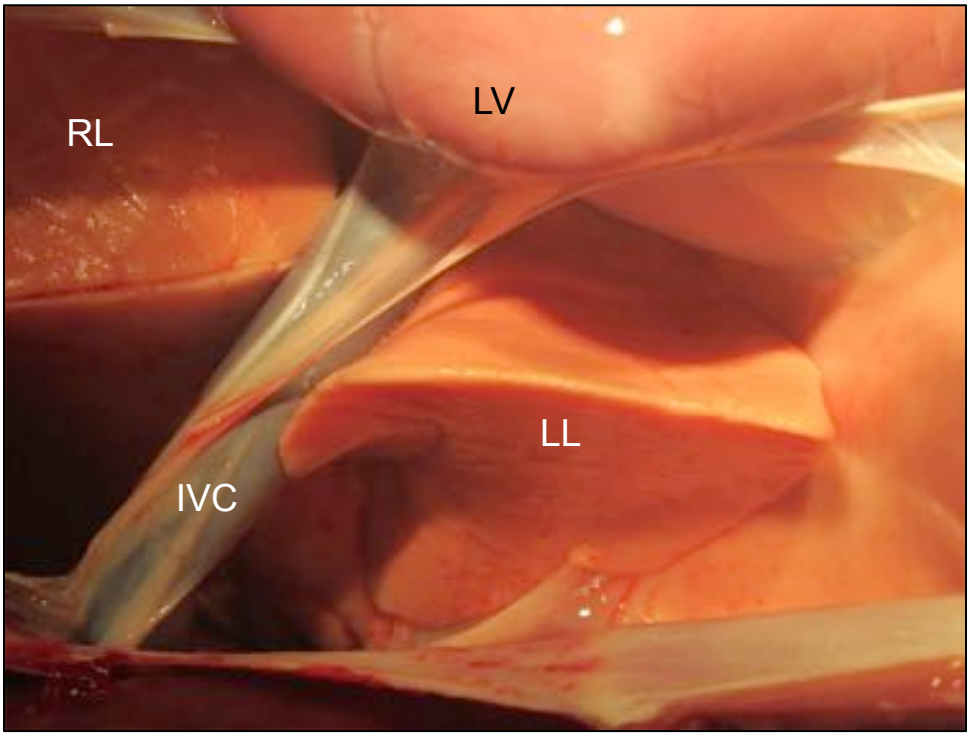

Figure 4, swine 280. Immediately after Fig. 3 image, chest was opened to evaluate heart. The inferior vena cava (IVC) and cardiac chambers were distended with blood. LV = left ventricle (apex). LL = left lung; RL = right lung.

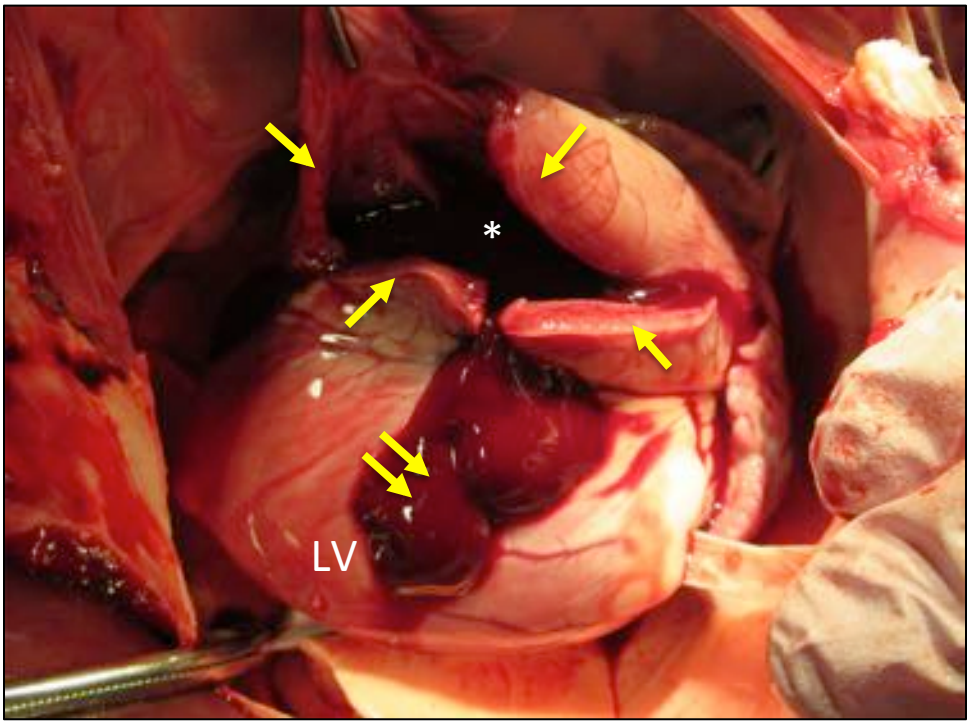

Figure 5, swine 280. View of heart. The wall of the right ventricle has been incised, exposing RV chamber (*) which was filled with clot (double arrows, extruded from RV chamber). Arrows indicated retracted wall of RV. LV = left ventricle, apex. Superior is at upper right of image.

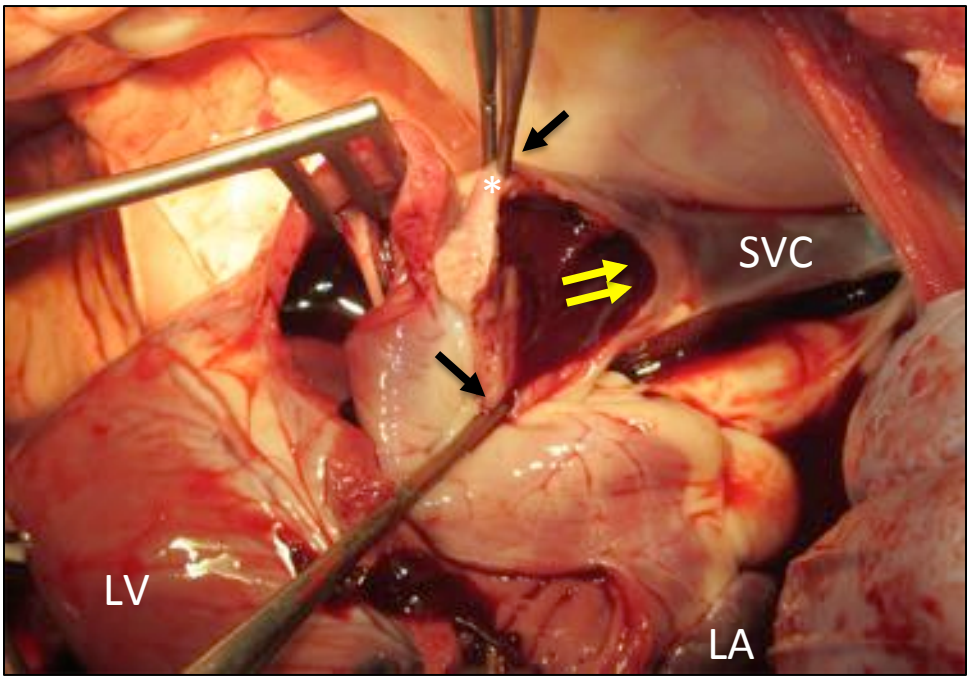

Figure 6, swine 280. View of heart. The right atrium has been opened, and its walls are being retracted with two forceps (black arrows). The right atrium and superior vena cava (SVC) were filled with clot (double yellow arrows). LV = apex of left ventricle; LA = left atrium. Superior is at right of image.

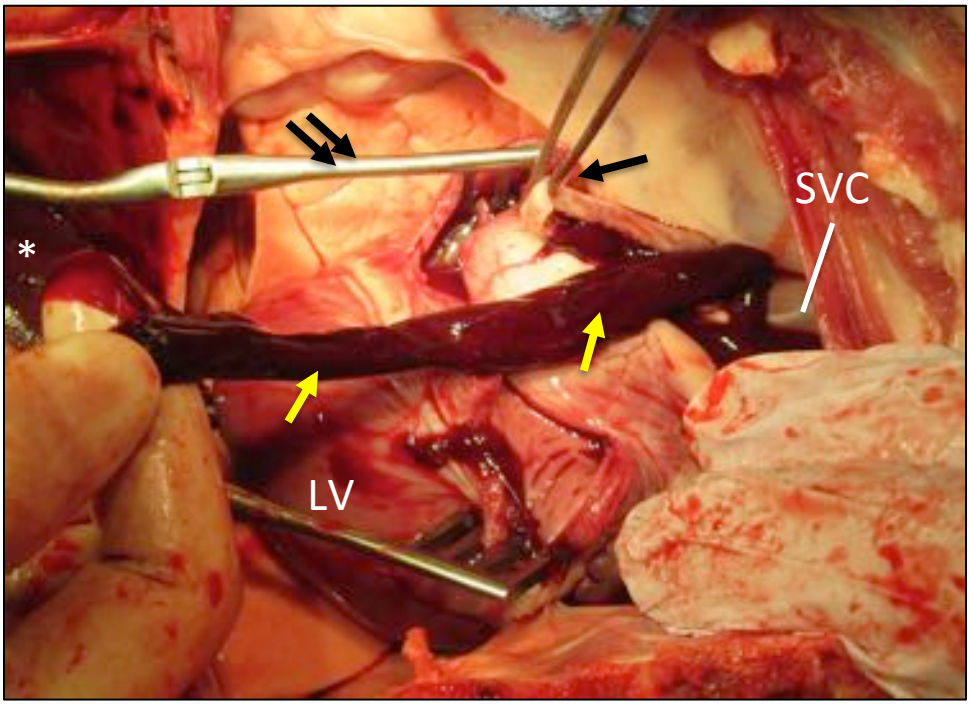

Figure 7, swine 280. View of heart. A long well-formed clot (yellow arrows) has been extruded from the SVC. Forcep (black arrow) is shown retracting wall of opened right atrium. Walls of RV are being held open with Wheatlander retractor (double black arrows). Superior is at right of image.

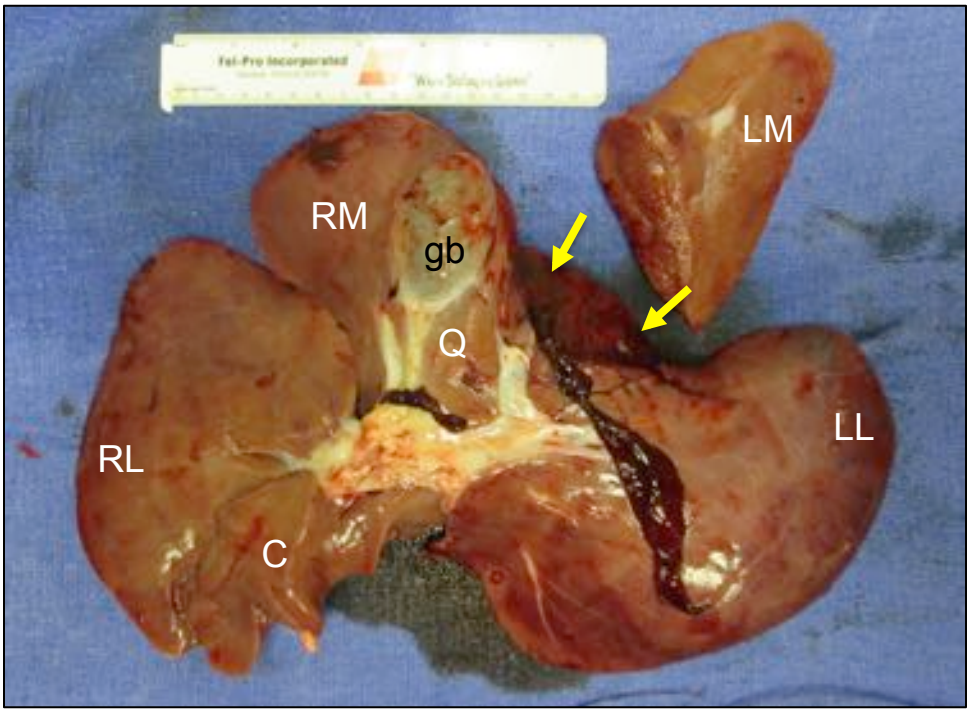

Figure 8, swine 280. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with adherent clot/foam. Anterior is at top of image.

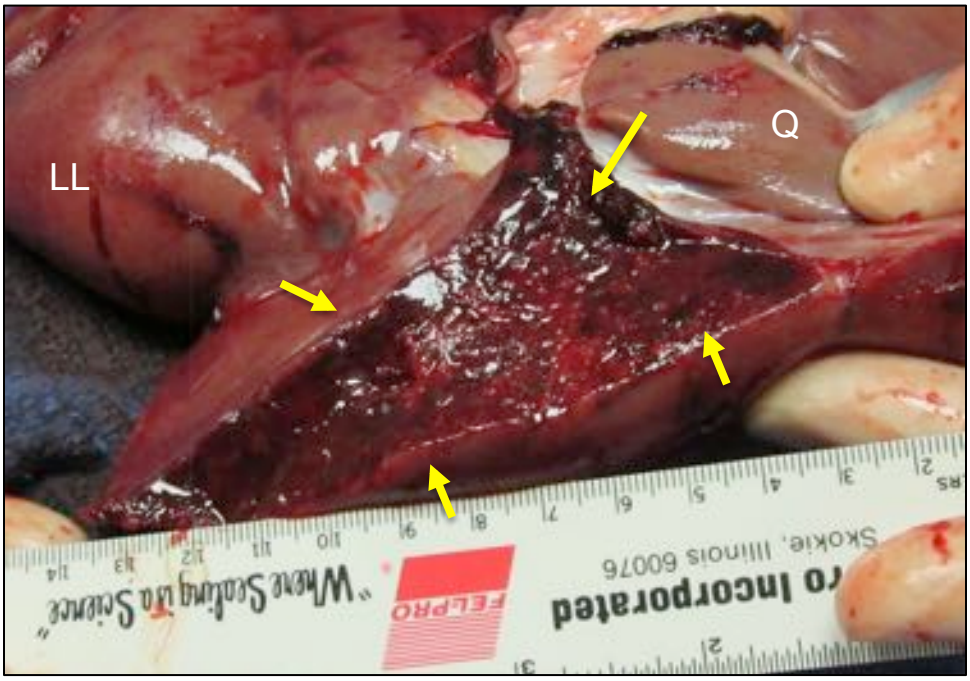

Figure 9, swine 280. Close-up of resection site (base of LM lobe, arrows), covered with thin layer of clot & foam. Superior is at bottom of image.

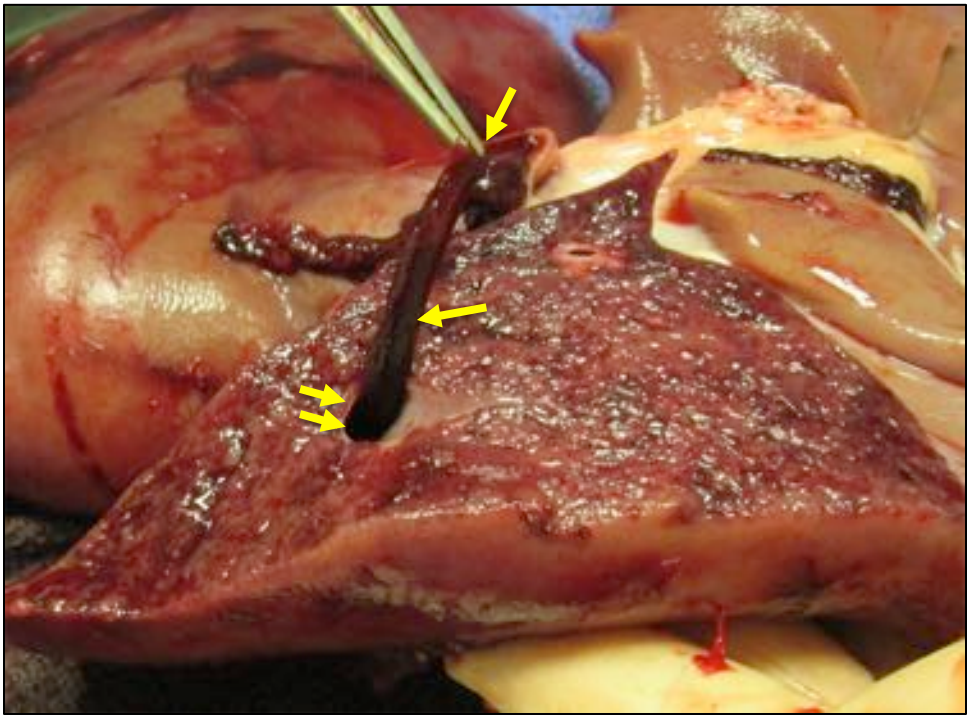

Figure 10, swine 280. Similar view as Fig. 9, but clot over injury site has been wiped away. Forceps is shown extruding a long well-formed clot (single arrows) from the orifice of the hepatic vein (double arrows) to the LM lobe.

I. OVERVIEW
Date: May 08, 2015
Swine no: 281
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam only (**no** biologics)
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Ragusa

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: Male
Date swine received from UNL Mead: 05/04/2015
Pre-procedure wt: 34.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor (was malfunctioning in this subject, no IAP data available)

Initial VS

- HR: 99
- MAP:91
- Temp: 38.1
- EtCO2: 40

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:15 AM
Spleen wt: 283.3 gm
LR (22°C) infused after splenectomy: 850 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 283.3 + 30 + 8.1 = 321.4 mL
- LR (22°C) infused (spleen replacement + incidental): 850 + 100 = 950 mL

Pre-injury VS

- HR: 98
- MAP: 97
- Temp: 37.5

- EtCO2 : 42
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 08:38 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 0 mg Factor XIII, and 0 units thrombin (i.e., **no** biologics)

Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 575.9 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 80 mm Hg

Resuscitation fluid: warm LR, 1.7 L preset maximum (50 mL/kg), given at constant rate of 9.6 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 08:40AM (within 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 08:50 AM

10 min post-injury VS

- HR: 186
- MAP: 34
- Temp: 37.3
- EtCO2: 42
- IAP: 88 (not accurate)

Blood draw no. 3: (30 min post-injury): 09:05 AM

30 min post-injury VS

- HR: 76
- MAP: 7
- Temp: 37.3
- EtCO2: 7

- IAP: 81 (not accurate)

Survival at 180 min? No
Target MAP attained ? No
Time of death: 9:10 AM
Cause of death: exsanguination
Interval from injury to death: 32 min

Post-treatment fluid data:

- Blood loss: 907.8 mL (suction) + 757.1 mL (clot + lap pads) + 60 mL (phlebotomies) = 1724.9 mL
- IV fluid given: LR (37°C): 200 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP monitor not functional today). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a ++ clots were seen (see Figs). Minor ischemic areas over small intestine were noted, not bad today. Injury site was covered with a large mass of foam + clot (see Figs), but this was not very adherent to the wound surface and was easily removed.
Volume foam recovered: 361.9 gms (see Figs)
Heart: RV and IVC distended; full of liquid blood (no air, no clot, no foam embolism).
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 102.7g (resected LM lobe) + 816.4 (remaining liver) = 919.1 g
Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam, no fibrinogen (N = 2 for this Rx; but not counting aberrant result from #280, really only N = 1). Expired from apparent exsanguination at 32 minutes with 1.7 L blood loss. Lump of clot and foam over the injury site, but not very adherent. No cardiac or central venous pathology.

This subject had no problems with pre-injury hypotension, in contrast to the previous subject (#280). Today we used a different lot of LR (Baxter #969634). So the data obtained today should be fairly believable.

VII. PLAN

Continue with this series on Wed 5/13 (alginate, no FI).

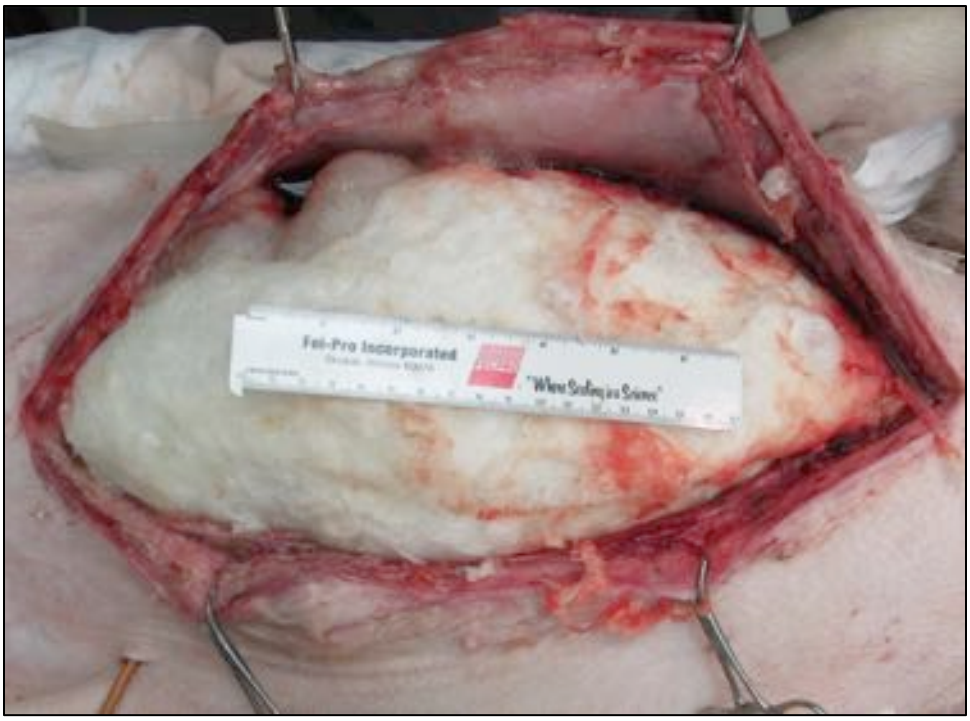

Figure 1, swine 281. Overhead view of re-opened abdomen immediately after expiration (~32 min after injury). Cephalad is to right. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision. No gas pockets.

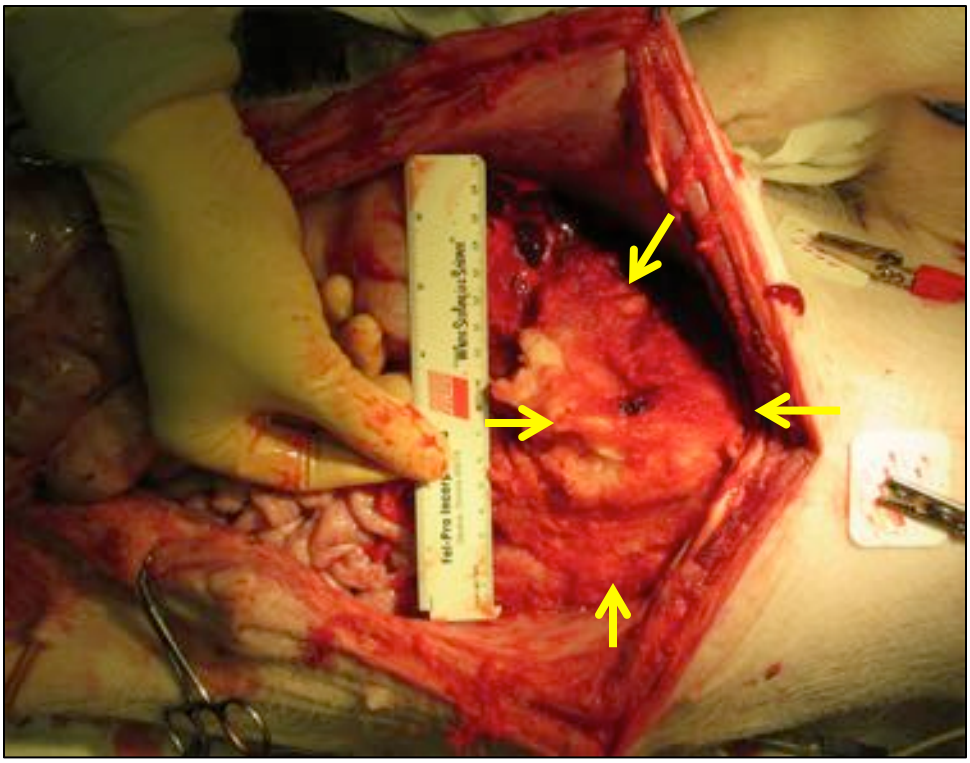

Figure 2, swine 281. View of upper abdomen after most of whitish (nonbloody) foam mass removed. Mass of foam mixed with blood/clot (arrows) was covering & lightly adherent to injury site. Cephalad is to the right.

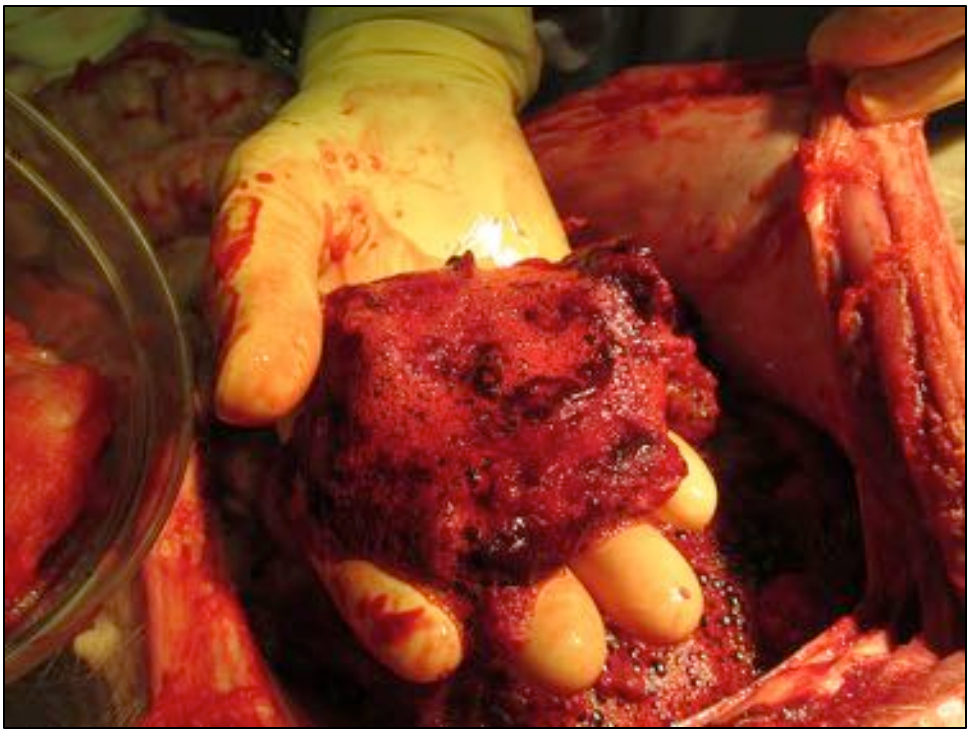

Figure 3, swine 281. Removal of foam shown in Fig. 2. This animal had lots of gas bubbles within the foam. Cephalad is at upper right of image.

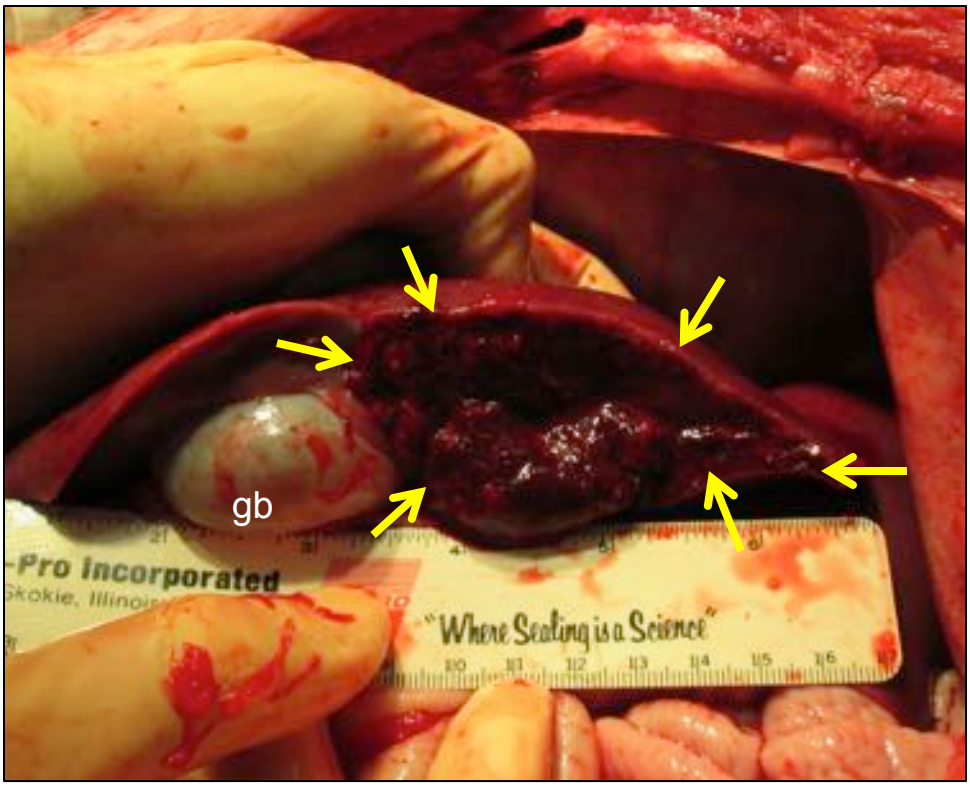

Figure 4, swine 281. Injury site (base of LM lobe, arrows) after removal of all foam, which was not very adherent to the injury. gb = gallbladder. Cephalad is at top of image.

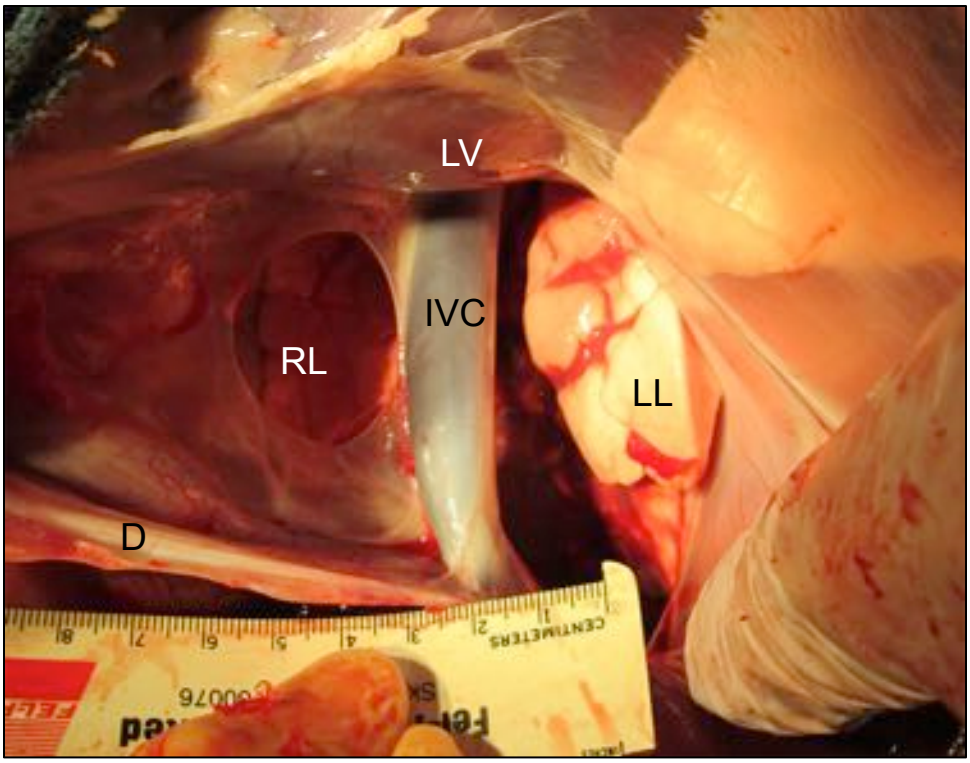

Figure 5, swine 281. Immediately after Fig. 4 image, chest was opened to evaluate heart. The inferior vena cava (IVC) and cardiac chambers were distended with blood. LV = left ventricle (apex). LL = left lung; RL = right lung; D = diaphragm (cut edge). Cephalad is at top of image.

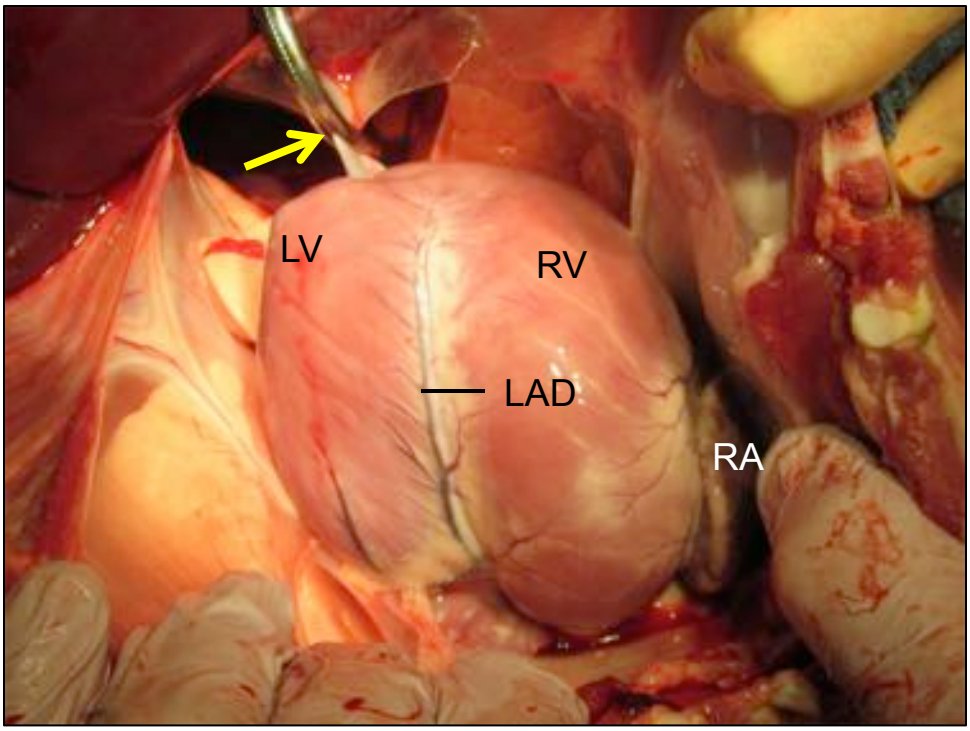

Figure 6, swine 281. View of heart. Right ventricle (RV) is distended. IVC has been clamped (arrow) to avoid postmortem migration of clot/foam into the heart (which would be a “false-positive”). LAD = left anterior descending artery. Cephalad is at bottom of image. Neither the RV nor right atrium (RA) contained any clot, foam, or gas.

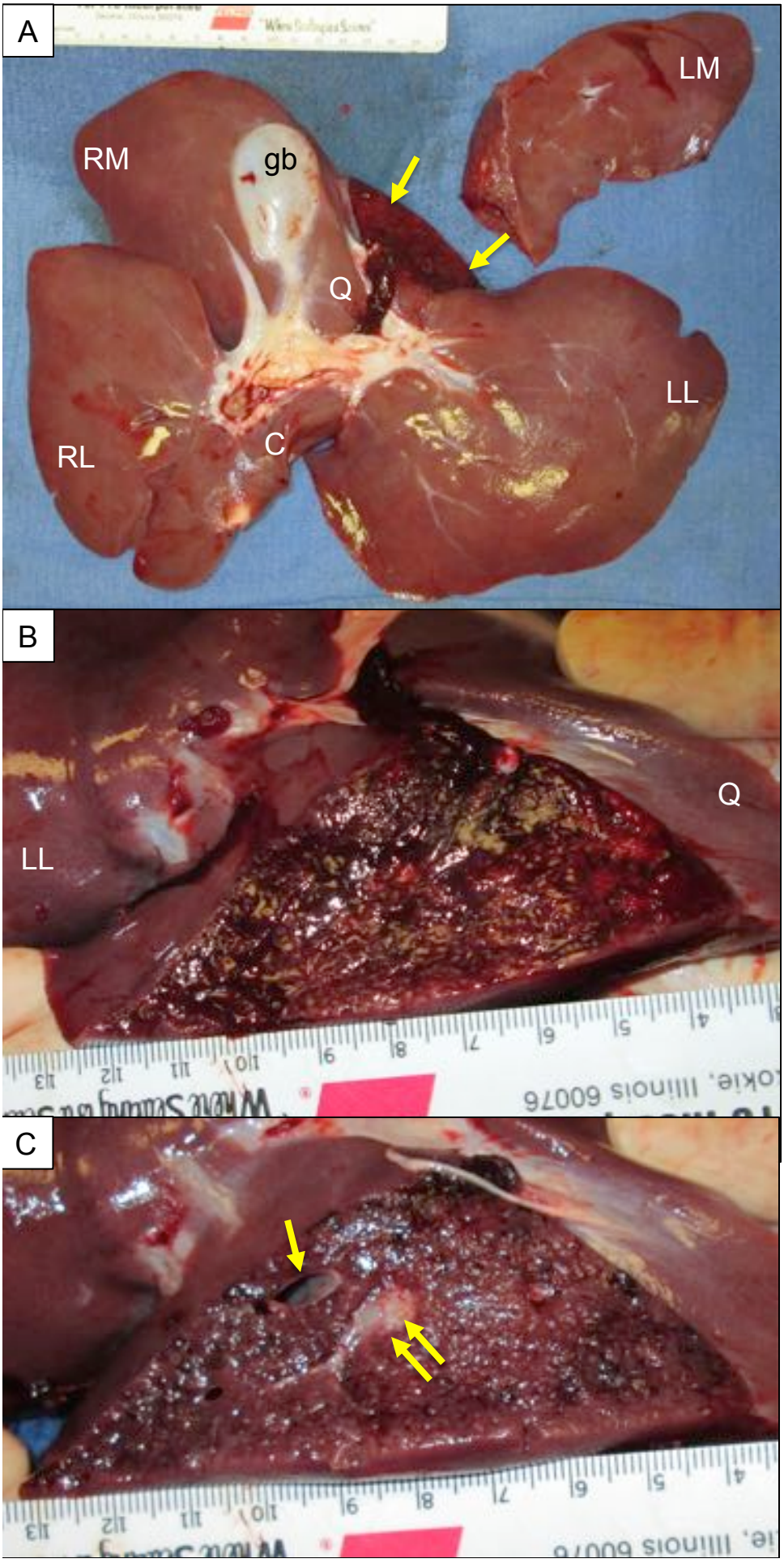

Figure 7, swine 280. Liver *ex vivo*. (A) Inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with adherent clot/foam. Anterior is at top of image. (B) Injury site at base of LM lobe (arrows in panel A). Site is covered with a thin layer of fibrin, no foam. Superior is at bottom of image. (C) Same view as panel B, but fibrin has been wiped away, revealing transected HV (single arrow) and PV (double arrow) branches to LM lobe.

I. OVERVIEW
Date: May 13, 2015
Swine no: 283
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam only. **No clotting factors**.
Personnel: Carlson, Cavanaugh, Hansen, Siford, Fatemi, Ismail, Ragusa, Fabian

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II. PRE-INJURY PHASE

Start time: 09:40 AM
Swine sex: Male
Date swine received from UNL Mead: 05/04/2015
Pre-procedure wt: 35.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 90
- MAP:114
- Temp: 39.0
- EtCO2: 38

Blood draw no. 1 (initial): 9:55 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 10:00 AM
Spleen wt: 278.1 gm
LR (22°C) infused after splenectomy: 850 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 278.1 + 30 + 56.2 = 364.3 mL
- LR (22°C) infused (spleen replacement + incidental): 850 + 80 = 930 mL

Pre-injury VS

- HR: 104
- MAP: 111
- Temp: 38.5

- EtCO2 : 39
- IAP: -1

III. INJURY & TREATMENT PHASE

Time of injury: 10:11 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 0 mg Factor XIII, and 0 units thrombin (i.e., no clotting factors)

Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 471.5 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 9.8 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 10:12AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 10:20 AM

10 min post-injury VS

- HR: 163
- MAP: 31
- Temp: 38.1
- EtCO2: 20
- IAP: 9

Blood draw no. 3: (30 min post-injury): 10:38 AM

30 min post-injury VS

- HR: 109
- MAP: 10
- Temp: 37.6
- EtCO2: 8

- IAP: 3

Survival at 180 min? No
Target MAP attained ? No
Time of death: 10:38 AM
Cause of death: exsanguination?
Interval from injury to death: 27 min

Post-treatment fluid data:

- Blood loss: 700.3 mL (suction) + 664.5 mL (clot + lap pads) + 60 mL (phlebotomies) = 1424.8 mL
- IV fluid given: LR (37°C): 350 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~ 5 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and clots were seen (see Figs). Some ischemic areas over small intestine were noted. Injury site was covered with a large mass of foam + clot (see Figs), but this was not adherent to the wound site, so the dimensions of this mass were not measured. A thin film of fibrin was covering the wound site.
Volume foam recovered: 383.4 gms
Heart (RV & RA & IVC): examined (see Figs); no air/clot/foam emboli.
Number of hepatic veins lacerated: 1, to LM lobe (see Figs)
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 91.7g (resected LM lobe) + 758.2 (remaining liver) = 849.9 g
Tissue harvested: Liver specimen from wound site (see Figs)

VI. COMMENTS

Noncompressible injury treated with alginate foam, no clotting factors (N = 2 for this Rx). Survived 27 minutes with 1.4 L blood loss. Lump of clot and foam near the injury site, but not adherent.

I am curious as to cause of death in this subject. Other subjects in this series have lost the same amount of blood, but survived the 3 h observation period without difficulty. Perhaps the LR infusion was harmful; i.e., similar to Swine #280 (see below). The mortality of this injury without any treatment other than hypotensive resuscitation is ~50%. If we find that the mortality in the alginate foam/no factors group is 100%, then we will need to consider whether the alginate foam on its own is harmful, i.e., increasing mortality. This may require additional control groups; we will have to wait & see.

This subject was given LR for splenic replacement from the same lot (#C968966) that has given us trouble in the past with hypotension. While this subject did not become overtly hypotensive with the LR, the MAP did drop 10-15 mm Hg with the onset of the LR replacement. I would not predict that the LR infusion would drop the BP. We will stop using this particular lot of LR, and begin carefully observing the MAP trend with LR splenic replacement.

VII. PLAN

Continue with this series on Fri 5/15 (alginate foam, no FI).

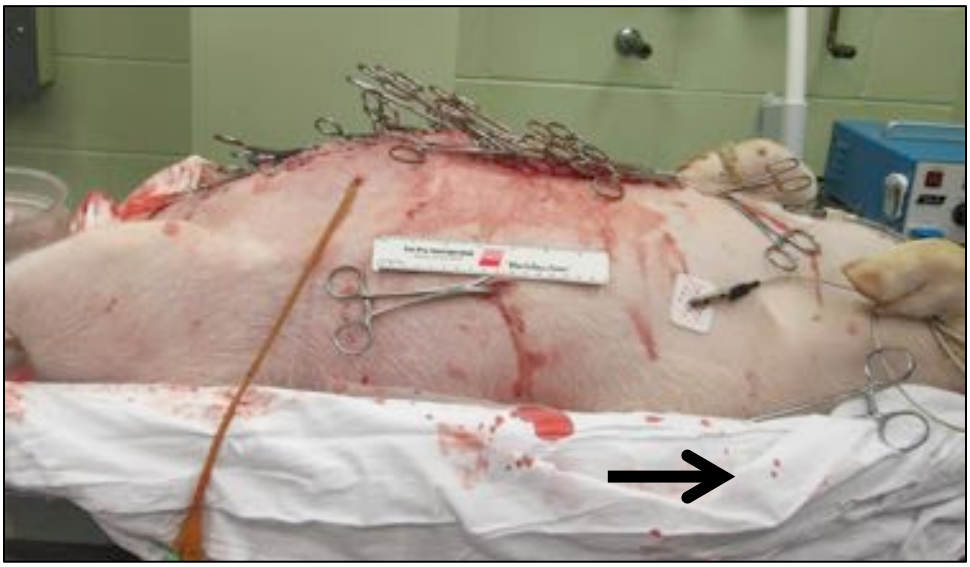

Figure 1, swine 283. Lateral view of swine just after expiration (~30 min after injury). Black arrow = cephalad.

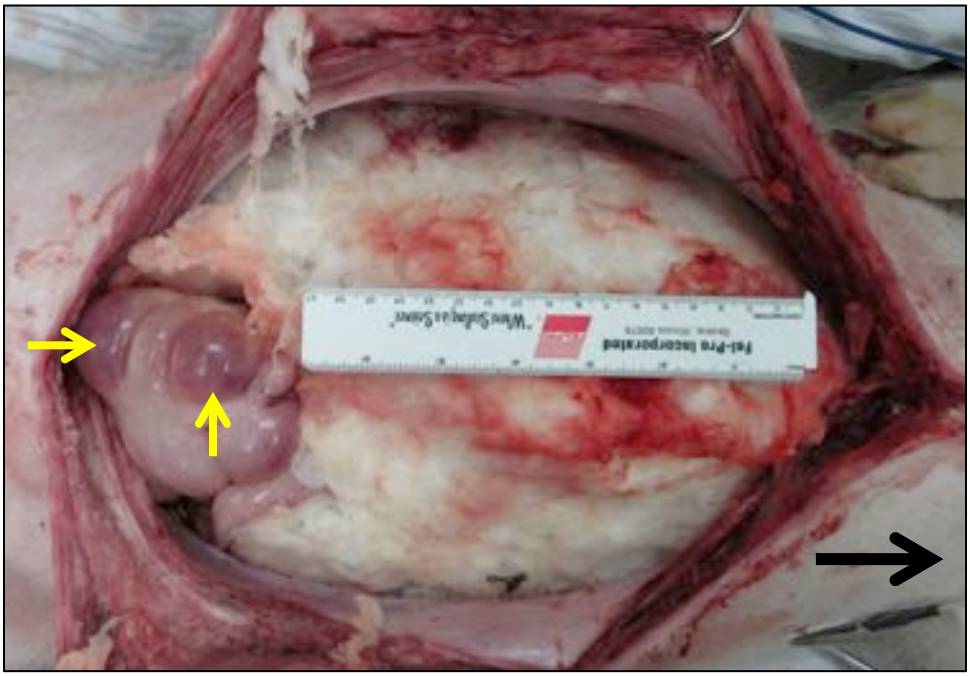

Figure 2, swine 283. Overhead view of re-opened abdomen immediately after expiration (~30 min after injury). Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision. No gas pockets. Note some surface changes to intestines (yellow arrows).

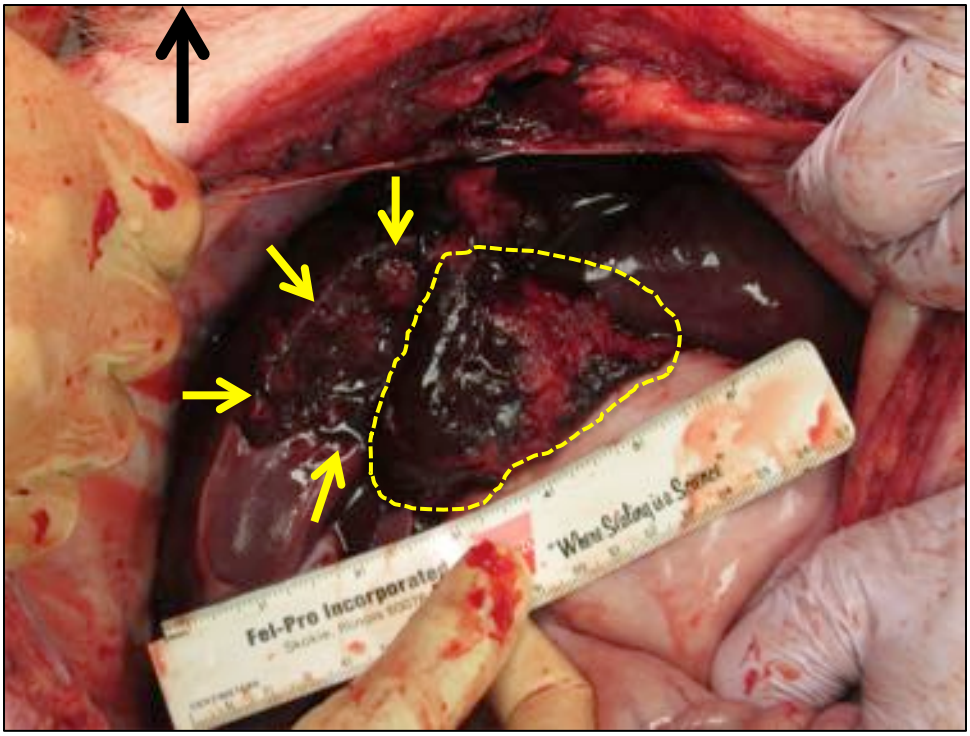

Figure 3, swine 283. View of upper abdomen after most of whitish (nonbloody) foam mass removed. Injury site indicated with yellow arrows. Black arrow = cephalad. There was a large mass of clot/foam (encircled) near the injury site, but this was not adherent to the wound, so its dimensions were not measured.

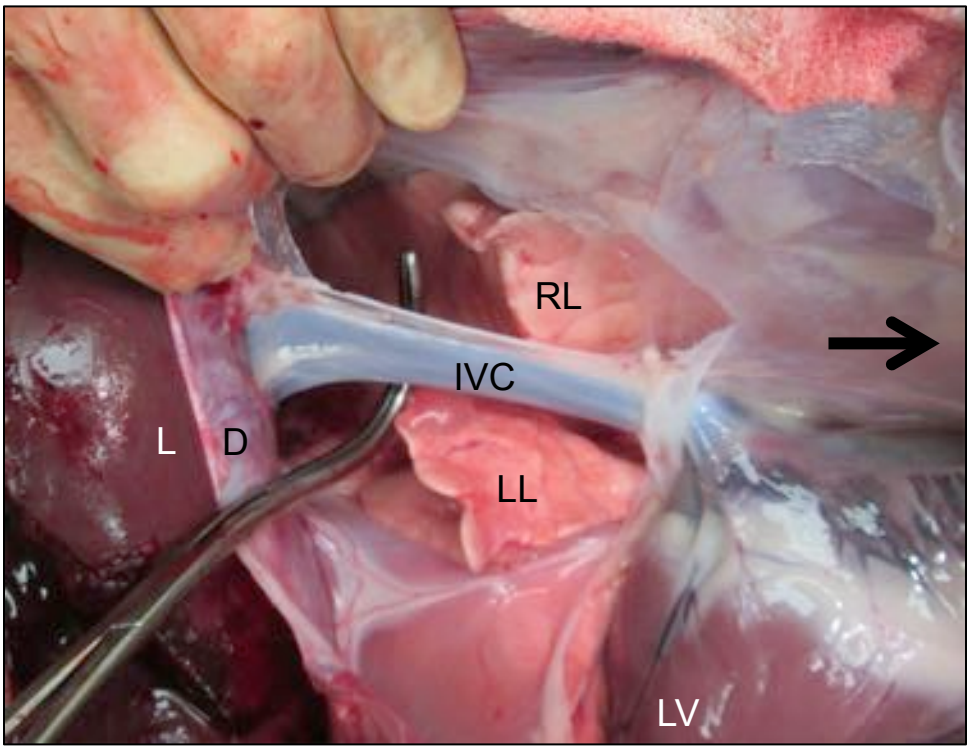

Figure 4, swine 283. Immediately after Fig. 3 image, chest was opened to evaluate heart. The inferior vena cava (IVC) did not contain any visible gas, clot, or debris. IVC and heart were not distended. LV = left ventricle (apex). LL = left lung; RL = right lung; D = diaphragm (cut edge); L = liver. Black arrow = cephalad.

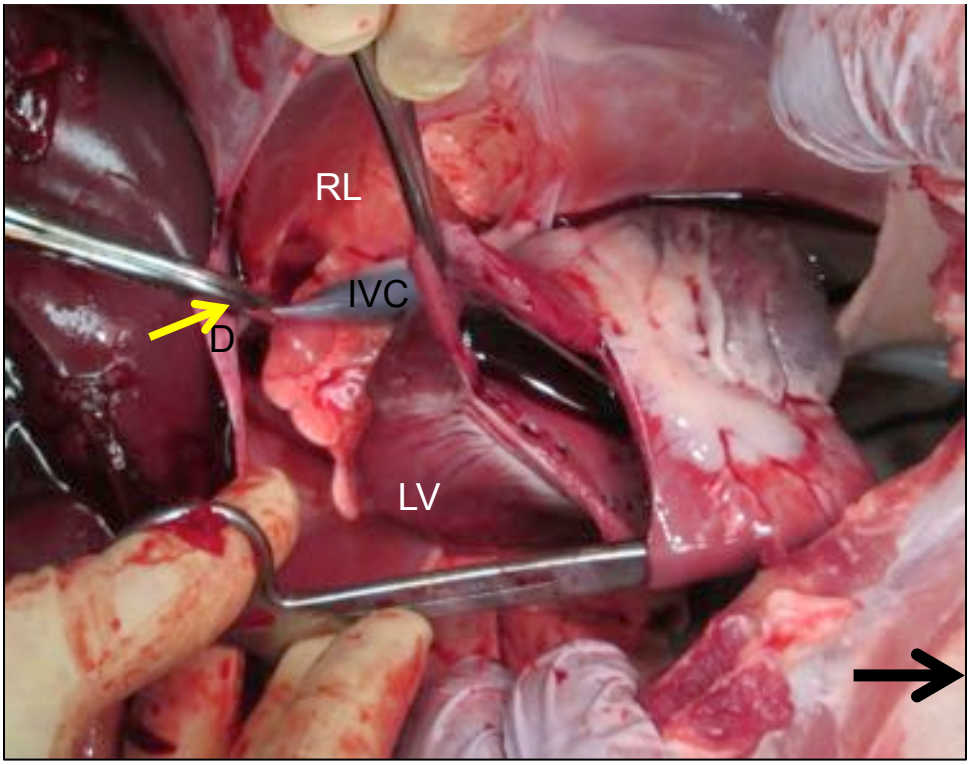

Figure 5, swine 283. Right ventricle has been opened (cut walls being stretched by instruments), revealing unclotted blood only without gas/clot/foam emboli. IVC has been clampled (yellow arrow) to prevent postmortem emboli during cardiac exploration. Black arrow = cephalad.

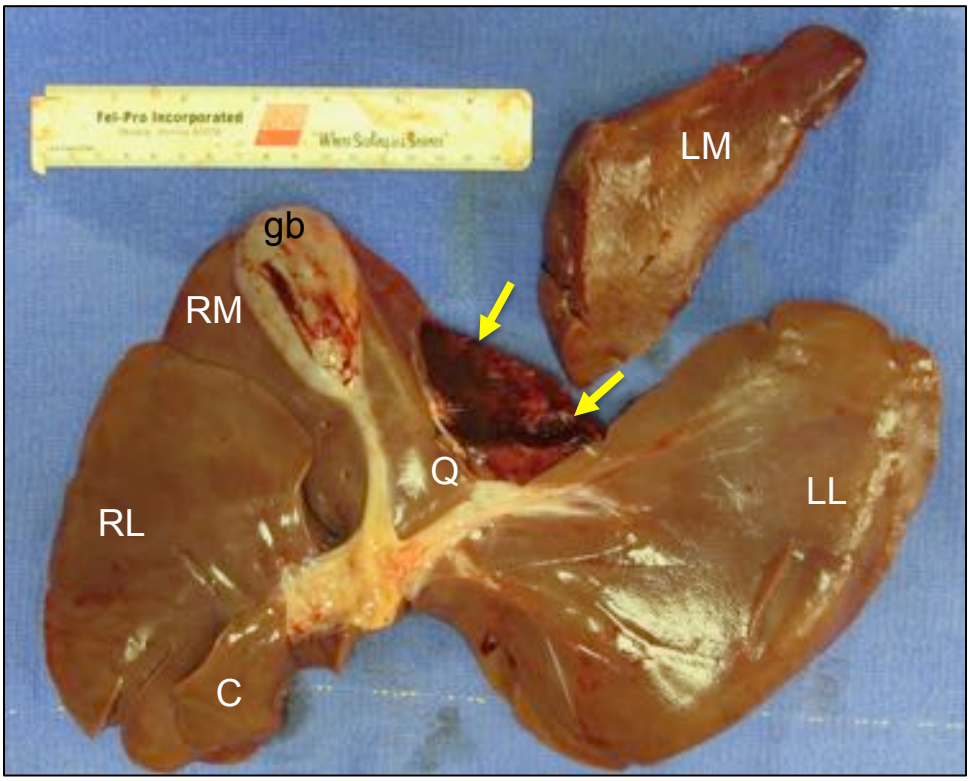

Figure 6, swine 283. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with thin film of clot. Anterior is at top of image.

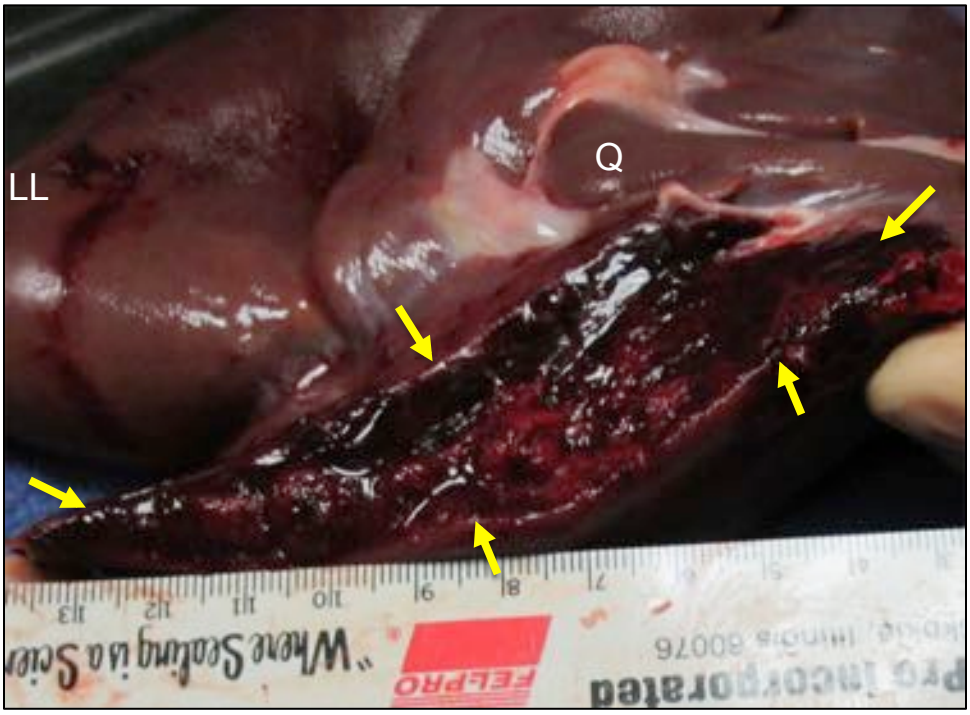

Figure 7, swine 283. Close-up of resection site (base of LM lobe, yellow arrows), covered with thin layer of clot. Superior is at bottom of image.

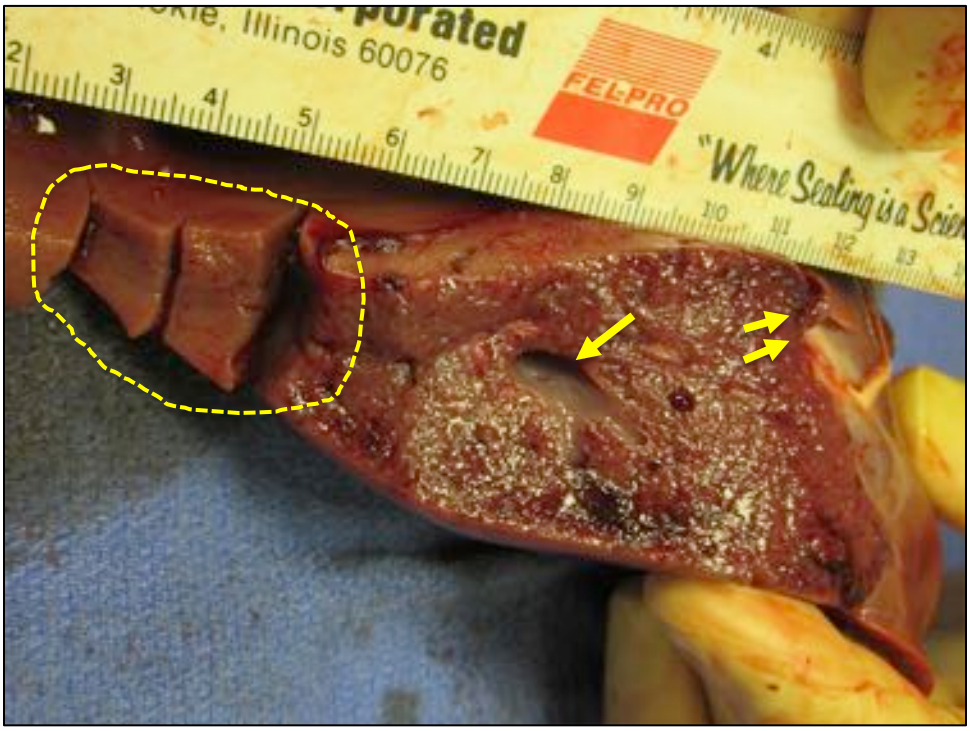

Figure 8, swine 283. Similar view as Fig. 7, but clot over injury site has been wiped away. Orifice of the hepatic vein to the LM lobe indicated with single yellow arrow. Double arrow indicates site of portal vein branch (collapsed) to LM lobe. Site of biopsy of wound surfaced encircled (for FFPE H&E histology).

I. OVERVIEW
Date: May 15, 2015
Swine no: 284
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam only (no clotting factors)
Personnel: Carlson, Hansen, Siford, Fatemi, Ragusa

II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: Male
Date swine received from UNL Mead: 05/04/2015
Pre-procedure wt: 36.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 80
- MAP:106
- Temp: 38.0
- EtCO2: 36

Blood draw no. 1 (initial): 8:10 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:15 AM
Spleen wt: 266.3 gm
LR (22°C) infused after splenectomy: 800 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 266.3 + 30 + 0 = 296.3 mL
- LR (22°C) infused (spleen replacement + incidental): 800 + 250(ear vein/jugular drip)= 1050 mL

Pre-injury VS

- HR: 71
- MAP: 110
- Temp: 37.5

- EtCO2 : 39
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:37 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through a separate stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: **0 mg** Fibrinogen, 0 mg Factor XIII, and 0 units thrombin (i.e., no clotting factors)

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. IAP briefly attained 30 mm Hg several min after injection began, then slowly decreased.

Total mass injected: 999.4 g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:41AM (within 4 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:46 AM

10 min post-injury VS

- HR: 100
- MAP: 63
- Temp: 37.4
- EtCO2: 44
- IAP: 12

Blood draw no. 3: (30 min post-injury): 9:05 AM

30 min post-injury VS

- HR: 95
- MAP: 37

- Temp: 37.1
- EtCO2: 34
- IAP: 7

Blood draw no. 4: (60 min post-injury): 9:35AM

60 min VS
- HR: 102
- MAP: 40
- Temp: 36.5
- EtCO2: 36
- IAP: 6

Blood draw no. 5: (90 min post-injury): 10:10AM

90 min post-injury VS
- HR: 105
- MAP: 40
- Temp: 36.0
- EtCO2: 36
- IAP: 3

Blood draw no. 6: (120 min post-injury): 10:35AM

120 min post-injury VS
- HR: 100
- MAP: 48
- Temp: 35.6
- EtCO2: 36
- IAP: 2

Blood draw no. 7: (150 min post-injury): 11:05AM

150 min post-injury VS
- HR: 94
- MAP: 42
- Temp: 35.3
- EtCO2: 35
- IAP: 2

Blood draw no. 8: (180 min post-injury): 11:35AM

180 min post-injury VS
- HR: 87
- MAP: 48
- Temp: 34.9
- EtCO2: 35
- IAP: 2

Survival at 180 min? Yes
Target MAP attained ? 4 minutes
Time of death: 11:38 AM
Cause of death: Exsanguination (intentional with euthanasia)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 1002.9 mL (suction) + 310.7 mL (clot + lap pads) + 210 mL (phlebotomies) = 1523.6 mL
- IV fluid given: LR (37°C): 1875 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; modestly tense (IAP ~ 2 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of clotted & unclotted blood were seen (see Figs). Large areas of intestinal discoloration were noted, see Figs. Injury site was covered with a large mass of foam + clot (see Figs). No active bleeding. This mass fell off during the liver explantation phase. The amorphous mass of foam+clot measured approx. 15 x 12 x 3 cm (see Figs).
Volume foam recovered: 610.00 gms (see Figs)
Heart: examined (RA & RV); no emboli; liquid blood only.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 100.7g (resected LM lobe) + 870.3 (remaining liver) = 971.0 g
Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam, no clotting factors (N = 3 for this Rx). Survived 180 minutes with 1.5 L blood loss. Lump of clot and foam covering and adherent to injury site, with no active bleeding at 3 h. Note that this subject lost 1.5 L of blood, about the same as the other subjects treated with alginate foam only that died within the 1st h post-injury.

VII. PLAN

Continue with this series in June, after ISR meeting.

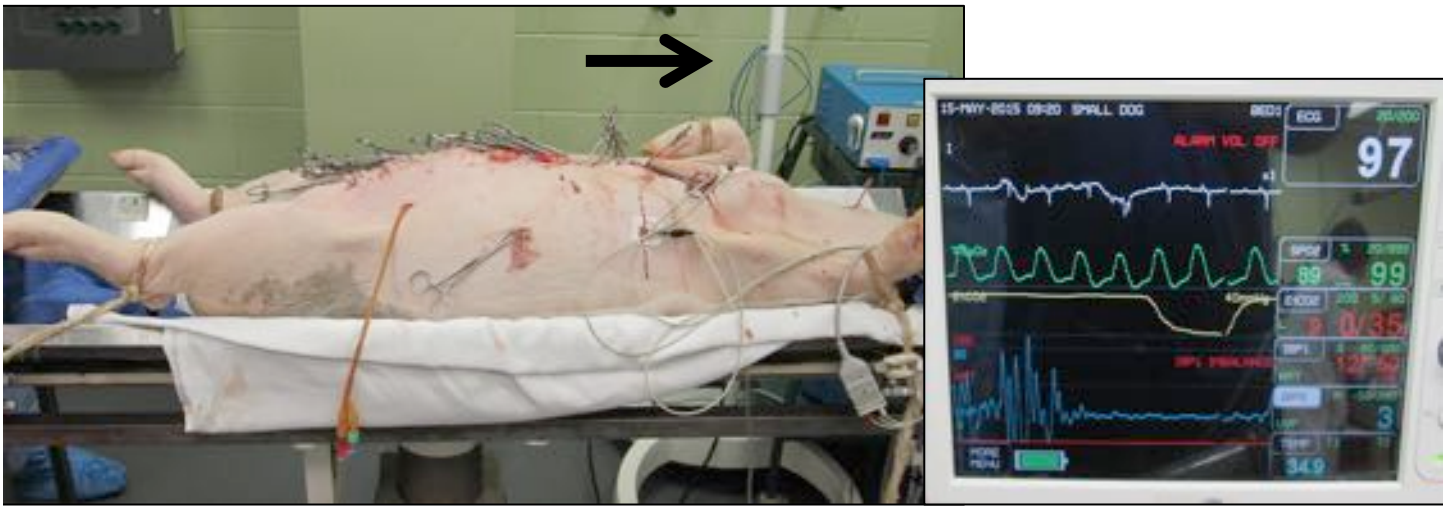

Figure 1, swine 284. Lateral view of swine at end of 3 h observation period. Subject alive and stable with pulse = 97 BPM, O2 Sat = 99%, EtCO2 = 35 mm Hg, MAP = 48 mm Hg, T = 34.9˚C. Black arrow = cephalad.

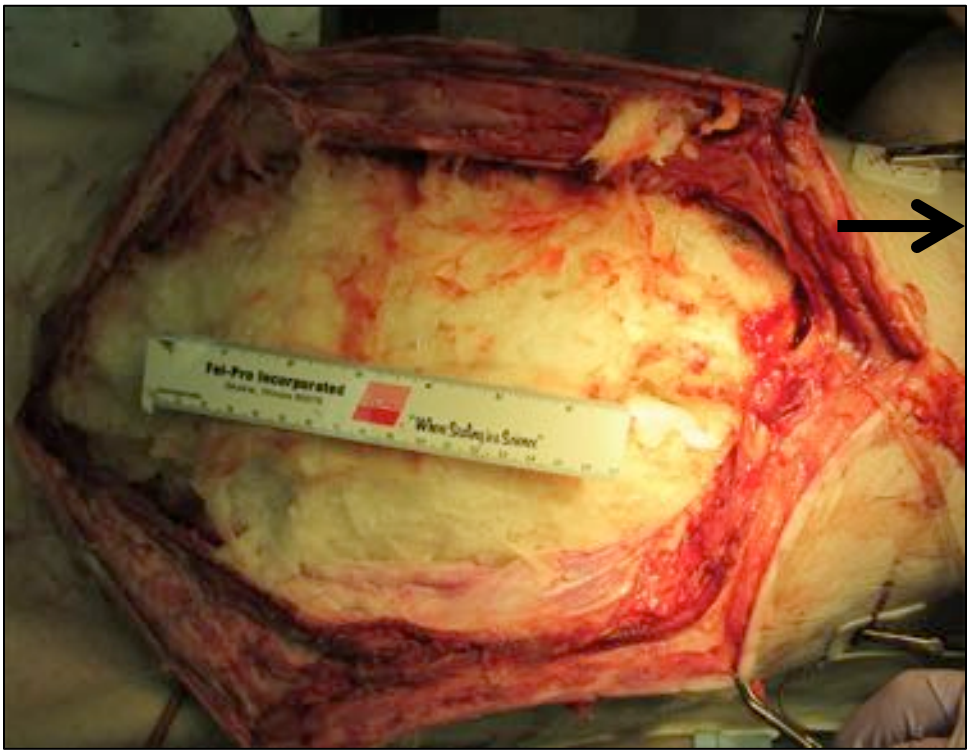

Figure 2, swine 284. Overhead view of re-opened abdomen immediately after Fig. 1; subject still alive. Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision. No gas pockets.

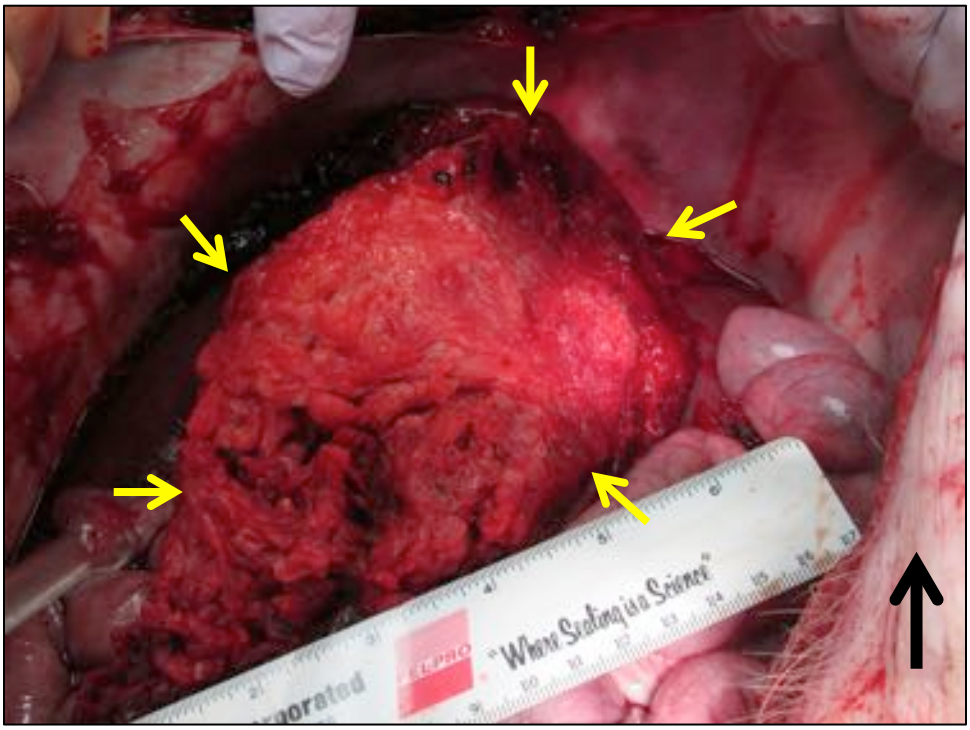

Figure 3, swine 284. View of upper abdomen after most of whitish (nonbloody) foam mass removed. Subject still alive. Black arrow = cephalad. There was a large mass of clot/foam (yellow arrows) covering the injury site, lightly adherent to the wound, but became dislodged as the liver was explanted.

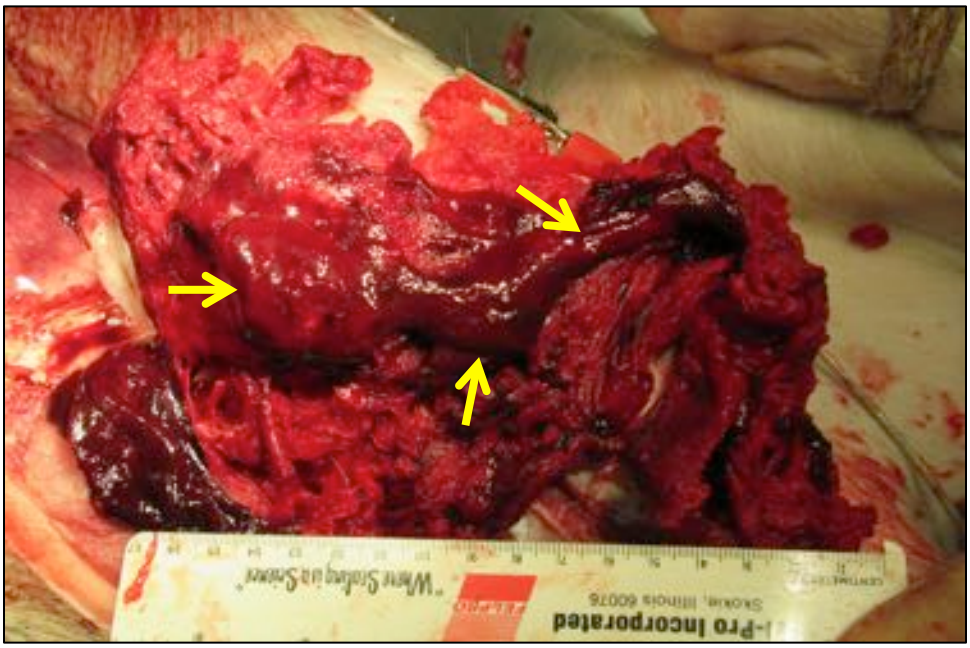

Figure 4, swine 284. Liver was explanted (see Fig. 6); mass of foam+clot covering jury site was disloged during explantation, and this mass is shown here. Clumps of clot (yellow arrows) interspersed with foam. The side that facing the wound surface is now facing the camera.

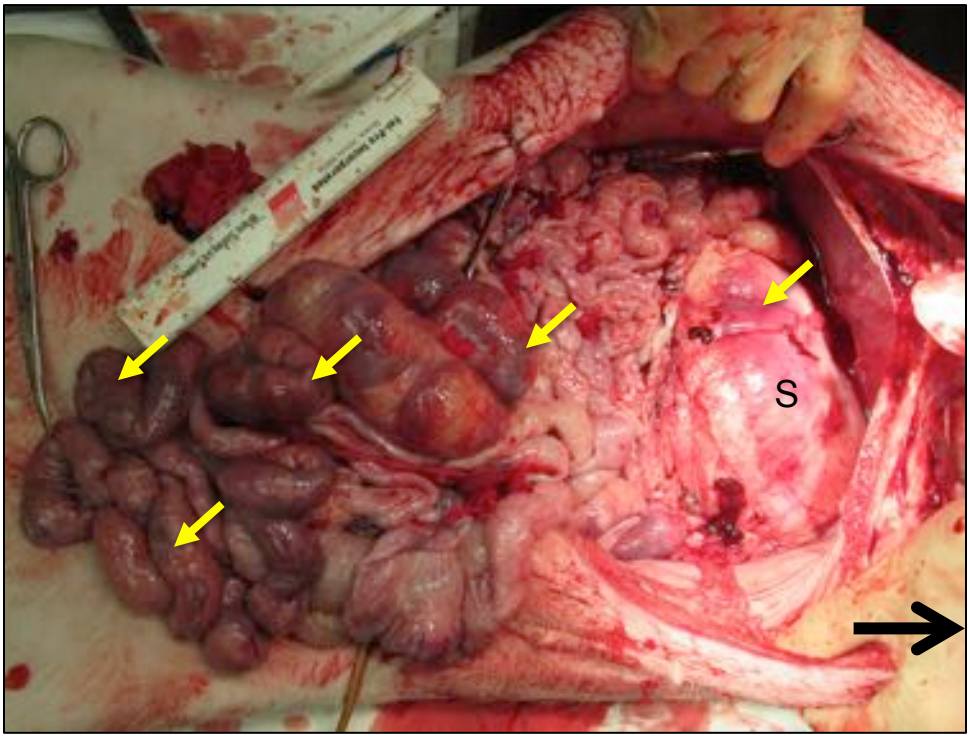

Figure 5, swine 284. View of abdomen after liver has been explanted. Note numerous areas of intestinal discoloration (yellow arrows). S = stomach. Black arrow = cephalad.

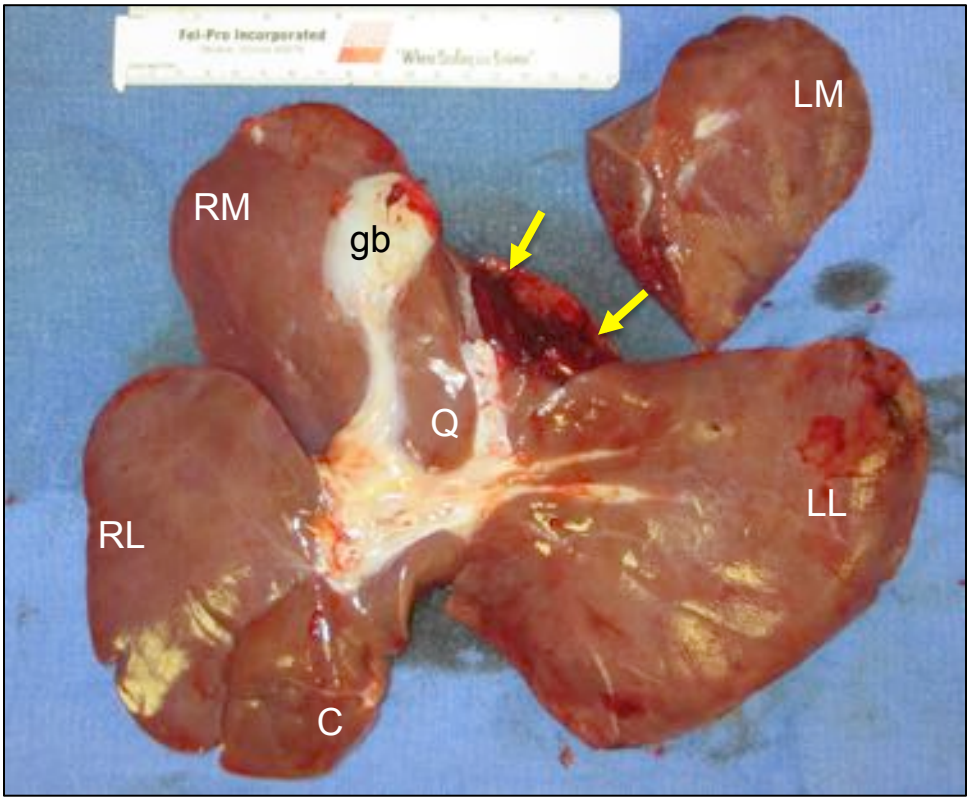

Figure 6, swine 284. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with thin film of clot. Anterior is at top of image.

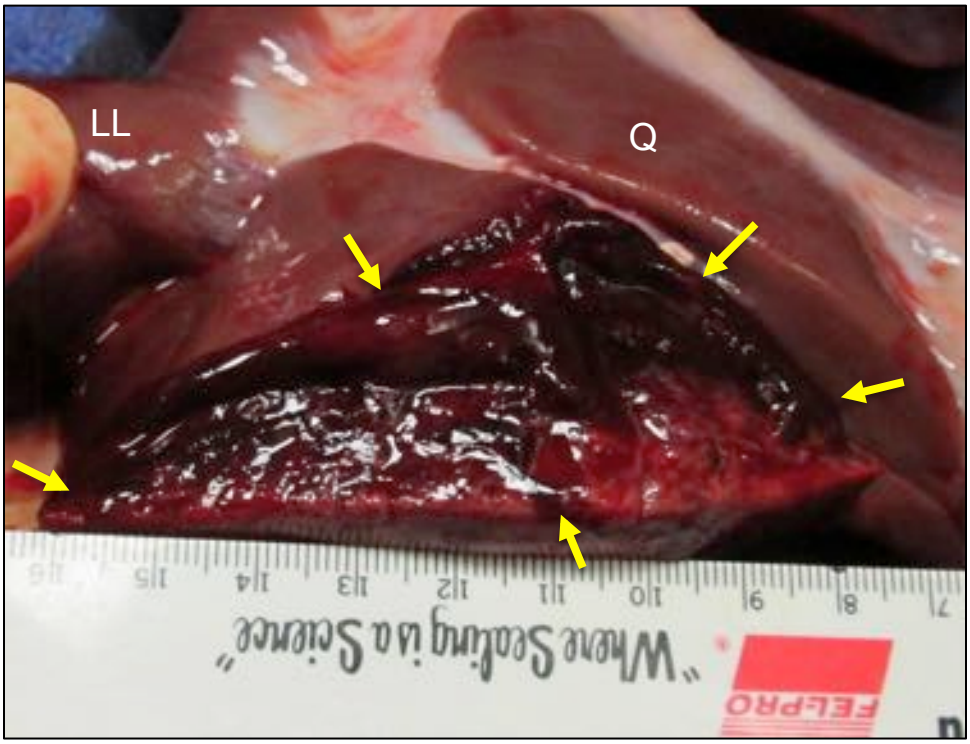

Figure 7, swine 284. Close-up of resection site (base of LM lobe, yellow arrows), covered with thin layer of clot. Superior is at bottom of image.

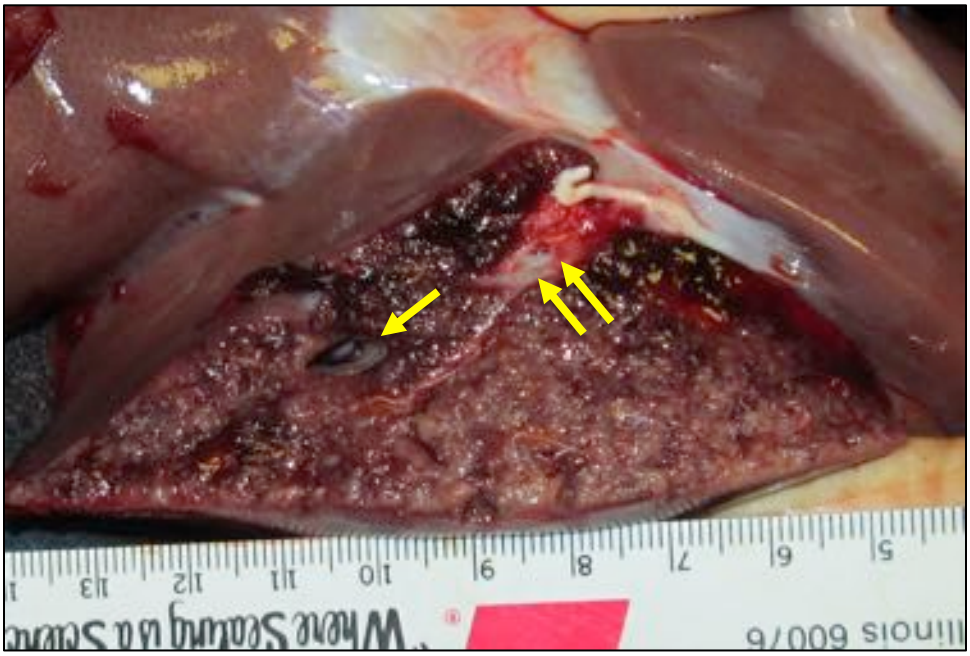

Figure 8, swine 284. Similar view as Fig. 7, but clot over injury site has been wiped away. Orifice of the hepatic vein to the LM lobe indicated with single yellow arrow. Double arrow indicates site of portal vein branch (collapsed) to LM lobe.

I. OVERVIEW
Date: June 03, 2015
Swine no: 289
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam and Fibrin Sealant
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Fabian, Spretz, Johnson

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II. PRE-INJURY PHASE

Start time: 08:00 AM
Swine sex: Male
Date swine received from UNL Mead: 06/01/2015
Pre-procedure wt: 38.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 99
- MAP:134
- Temp: 39.1
- EtCO2: 43

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:23 AM
Spleen wt: 340.4 gm
LR (22°C) infused after splenectomy: 1025 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 340.4 + 30 + 31.1 = 401.5 mL
- LR (22°C) infused (spleen replacement + incidental): 1025 + 75(ear vein/jugular drip)= 1100 mL

Pre-injury VS
- HR: 124
- MAP: 131

- Temp: 38.2
- EtCO2 : 46
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:40 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figs). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: full complement of FS (pdhFI, rhFIIa, rhFXIIIa)

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. Subjectively, the injection contents went into the abdomen quite quickly compared to previous subjects, and the subject's MAP jumped up to 50 mm Hg momentarily before dropping down to ~30 at 3-4 min after injection. The subject developed subcutaneous emphysema in multiple places, primarily in the bilateral groins.

Total mass injected: 420.3 g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.6 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:41AM (within 1 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:50 AM

10 min post-injury VS

- HR: 131
- MAP: 42
- Temp: 38.7
- EtCO2: 45
- IAP: 12

Blood draw no. 3: (30 min post-injury): 9:10 AM

30 min post-injury VS

- HR: 125
- MAP: 62
- Temp: 38.0
- EtCO2: 47
- IAP: 5

Blood draw no. 4: (60 min post-injury): 9:40AM

60 min VS
- HR: 130
- MAP: 61
- Temp: 37.5
- EtCO2: 45
- IAP: 1

Blood draw no. 5: (90 min post-injury): 10:10AM

90 min post-injury VS
- HR: 124
- MAP: 75
- Temp: 37.1
- EtCO2: 42
- IAP: 1

Blood draw no. 6: (120 min post-injury): 10:40AM

120 min post-injury VS
- HR: 127
- MAP: 85
- Temp: 36.9
- EtCO2: 53
- IAP: 1

Blood draw no. 7: (150 min post-injury): 11:10AM

150 min post-injury VS
- HR: 145
- MAP: 63
- Temp: 36.6
- EtCO2: 63
- IAP: 0

Blood draw no. 8: (180 min post-injury): 11:40AM

180 min post-injury VS
- HR: 140
- MAP: 64
- Temp: 36.3
- EtCO2: 50

- IAP: 0

Survival at 180 min? Yes
Target MAP attained ? Briefly, less than 1 minute
Time of death: 11:45 AM
Cause of death: Exsanguination
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 512.8mL (suction) + 347.8 mL (clot + lap pads) + 210 mL (phlebotomies) = 1,070.6 mL
- IV fluid given: LR (37°C): 1,925 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; not very tense (IAP 0-1 mm Hg). Upon re-opening abdomen, pocket of butane gas present underneath incision; then, a subjectively decreased volume of foam (compared to previous subjects) by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Only very minor ischemic areas over intestine were noted. Injury site was covered with a large mass of foam + clot (see Figs). The foam fell off the injury site during liver explantation, but the site was still covered with a heavy layer of clot.
Clot-injury site adhesion score = 4
Volume foam recovered: 212.4 g, less volume than typical (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 81.4g (resected LM lobe) + 1082.7 (remaining liver) = 1164.1 g

Tissue harvested: (i) Jelly like clot, (ii) liver from resected lobe, (iii) liver from injury site

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VI. COMMENTS

Noncompressible injury treated with alginate foam, no fibrinogen (N = 4 for this Rx). Survived 180 minutes with ~1.1 L blood loss. Lump of clot and foam covering and adherent to injury site, with no obvious active bleeding at 3 h.

VII. PLAN

Continue with this series on June 5th, treatment with Ca alg foam + FS.

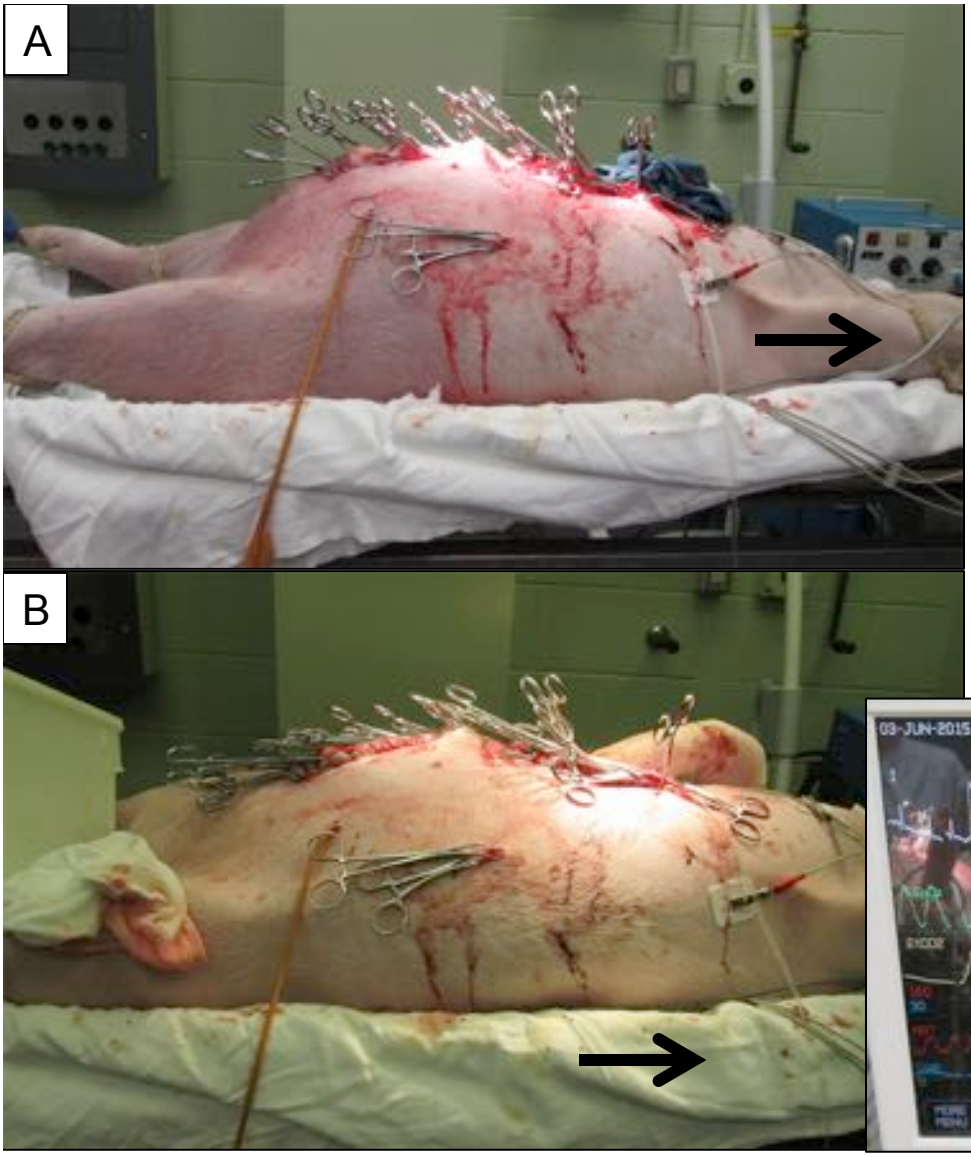

Figure 1, swine 289. Lateral view of swine during procedure. (A) At 3 min after injection completed; IAP ~30 mm Hg with sq emphysema. (B) At end of 3 h observation period; IAP decreased gradually, to 0-1 mm Hg in this image. Subject alive and stable with pulse = 119 BPM, O2 Sat = 99%, EtCO2 = 50 mm Hg, MAP = 60 mm Hg, T = 36.3˚C (see monitor inset). Black arrow = cephalad.

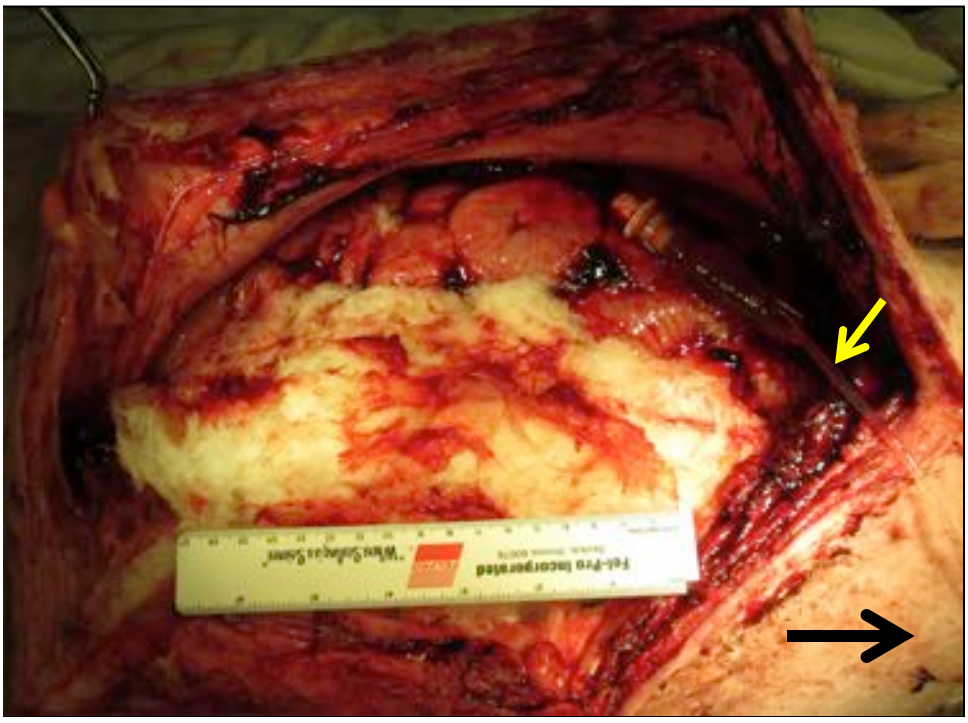

Figure 2, swine 289. Overhead view of re-opened abdomen immediately after Fig. 1B; subject still alive. Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision. Volume of foam was qualitatively lower in this subject than in previous subjects. Yellow arrow = line to IAP monitor.

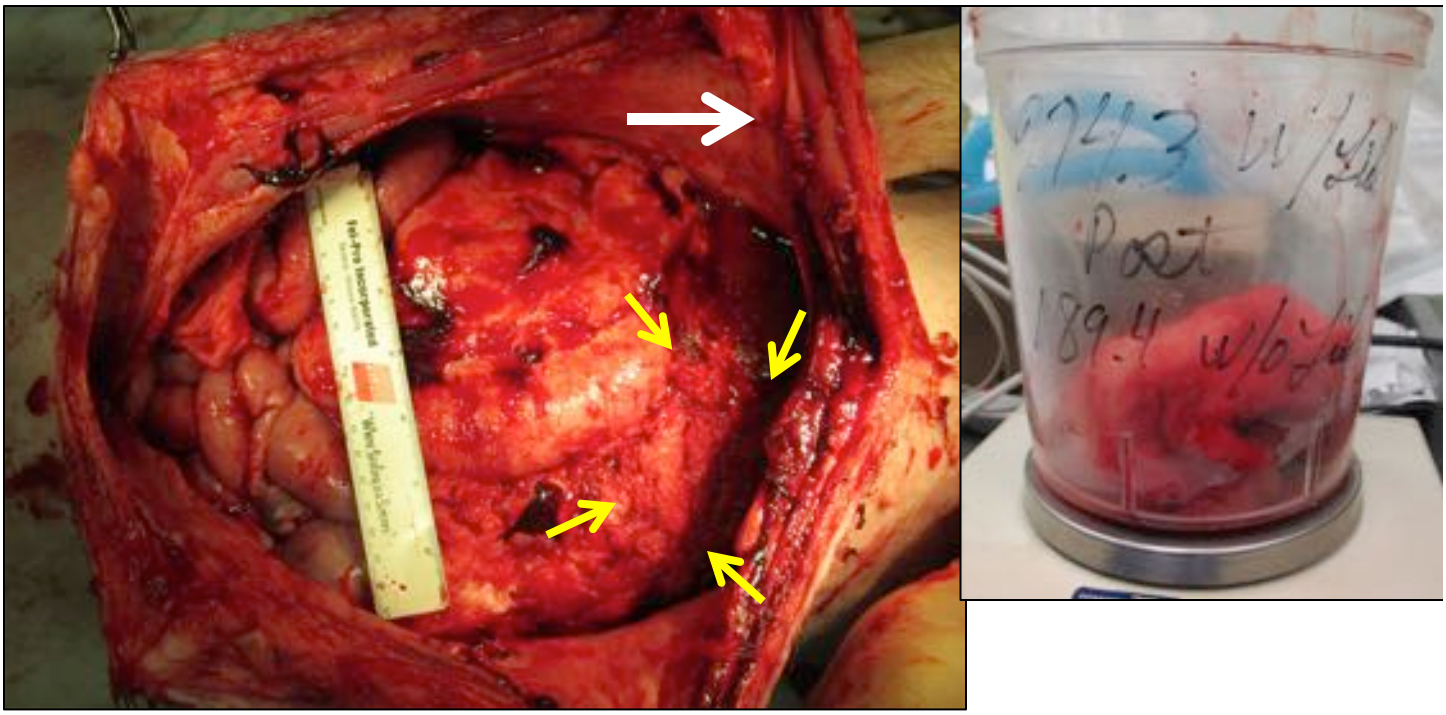

Figure 3, swine 284. View of upper abdomen after most of whitish (nonbloody) foam mass removed. Subject still alive. White arrow = cephalad. There was a large mass of clot/foam (yellow arrows) covering the injury site, adherent to the wound; became dislodged during liver explantation. Right: volume of foam removed (noticeably less than in previous subjects).

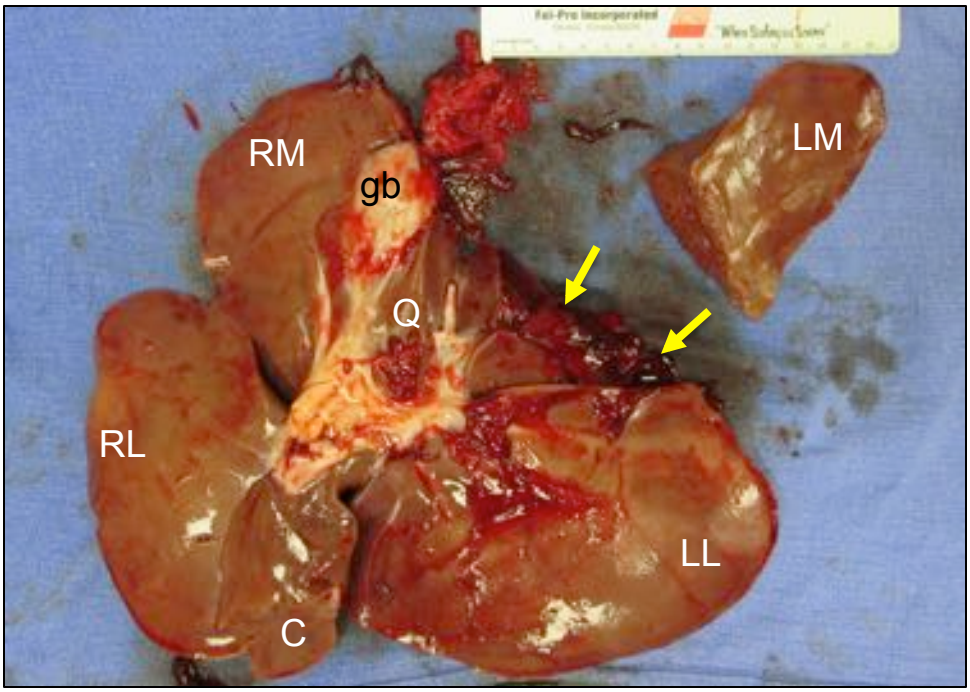

Figure 4, swine 289. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection site, covered with heavy clot. Anterior is at top of image.

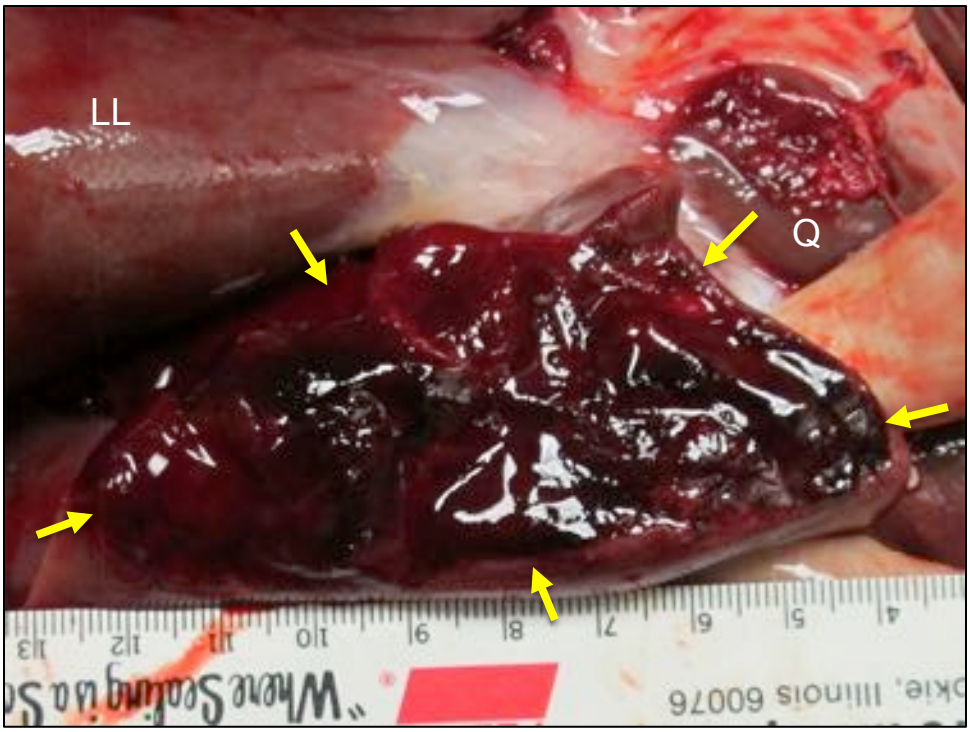

Figure 5, swine 289. Close-up of resection site (base of LM lobe, yellow arrows), covered with heavy layer of clot. Superior is at bottom of image.

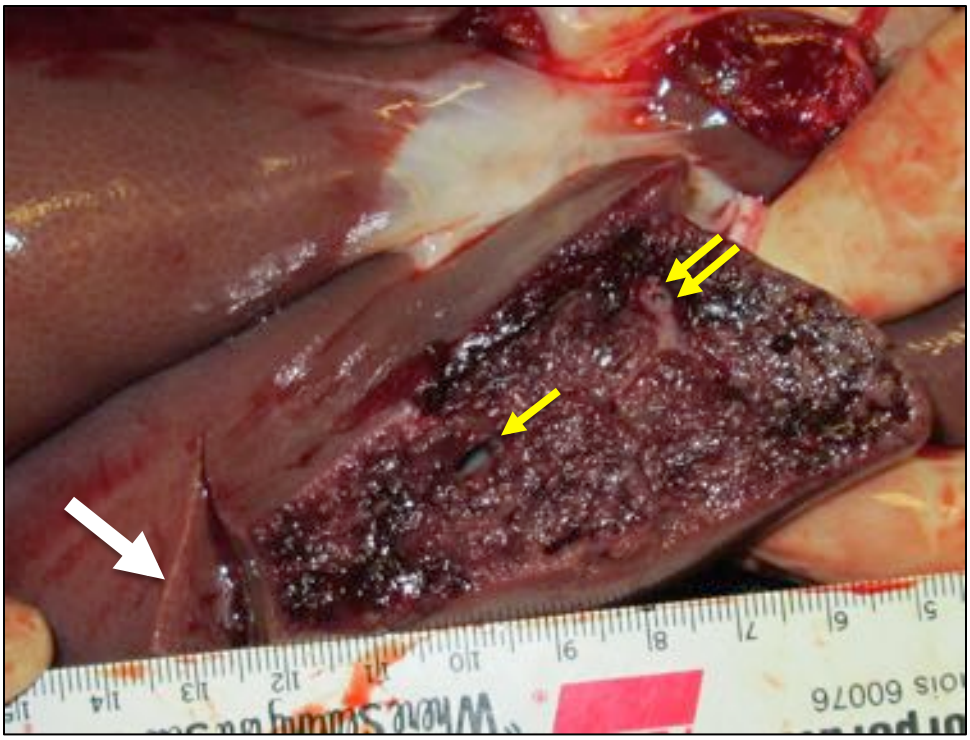

Figure 6, swine 289. Similar view as Fig. 5, but clot over injury site has been wiped away. Orifice of the hepatic vein to the LM lobe indicated with single yellow arrow. Double arrow indicates site of portal vein branch to LM lobe. White arrow = site of wound site specimen harvest for IHC.

I. OVERVIEW
Date: June 05, 2015
Swine no: 290
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam and Fibrin Sealant
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Fabian, Spretz, Johnson

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II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: Male
Date swine received from UNL Mead: 06/01/2015
Pre-procedure wt: 35.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 90
- MAP:113
- Temp: 37.5
- EtCO2: 39

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:12 AM
Spleen wt: 232.7 g
LR (22°C) infused after splenectomy: 700 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 232.7 + 30 + 3.2 = 265.9 mL
- LR (22°C) infused (spleen replacement + incidental): 700 + 60(ear vein/jugular drip) + 500 (LR infused prior to splenectomy, low MAP) = 1260 mL

Pre-injury VS
- HR: 94

- MAP: 104
- Temp: 37.2
- EtCO2 : 42
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:29 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figs). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: full complement of FS (pdhFI, rhFIIa, rhFXIIIa).

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 470.2 g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 85

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 9.9 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:31 (within 2 min of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:40 AM

10 min post-injury VS

- HR: 201
- MAP: 38
- Temp: 37.0
- EtCO2: 38
- IAP: 19

Blood draw no. 3: (30 min post-injury): 9:00 AM

30 min post-injury VS

- HR: 173
- MAP: 23

- Temp: 36.3
- EtCO2: 27
- IAP: 8

Blood draw no. 4: (60 min post-injury): 9:30AM

60 min VS
- HR: 158
- MAP: 21
- Temp: 35.3
- EtCO2: 30
- IAP: 4

Blood draw no. 5: (90 min post-injury): 10:00AM

90 min post-injury VS
- HR: 146
- MAP: 22
- Temp: 35.1
- EtCO2: 37
- IAP: 2

Blood draw no. 6: (120 min post-injury): 10:30AM

120 min post-injury VS
- HR: 138
- MAP: 23
- Temp: 34.9
- EtCO2: 36
- IAP: 2

Blood draw no. 7: (150 min post-injury): 11:00AM

150 min post-injury VS
- HR: 137
- MAP: 21
- Temp: 34.5
- EtCO2: 32
- IAP: 2

Blood draw no. 8: (180 min post-injury): 11:30AM

180 min post-injury VS
- HR: 62
- MAP: 13
- Temp: 33.9
- EtCO2: 10
- IAP: 2

Survival at 180 min? Yes
Target MAP attained ? Less than 2 minutes
Time of death: 11:30 AM
Cause of death: Exsanguination (not euthanasia; animal expired right at 180 min)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 857mL (suction) + 500.9mL (clot + lap pads) + 210 mL (phlebotomies) = 1567.9 mL
- IV fluid given: LR (37°C): 1800 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; not really tense (IAP 0-1 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Minimal ischemic areas over intestines were noted. Injury site was actively bleeding at re-exploration, even though subject had been declared dead. There was no foam adherent to the injury site; only a small amount of clot was present, but it was not hemostatic (see Figs).
Clot-injury site adhesion score = 0
Volume foam recovered: 481.7 g (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 133.3g (resected LM lobe) + 834.3 (remaining liver) = 967.6 g

Tissue harvested: (i) liver from resected lobe, (ii) liver from injury site.

VI. COMMENTS

Noncompressible injury treated with alginate foam, no fibrinogen (N = 5 for this Rx). Subject's MAP dropped rapidly after injury to 20's, and (remarkably) remained there for entire 3 h observation period. Typically when we see a MAP drop that low that fast, it means that subject will expire within 1 h. But this subject did not… subject barely survived to 180 min, and then died right at end of 3 h observation period, with ~1.6 L blood loss. There was no obvious hemostatic effect of the treatment at autopsy… not sure what happened in this subject. This result will mess up our statistics. Now we will need a larger N to determine whether today's result was a fluke result, or an actual/trusted result.

VII. PLAN

Continue with this protocol on Wed June 17, 2015: Alg foam + rFIIa/rFXIIIa.

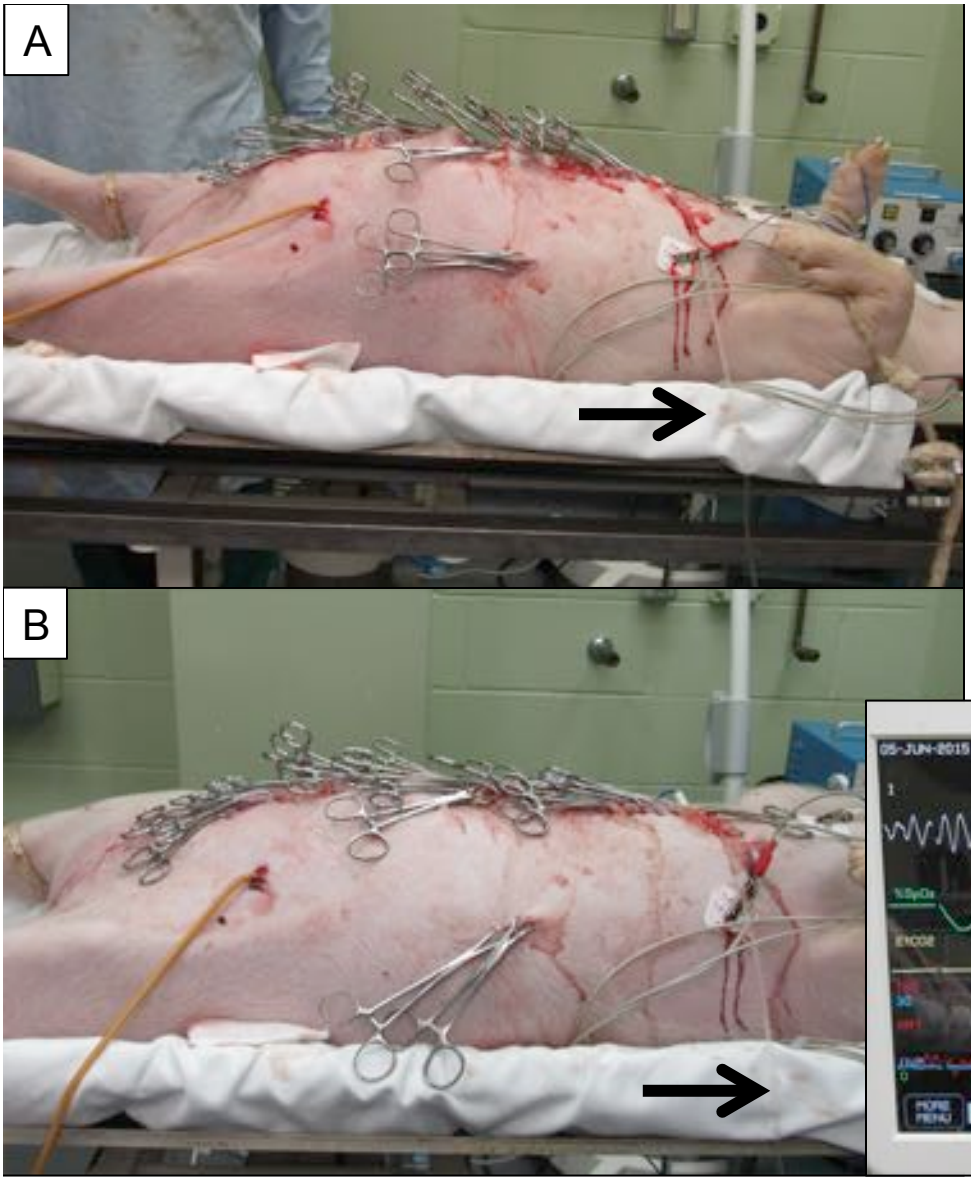


Figure 1, swine 290. Lateral view of swine during procedure. (A) At 3 min after injection completed; IAP ~30 mm Hg with sq emphysema. (B) At end of 3 h observation period; IAP decreased gradually, to 0-1 mm Hg in this image. Subject nearly expired with HR = 62 BPM, O2 Sat = not readable, EtCO2 = 7 mm Hg, MAP = 13 mm Hg (still with pulse wave), T = 33.8˚C (see monitor inset). Black arrow = cephalad.

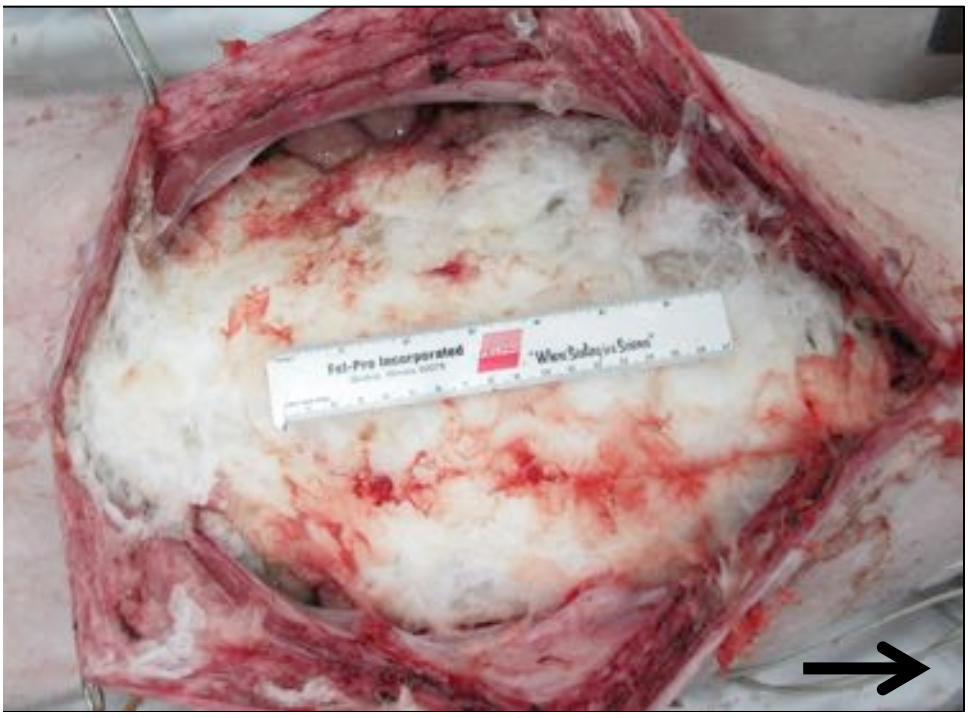


Figure 2, swine 290. Overhead view of re-opened abdomen immediately after Fig. 1B; subject expired right at 180 min time limit. Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision.

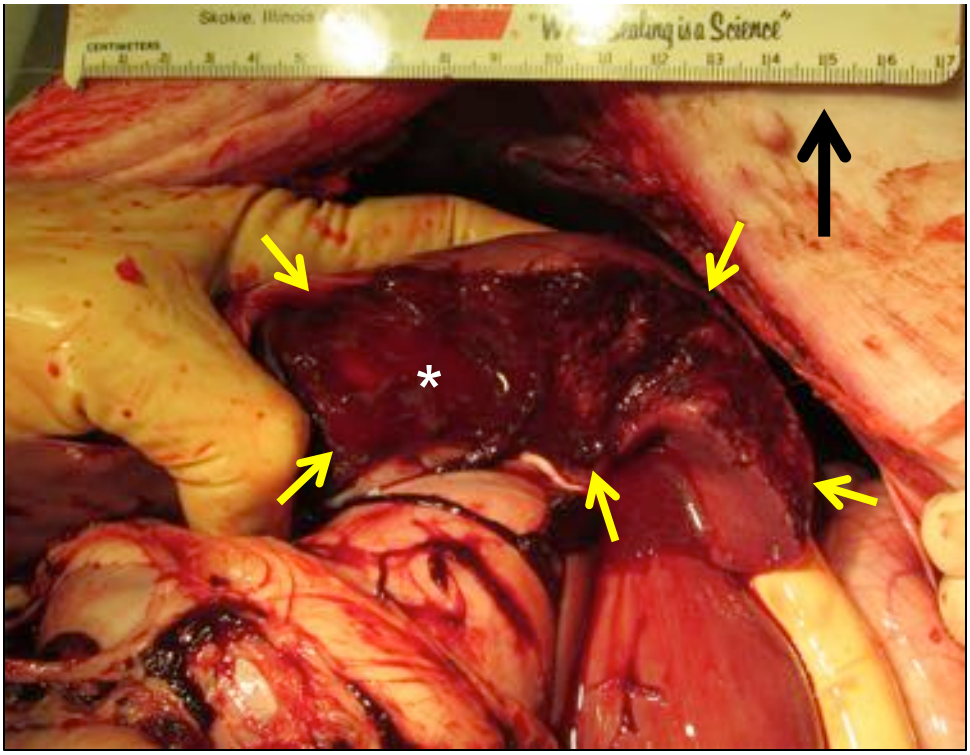


Figure 3, swine 290. View of upper abdomen after foam mass removed. Subject expired. None of the foam was adherent to the wound site (yellow arrows). There was blood actually flowing from the injury site, even though subject had expired several minutes prior to this photograph. There was some clot present at the wound site (asterisk), but this was not preventing hemorrhage. Black arrow = cephalad.

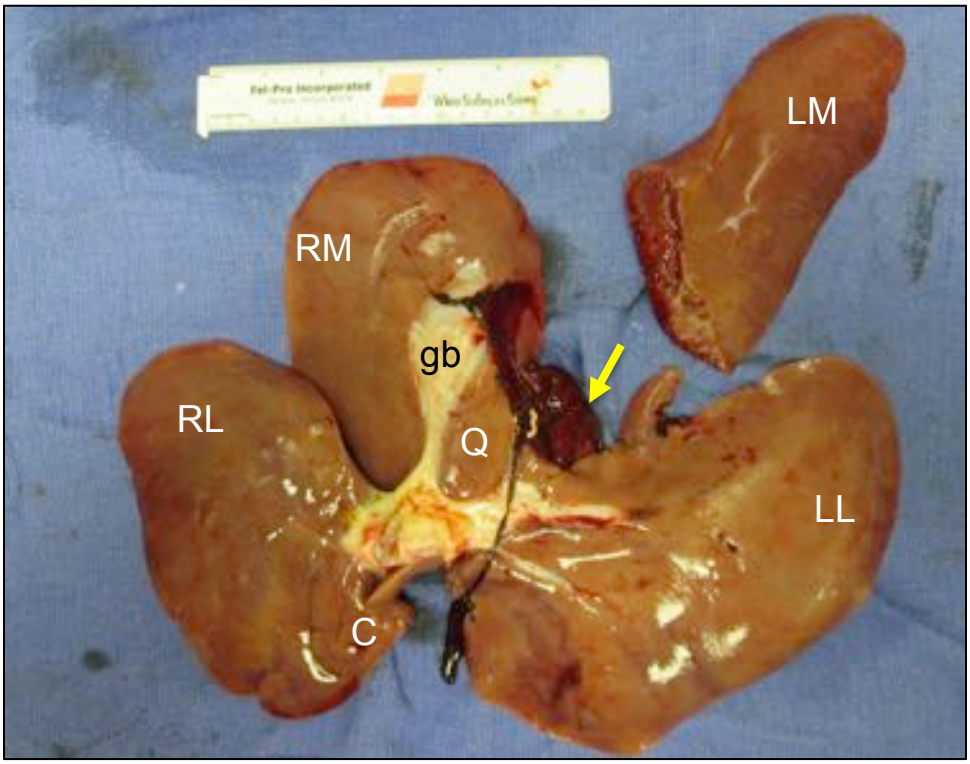


Figure 4, swine 290. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site. Anterior is at top of image.

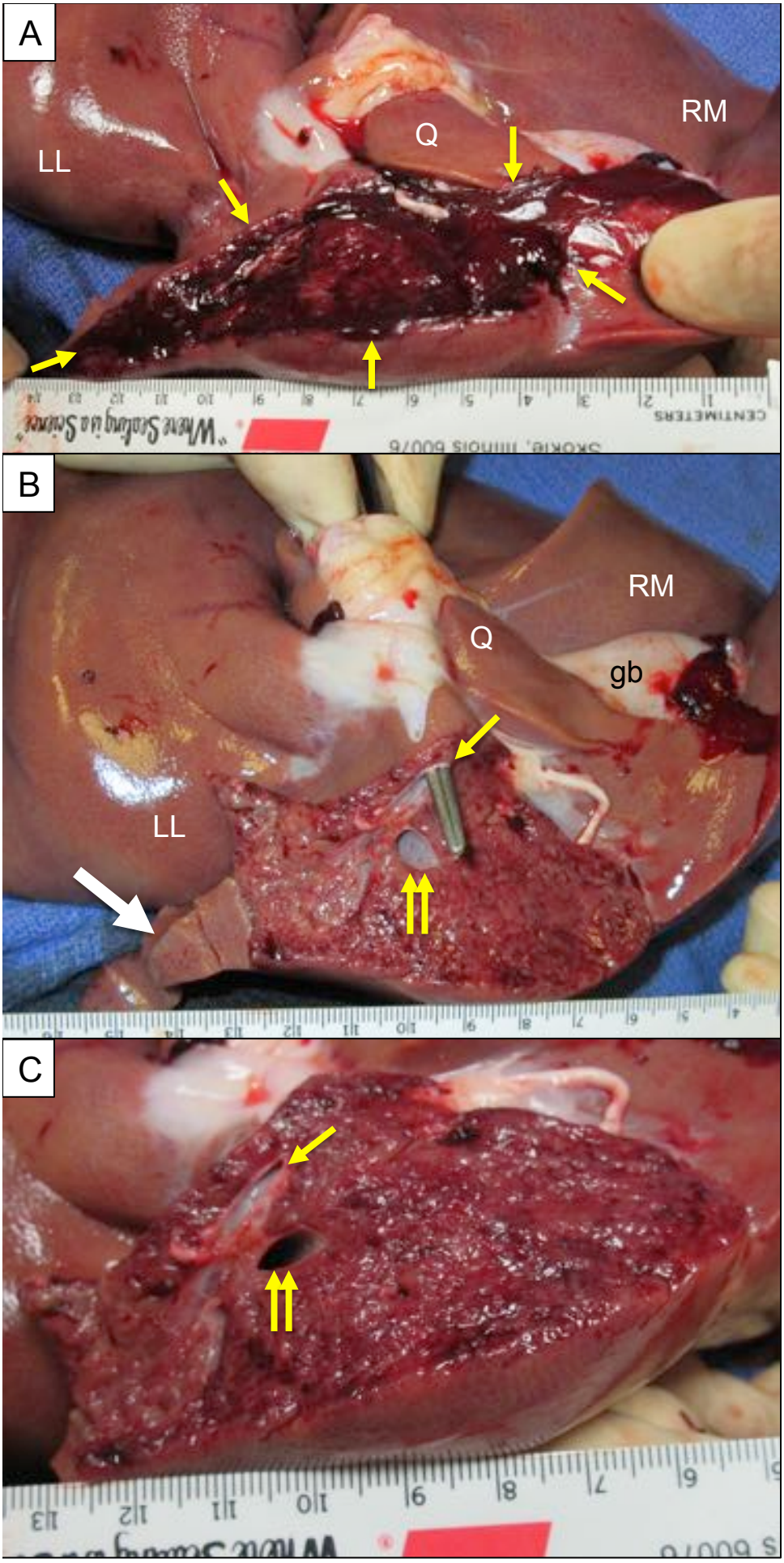


Figure 5, swine 290. (A) Close-up of resection site (base of LM lobe, yellow arrows), covered incompletely with clot. Superior is at bottom of image. (B) Similar view as in panel A, but clot over injury site has been wiped away. A forceps has been inserted into the portal vein at the porta hepatis, and the tips emerged from the transected end of the PV branch to the LM lobe (single yellow arrow). Orifice of the hepatic vein to the LM lobe indicated with double yellow arrow. Large white arrow = site of wound site specimen harvest for IHC. (C) Same view as in panel B, except that forceps has been removed.

I. OVERVIEW
Date: June 17, 2015
Swine no: 292
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam and Factors II/XIII
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Fabian, Spretz, Johnson, Zhou.

II. PRE-INJURY PHASE

Start time: 08:05 AM
Swine sex: Male
Date swine received from UNL Mead: 06/12/2015
Pre-procedure wt: 36.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 119
- MAP:125
- Temp: 38.1
- EtCO2: 39

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:25 AM
Spleen wt: 289.3 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 289.3 + 30 + 12.4(sponges) = 331.7 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 45(ear vein/jugular drip)= 945 mL

Pre-injury VS
- HR: 116
- MAP: 131

- Temp: 37.6
- EtCO2 : 45
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:39 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: Factors II & XIII; **no** Fibrinogen

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 610.4 g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:39AM (within 1 minute of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:50 AM

10 min post-injury VS

- HR: 180
- MAP: 56
- Temp: 37.5
- EtCO2: 48
- IAP: 26

Blood draw no. 3: (30 min post-injury): 9:10 AM

30 min post-injury VS

- HR: 128
- MAP: 62
- Temp: 37.3

- EtCO2: 43
- IAP: 17

Blood draw no. 4: (60 min post-injury): 9:40AM

60 min VS
- HR: 128
- MAP: 60
- Temp: 36.7
- EtCO2: 42
- IAP: 15

Blood draw no. 5: (90 min post-injury): 10:10AM

90 min post-injury VS
- HR: 122
- MAP: 63
- Temp: 36.3
- EtCO2: 41
- IAP: 14

Blood draw no. 6: (120 min post-injury): 10:40AM

120 min post-injury VS
- HR: 137
- MAP: 62
- Temp: 36.0
- EtCO2: 37
- IAP: 14

Blood draw no. 7: (150 min post-injury): 11:10AM

150 min post-injury VS
- HR: 133
- MAP: 61
- Temp: 35.9
- EtCO2: 36
- IAP: 13

Blood draw no. 8: (180 min post-injury): 11:40AM

180 min post-injury VS
- HR: 124
- MAP: 57
- Temp: 35.8
- EtCO2: 36
- IAP: 14 (the IAP monitor was re-zeroed after this reading, and the actual IAP was 3-4).

Survival at 180 min? Yes

Target MAP attained ? Less than 1 minute
Time of death: 11:35 AM
Cause of death: Exsanguination
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 642.0mL (suction) + 108.5 mL (clot + lap pads) + 210 mL (phlebotomies) = 960.5 mL
- IV fluid given: LR (37°C): 2000 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~4 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Some ischemic areas over small intestine were noted, along with generalized discoloration of colon. Injury site was covered with a large mass of foam + clot (see Figs), but this was not adherent to the injury site. There was no active hemorrhage when the foam was removed; the injury had sealed against the stomach (see Figs), which was distended. After liver explantation, a large clot was seen adherent to one end of the injury site, without any foam (see Figs).
Clot-injury adhesion score = 2 (out of 4).
Volume foam recovered: 550.9 g (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 137.5g (resected LM lobe) + 968.8 (remaining liver) = 1106.3 g

Tissue harvested: Liver and skin for Instron experiments.

VI. COMMENTS

Noncompressible injury treated with alginate foam + FII/FXIII. Survived 180 minutes with ~1 L blood loss. No foam was adherent to the injury site; some clot was adherent to one end of the site. The mechanism that stopped the bleeding in this stomach appeared to be a distended stomach that sealed up against the injury.

Also, note in the Figs that the degree of abdominal distension seen initially is greater than that seen at 3 h. So I would venture to say that the foam volume initially is much greater than the volume at 3 h. Whether this "shrinkage" is from continual intraabdominal compression or from some other mechanism, I don't know. But in recent subjects, we seem only to be retrieving <1 L of foam volume at the end of the 3 h incubation. The IAP in this subject was 50+ mm Hg initially, but drifted down to 3-4 at the 3 h time point.

VII. PLAN

Continue with this series on 06-19-15.

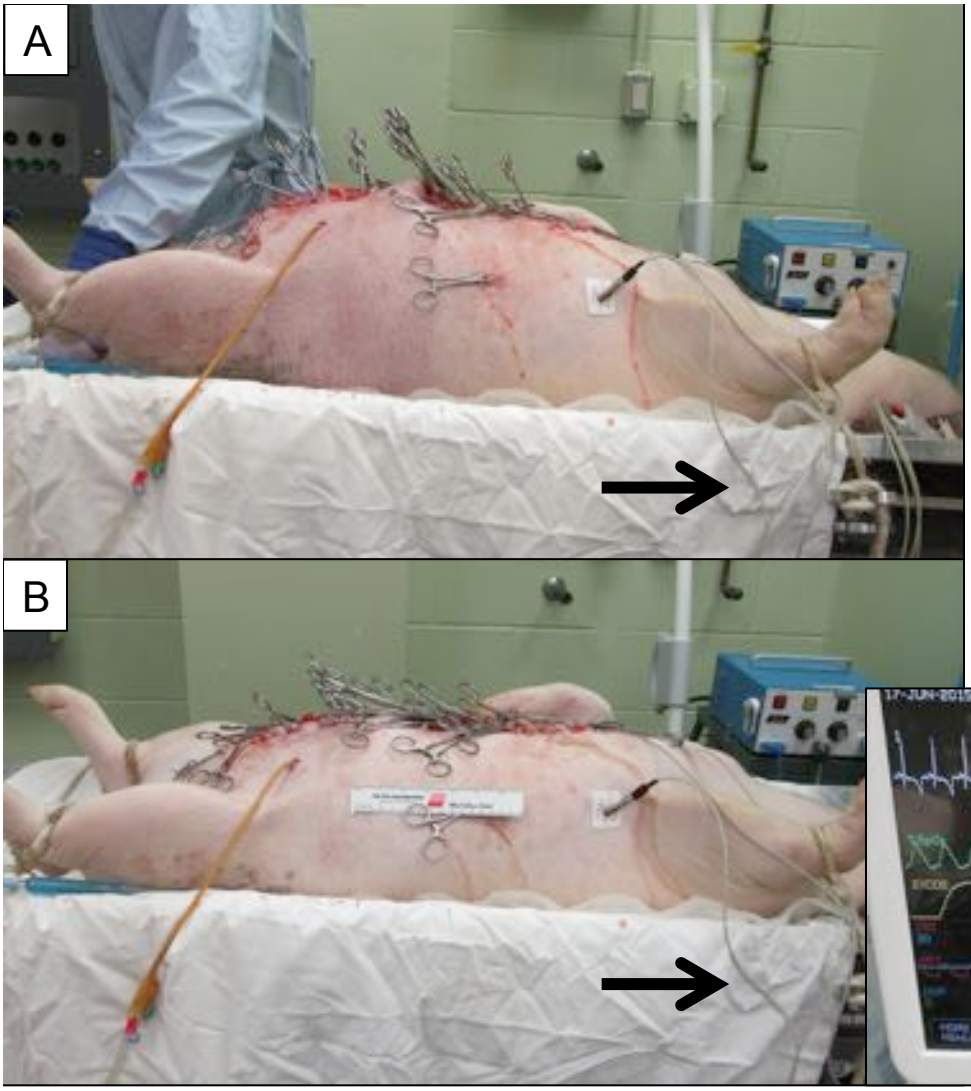


Figure 1, swine 292. Lateral view of swine during procedure. (A) At 3 min after injection completed; IAP ~50 mm Hg with sq emphysema. (B) At end of 3 h observation period; IAP decreased gradually, to ~4 mm Hg in this image (IAP monitor was reading 10 mm Hg above actual value). Subject stable with HR = 124 BPM, O2 Sat = 98%, EtCO2 = 36 mm Hg, MAP = 60 mm Hg, T = 35.8˚C (see monitor inset). Black arrow = cephalad. Note change in level of distension from t = 3 min to t = 180 min.

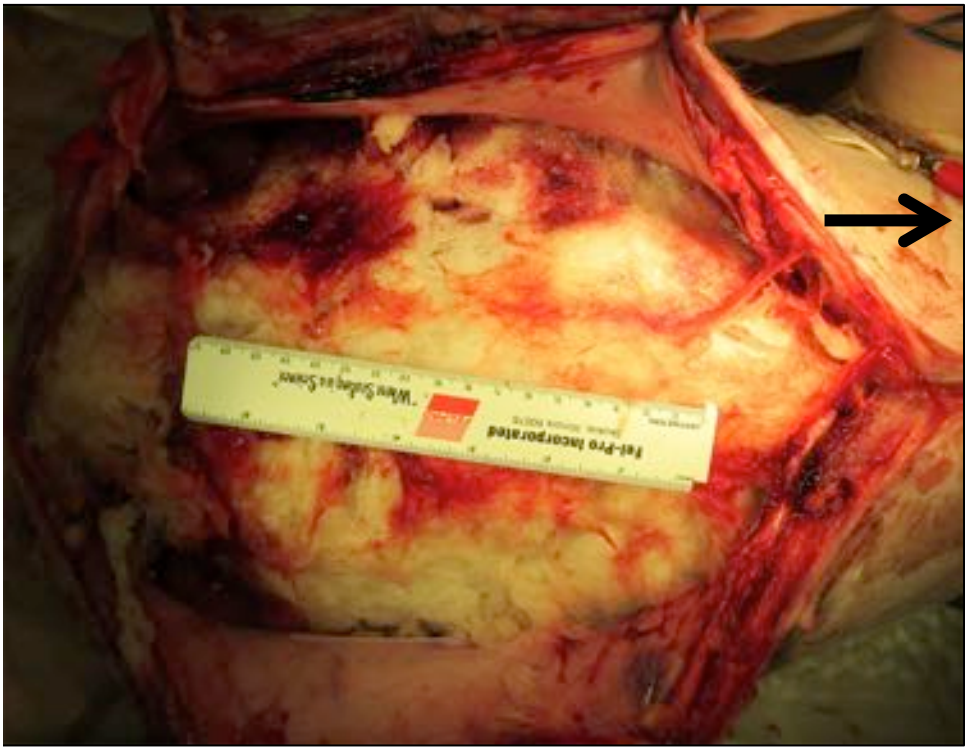


Figure 2, swine 292. Overhead view of re-opened abdomen immediately after Fig. 1B; subject still alive. Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision; no gas pockets.

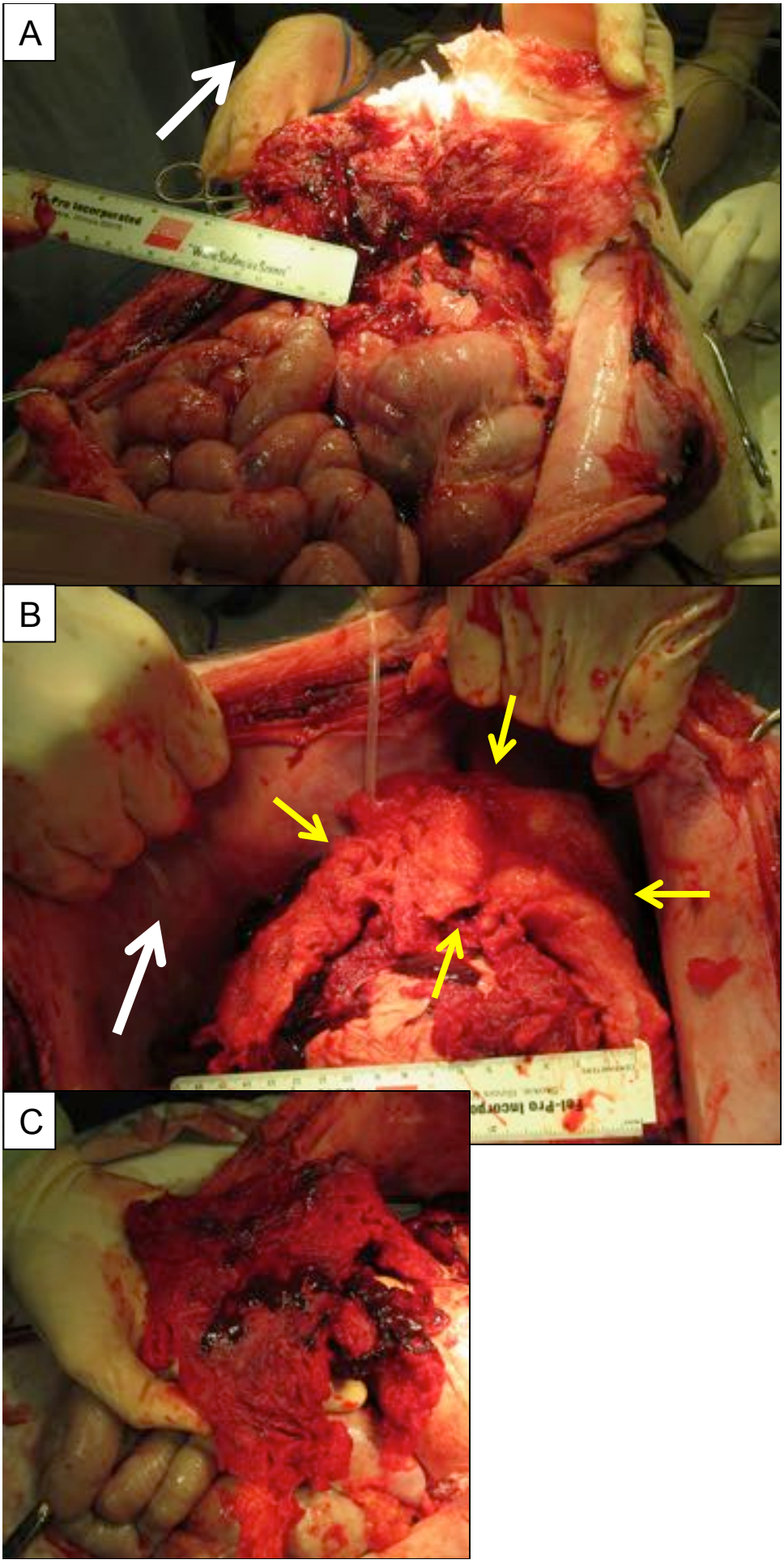


Figure 3, swine 292. Foam removal. (A) View toward head. Foam is being peeled out of the abdomen toward the head. White arrow = cephalad. Injury site is still covered by foam + blood. (B) Most of foam has been removed; site of injury still covered with mixture of foam + blood (yellow arrows). (C) Lump of foam mixed with blood that was overlying injury site. This lump was not adherent to the site, but fell off as the liver was mobilized. Aspect of lump facing the camera was adjacent to the wound site.

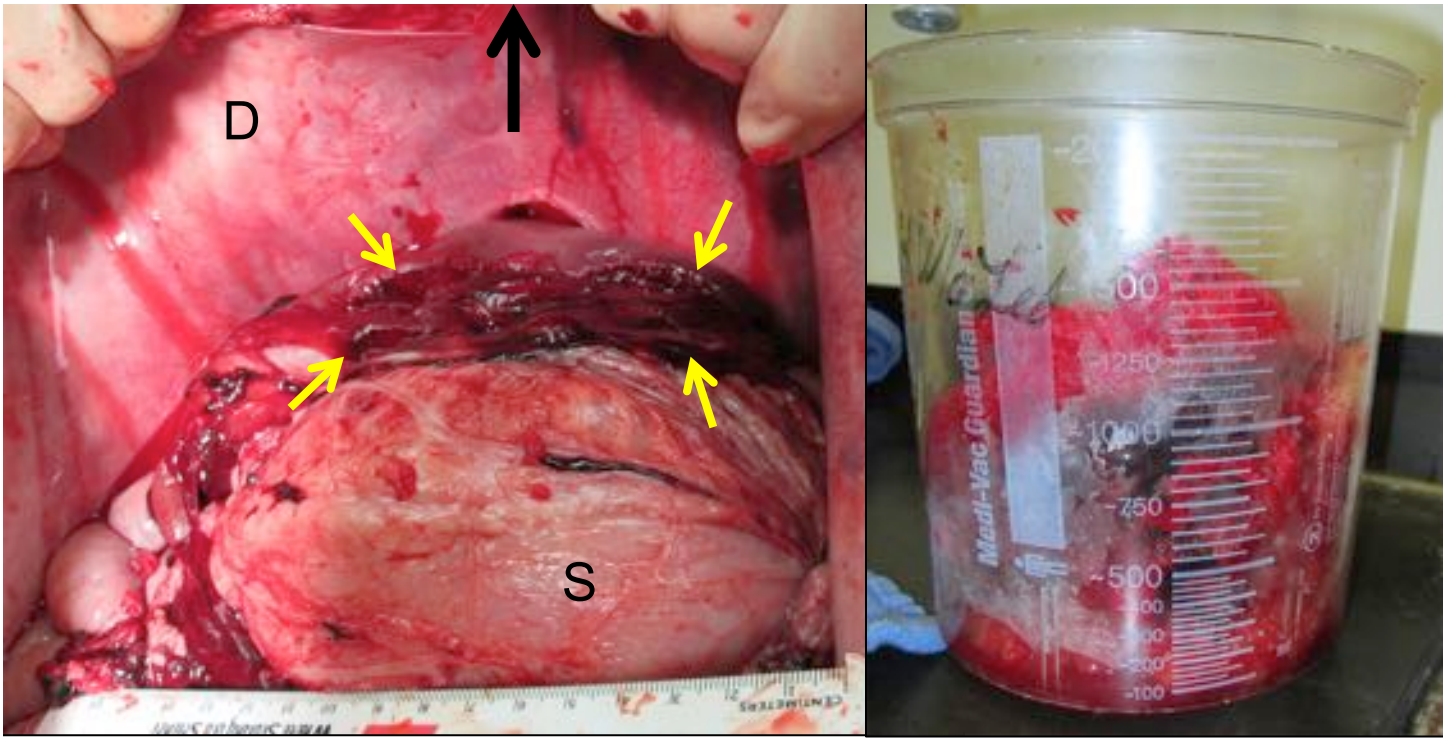


Figure 4, swine 292. Left: view of upper abdomen after foam mass removed. Subject still alive. None of the foam was adherent to the wound site (yellow arrows). There did not appear to be any active hemorrhage. Much of the wound site appeared to be compressed/tamponaded by the distended stomach (S). Black arrow = cephalad; D = diaphragm. Right: entire mass of foam from subject was placed into this 2 L beaker.

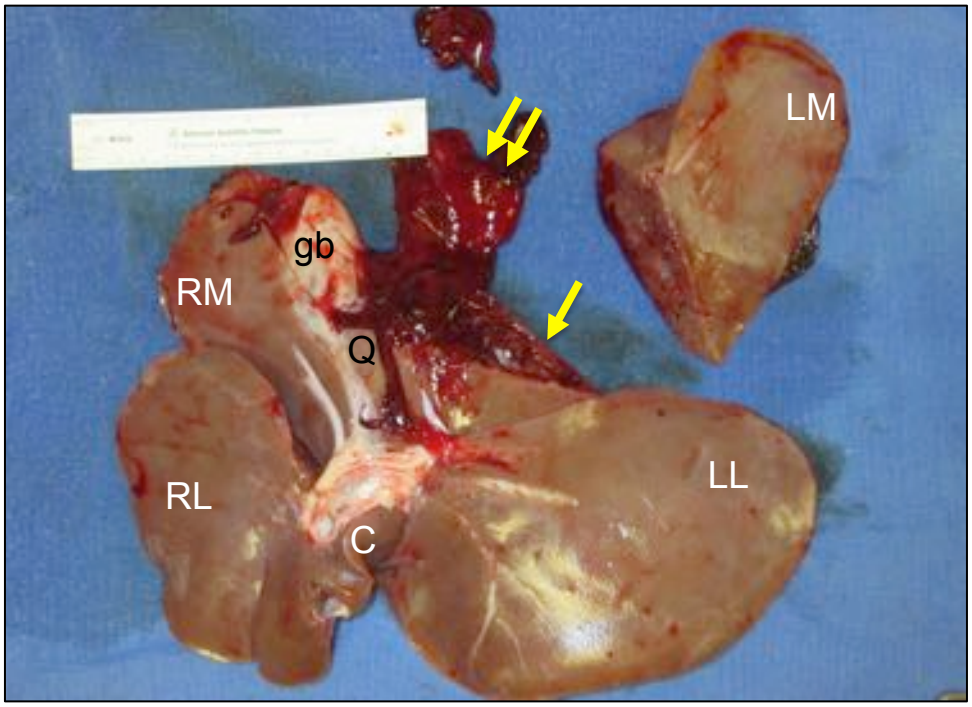


Figure 5, swine 292. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Single arrow = resection/injury site. Double arrow = clot adherent to injury site. Anterior is at top of image.

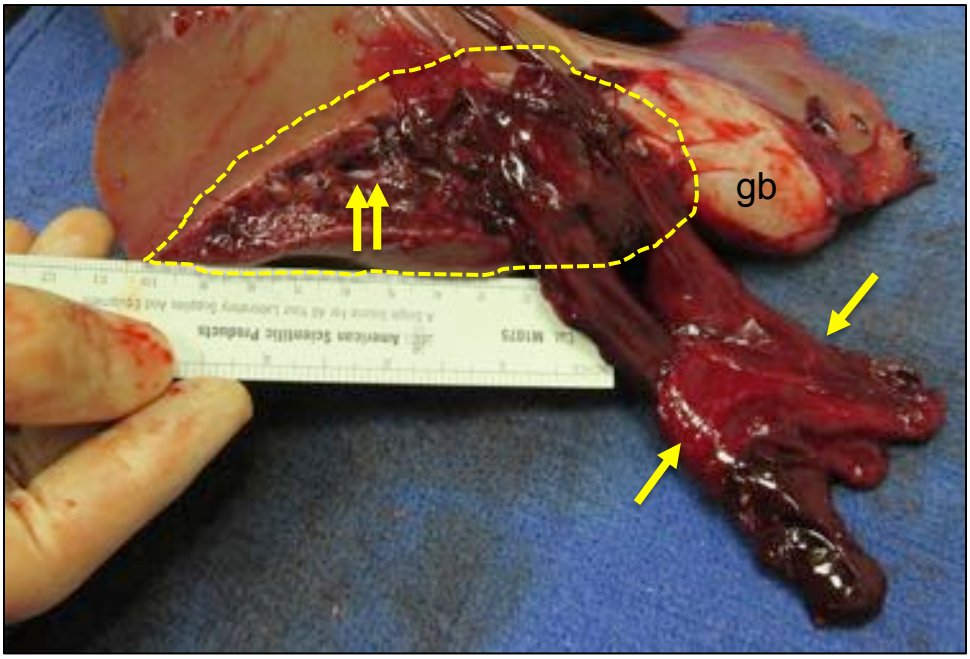


Figure 6, swine 292. Close-up of resection site (base of LM lobe, yellow dashed line), covered incompletely with clot. Superior is at bottom of image. Note large clot mass (yellow arrows) hanging off one end of the injury site. Note that transected hepatic vein (double arrow) is not covered by clot.

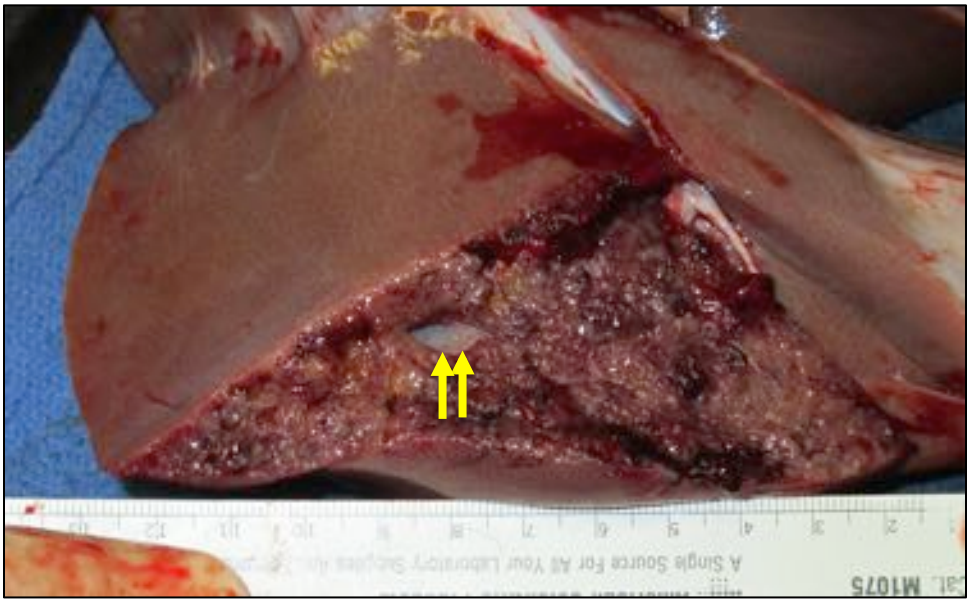


Figure 7, swine 292. Similar view as in panel A, but clot over injury site has been wiped away. Orifice of the hepatic vein to the LM lobe indicated with double yellow arrow.

I. OVERVIEW
Date: June 19, 2015
Swine no: 293
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam and Factors II/XIII
Personnel: Carlson, Hansen, Siford, Fatemi, Ismail, Fabian, Spretz, Johnson, Zhou.

II. PRE-INJURY PHASE

Start time: 07:48 AM
Swine sex: Male
Date swine received from UNL Mead: 06/18/2015
Pre-procedure wt: 50.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 84
- MAP:145
- Temp: 39.7
- EtCO2: 42

Blood draw no. 1 (initial): 8:07 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:17 AM
Spleen wt: 438 gm
LR (22°C) infused after splenectomy: 1325 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 438 + 30 + 7.6(sponges) = 475.6 mL
- LR (22°C) infused (spleen replacement + incidental): 1325 + 70(ear vein/jugular drip)= 1395 mL

Pre-injury VS
- HR: 101
- MAP: 143

- Temp: 38.8
- EtCO2 : 52
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:29 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: Factors II & XIII; **no** Fibrinogen.

Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left medial, LM) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 580.0 g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 115

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 14.0 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:30AM (within 1 minute of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:38 AM

10 min post-injury VS
- HR: 127
- MAP: 85
- Temp: 37.9
- EtCO2: 65
- IAP: 22

Blood draw no. 3: (30 min post-injury): 8:58 AM

30 min post-injury VS
- HR: 113
- MAP: 77
- Temp: 37.7

- EtCO2: 57
- IAP: 12

Blood draw no. 4: (60 min post-injury): 9:28AM

60 min VS
- HR: 102
- MAP: 70
- Temp: 37.4
- EtCO2: 50
- IAP: 9

Blood draw no. 5: (90 min post-injury): 9:58AM

90 min post-injury VS
- HR: 94
- MAP: 70
- Temp: 36.9
- EtCO2: 47
- IAP: 6

Blood draw no. 6: (120 min post-injury): 10:28AM

120 min post-injury VS
- HR: 83
- MAP: 84
- Temp: 36.4
- EtCO2: 42
- IAP: 4

Blood draw no. 7: (150 min post-injury): 10:58AM

150 min post-injury VS
- HR: 78
- MAP: 87
- Temp: 35.9
- EtCO2: 40
- IAP: 4

Blood draw no. 8: (180 min post-injury): 11:28AM

180 min post-injury VS
- HR: 77
- MAP: 92
- Temp: 35.3
- EtCO2: 35
- IAP: 3

Survival at 180 min? Yes

Target MAP attained ? 1 minute
Time of death: 11:30 AM
Cause of death: euthanasia by intentional exsanguination from IVC transection
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 482.6mL (suction) + 37.4 mL (clot + lap pads) + 210 mL (phlebotomies) = 730 mL
- IV fluid given: LR (37°C): 2555 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest re-exploration: abdomen distended; moderately tense (IAP ~ 3 mm Hg). Upon re-opening abdomen, foam by itself in the superficial region, then mixed with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Some focal areas of discoloration over intestines were noted. Injury site was covered with a large mass of foam + clot (see Figs), but this was not adherent to the wound site. When this mass was lifted up, a red clot covering the injury site was noted. No active hemorrhage noted. Note similar to all pigs in this series, the level of abdominal distension immediately after foam injection (~3 min after injury) was much greater than the level of distension at 3 h (see Figs). So the foam volume is modulated during the 3 h observation period.
Clot-injury adhesion score = 2 (out of 4)
Volume foam recovered: 364.3 g (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 96.6g (resected LM lobe) + 1449.7 (remaining liver) = 1546.3 g

Tissue harvested: Liver for Instron experiments.

VI. COMMENTS

Noncompressible injury treated with alginate foam + FII/FXIII, but no fibrinogen. Survived easily to 180 minutes with ~0.7 L blood loss. Lump of clot/foam covering injury site, but not adherent, with no active bleeding at 3 h. As we have observed before, the intraabdominal foam volume at t ~3 min is greater than at t = 180 min; i.e., the intraabdominal foam volume shrinks during the observation period, perhaps by compression/compaction, gas diffusion, or something else.

VII. PLAN

Next week on Thu & Fri (June 25th & 26th) we will be doing protocol #00827, the hepatic resection treated with PCL bandage + FS (FI/FII/FXIII), one subject on each day.

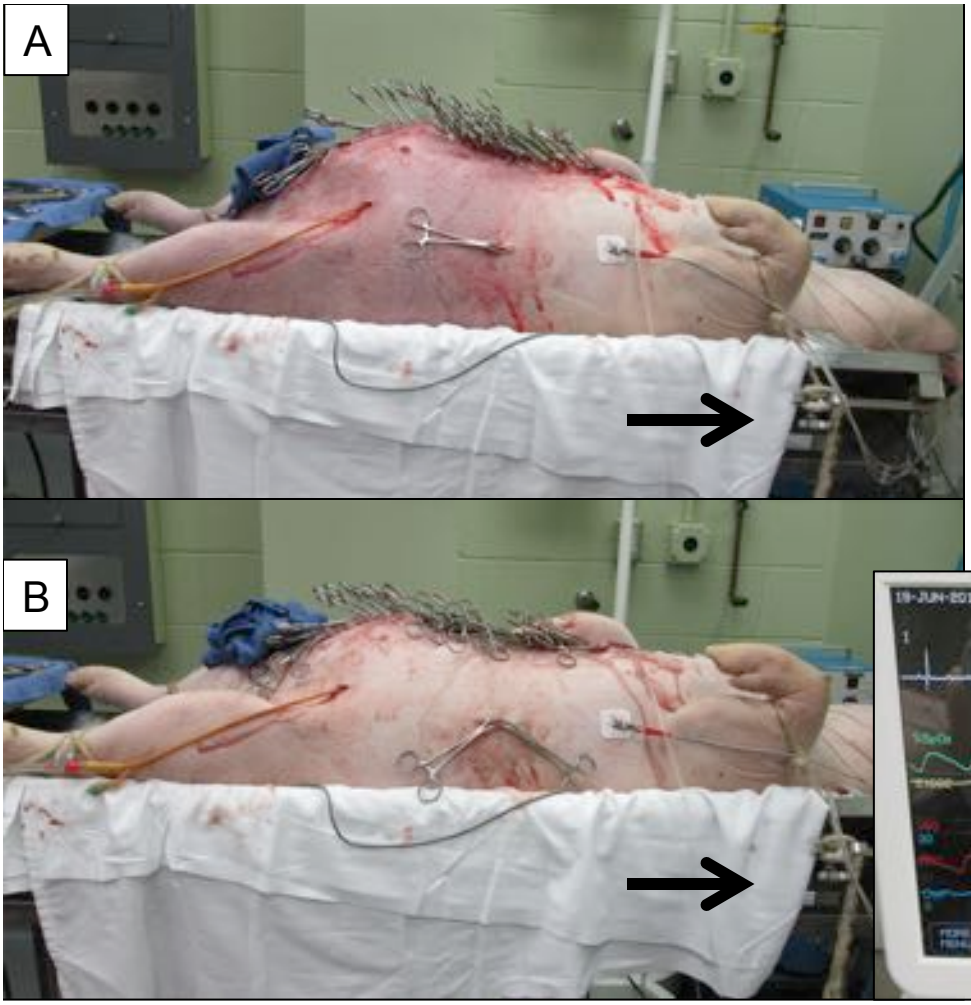


Figure 1, swine 293. Lateral view of swine during procedure. (A) At 3 min after injection completed; IAP ~55 mm Hg with sq emphysema, and discoloration of the inferior half of the body. (B) At end of 3 h observation period; IAP decreased gradually, to ~3 mm Hg in this image. Subject stable with HR = 77 BPM, O2 Sat = 97%, EtCO2 = 35 mm Hg, MAP = 89 mm Hg, T = 35.4˚C (see monitor inset). Black arrow = cephalad. Note change in level of distension from t = 3 min to t = 180 min.

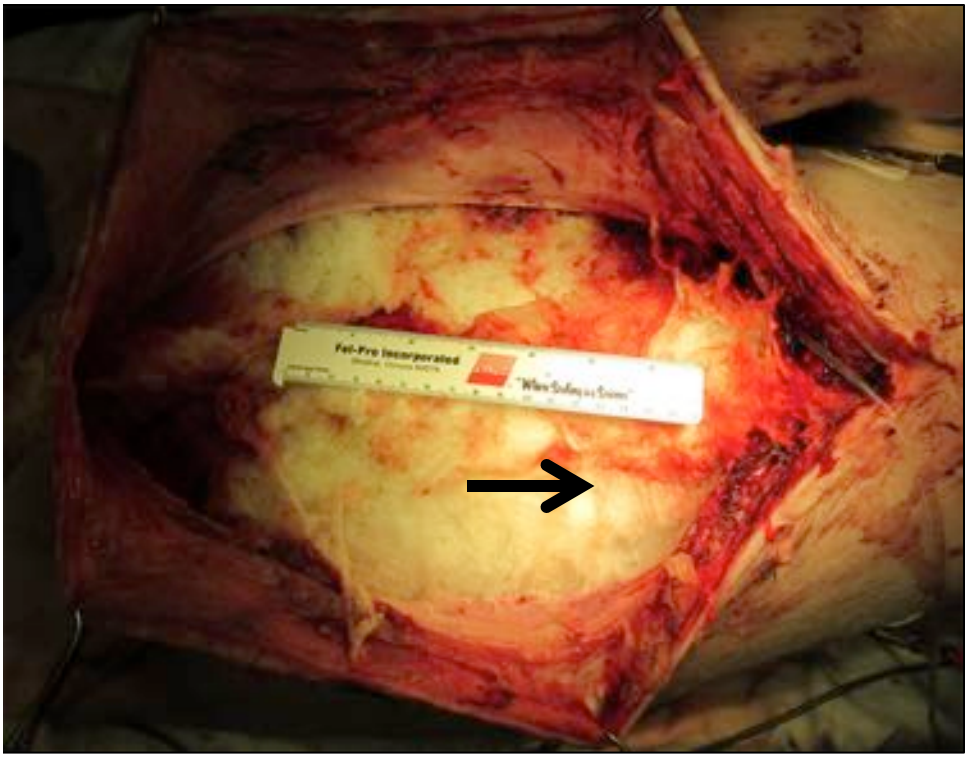


Figure 2, swine 293. Overhead view of re-opened abdomen immediately after Fig. 1B; subject still alive. Black arrow = cephalad. Foam is overlying the intestines. Small amount blood visible at edges of foam upon re-opening incision; small amount of gas escaped when incision re-opened.

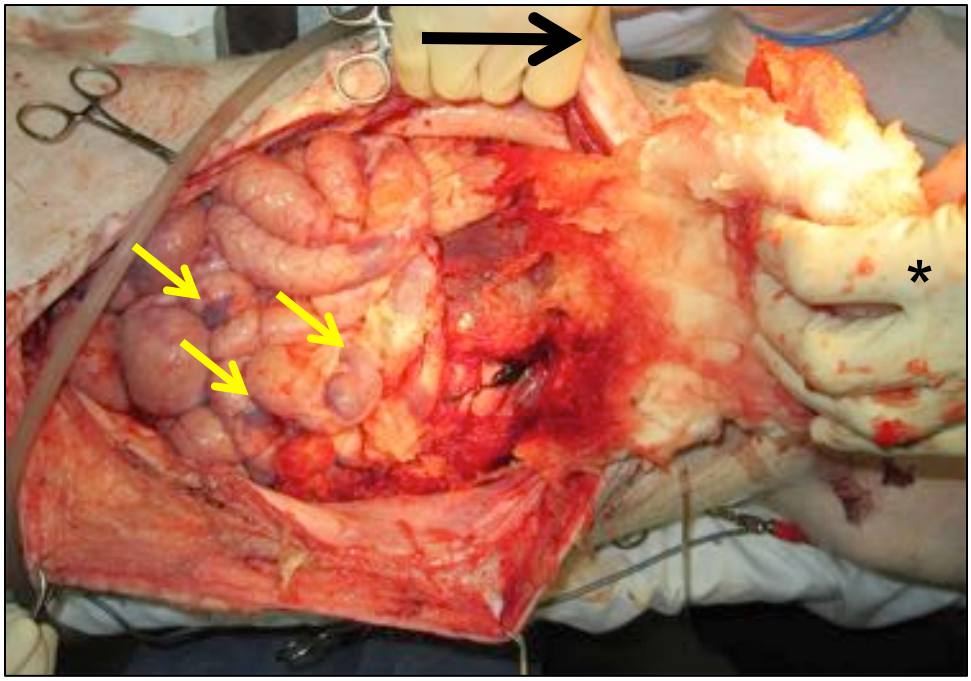


Figure 3, swine 293. Foam is being peeled out of abdomen by hand (asterisk). Black arrow = cephalad. Injury site is still covered by foam + blood. Note areas of intestinal discoloration on intestines (yellow arrows).

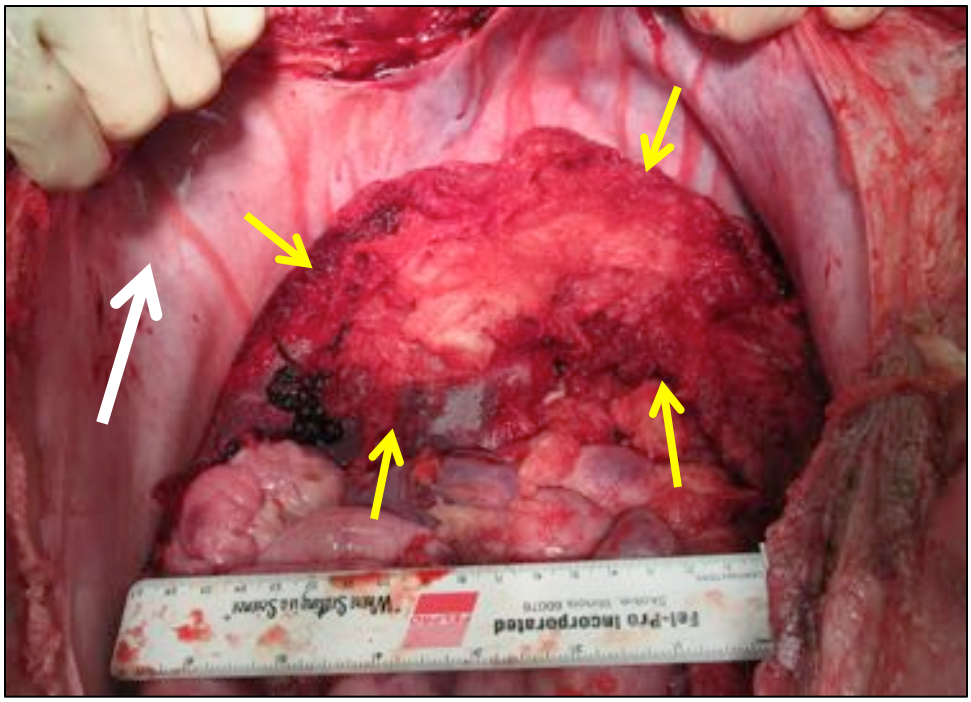


Figure 4, swine 293. Most of foam has been peeled out of abdomen, leaving a foam mass covering injury (yellow arrows). It appeared that this mass was adherent to the injury site, but the foam mass fell off during the subsequent liver explantation, so the mass was not adherent at all. White arrow = cephalad.

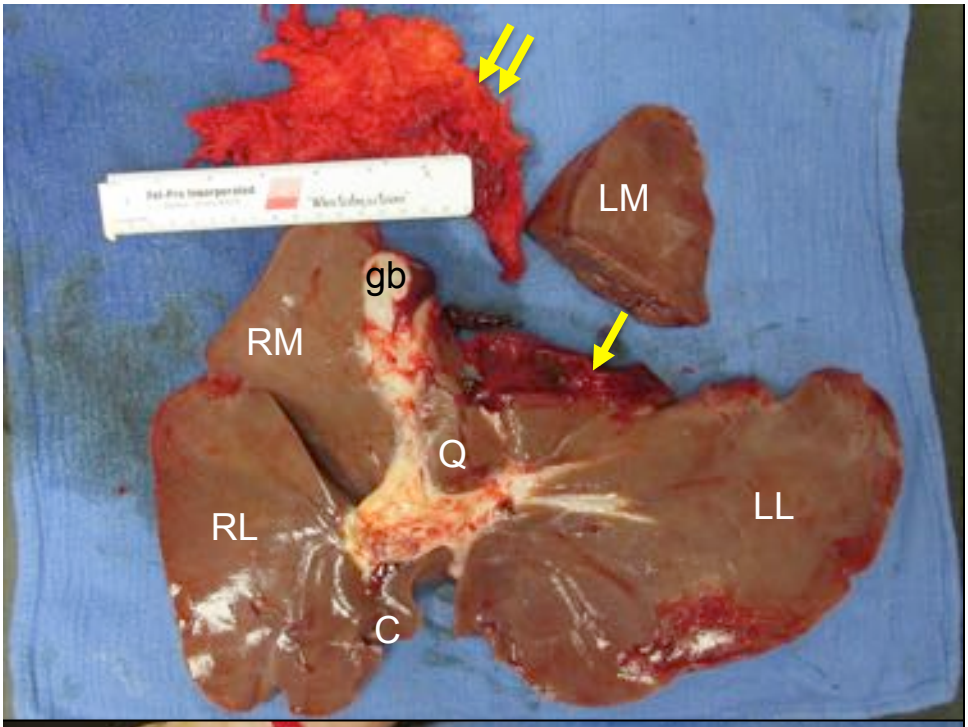


Figure 5, swine 293. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Single arrow = resection/ injury site. Double arrow = foam mass from Fig. 4 that was adjacent to but not adherent to injury site. Anterior is at top of image.

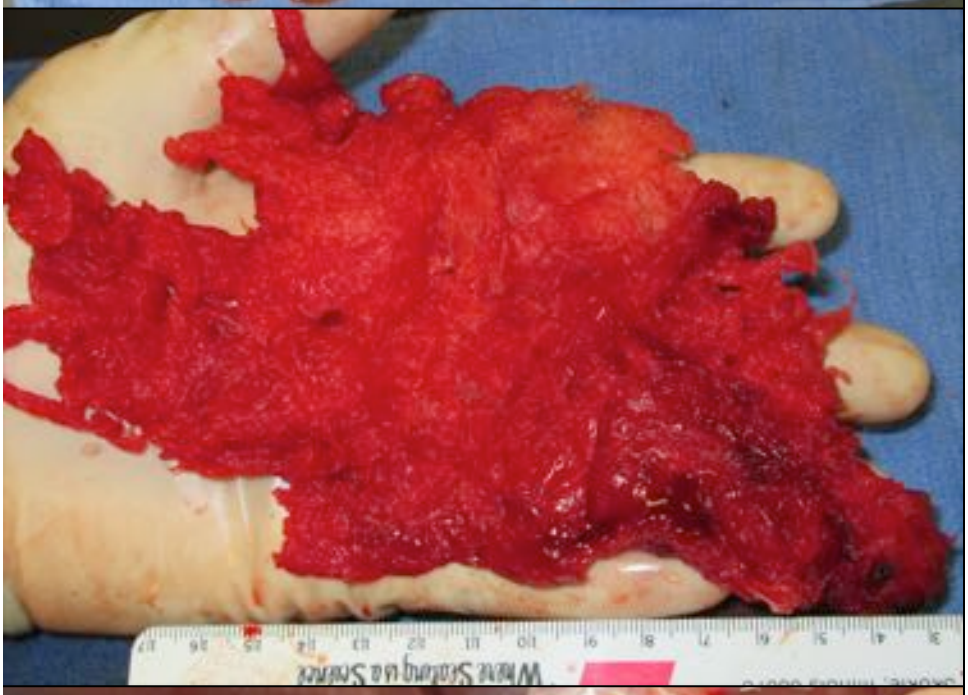


Figure 6, swine 293. Close-up of foam mass from Fig. 5.

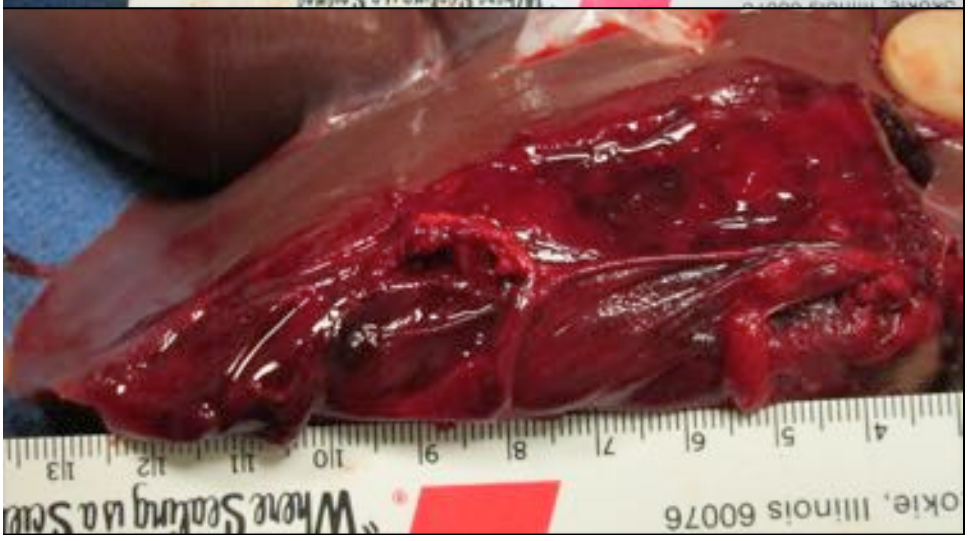


Figure 7, swine 293. Close-up of injury site from Fig. 5. Entire site was covered with clot.

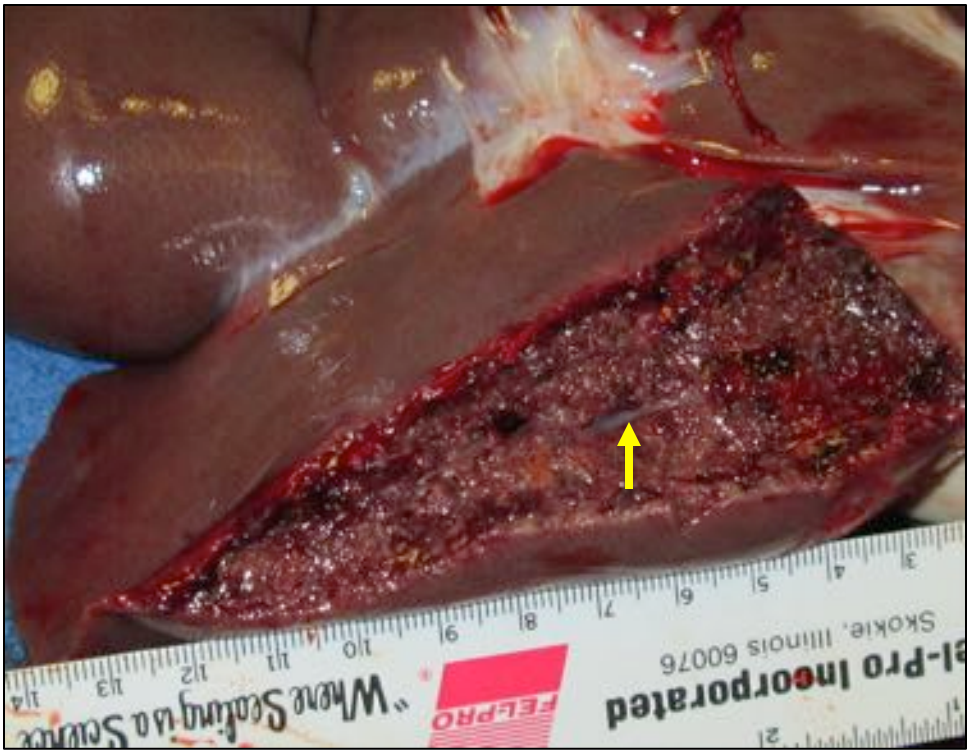


Figure 8, swine 293. Similar view as in Fig. 7, but clot over injury site has been wiped away. Orifice of the hepatic vein (collapsed) to the LM lobe indicated with yellow arrow.

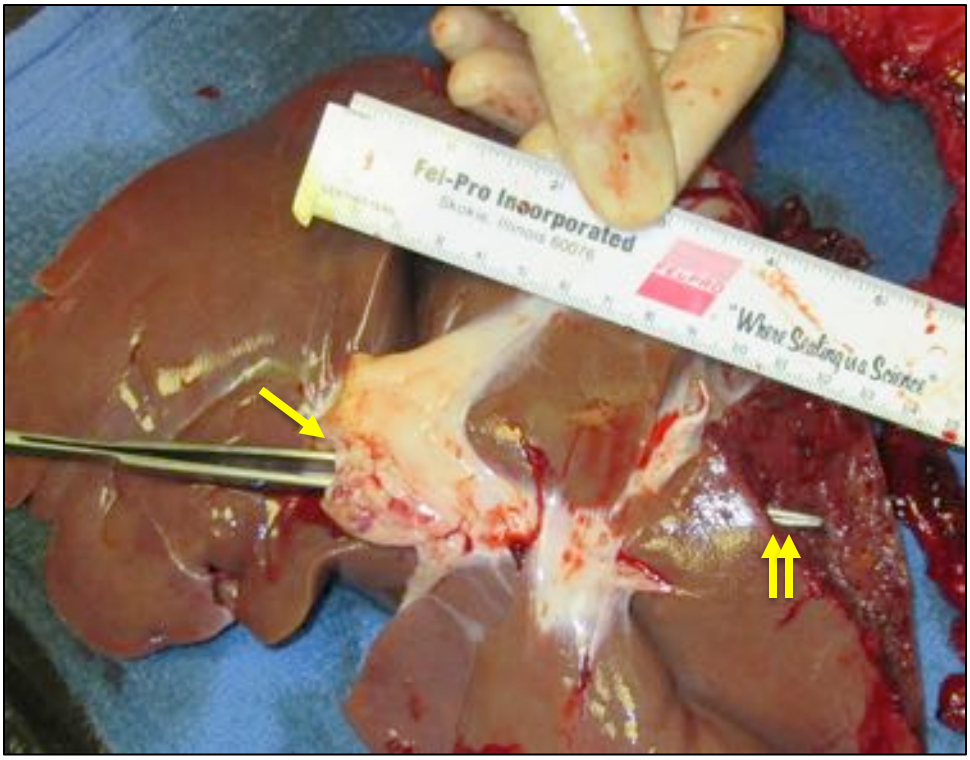


Figure 9, swine 293. Inferior view of liver. Forceps has been inserted into main portal vein at the port hepatis (single yellow arrow) and tips emerge out the orifice of transected PV branch to LM lobe.

I. OVERVIEW
Date: July 29, 2015
Swine no: 299
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate foam and Factors II/XIII
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz

II. PRE-INJURY PHASE

Start time: 07:45 AM
Swine sex: Male
Date swine received from UNL Mead: 07/24/2015
Pre-procedure wt: 36.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 101
- MAP:126
- Temp: 38.7
- EtCO2: 39

Blood draw no. 1 (initial): 7:55 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:08 AM
Spleen wt: 319.6 gm
LR (22°C) infused after splenectomy: 1000 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 319.6 + 30 + 0.3(sponges) = 319.9 mL
- LR (22°C) infused: 1,000 (spleen replacement + incidental) + 150 (ear vein/jugular drip) = 1,150 mL

Pre-injury VS

- HR: 101
- MAP: 114

- Temp: 37.9
- EtCO2 : 42
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:35 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through a separate stab incision in the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the mid abdomen.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 160 mL/min 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: Factors II & XIII; **no** Fibrinogen.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. ***Note****: due to pump malfunction, very little (if any) calcium was injected with the alginate during this procedure. That is, only alginate + Factors II and XIII made into the abdomen. The abdomen became tense very rapidly, much more rapidly than in previous cases, and I stopped injection after perhaps 10-15 sec. IAP upon completion of injection was in the upper 20's. I am pretty sure that most of the abdominal distension was caused by the butane carrier gas, and not the alginate. There was a strong smell of butane gas, much heavier than we have noted in the past.*

Total mass injected: 569.0g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 95

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:36AM (within 1 minute of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:45 AM

10 min post-injury VS

- HR: 87
- MAP: 76
- Temp: 37.6
- EtCO2: 33
- IAP: 10

Blood draw no. 3: (30 min post-injury): 9:05 AM

30 min post-injury VS
- HR: 88
- MAP: 79
- Temp: 36.7
- EtCO2: 33
- IAP: -1

Blood draw no. 4: (60 min post-injury): 9:35AM

60 min VS
- HR: 83
- MAP: 91
- Temp: 35.7
- EtCO2: 33
- IAP: -2

Blood draw no. 5: (90 min post-injury): 10:05AM

90 min post-injury VS
- HR: 82
- MAP: 92
- Temp: 35.0
- EtCO2: 32
- IAP: -3

Blood draw no. 6: (120 min post-injury): 10:35AM

120 min post-injury VS
- HR: 81
- MAP: 114
- Temp: 34.6
- EtCO2: 30
- IAP: -3

Blood draw no. 7: (150 min post-injury): 11:05AM

150 min post-injury VS
- HR: 78
- MAP: 114
- Temp: 34.2
- EtCO2: 29
- IAP: -2

Blood draw no. 8: (180 min post-injury): 11:35AM

180 min post-injury VS
- HR: 80

- MAP: 159
- Temp: 33.8
- EtCO2: 28
- IAP: -3

Survival at 180 min? Yes
Target MAP attained ? Yes, **75-90 min duration (longest such duration we have seen with this model)**
Time of death: 11:42 AM
Cause of death: Exsanguination from intentional transection of supradiaphragmatic IVC (euthanasia)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 526.1(clot + lap pads+ foam residue) + 210 mL (phlebotomies) = 736.1 mL
- IV fluid given: LR (37°C): 1025mL (infused) + 100mL (ear vein) + 100mL (jugular drip) = 1,225 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen scaphoid, IAP = 0. Very little blood. That which was present was a viscous/nonclotted material, apparently blood mixed with the alginate. This could not be suctioned 2˚ its viscosity, so it was removed with lap pads. There was a small pocket of white foam in the infrahepatic region, not associated with the wound (see Figs). A small amount of clot was removed (see Figs). Injury site was covered with a thinnish (several mm) layer of clot; no active hemorrhage. *Overall, this was the least amount of hemorrhage I have seen with this model*. No surface changes noted to any internal organs.
Volume foam recovered: scant (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 2 branches, to LM lobe
Other: none
Ex vivo liver wt: 95.3g (resected LM lobe) + 890.7 (remaining liver) = 985.7 g
Tissue harvested: Liver for Instron experiments.

VI. COMMENTS

Interesting day. Noncompressible injury treated with alginate foam and FII/FXIII, unintentionally injected without the 1 *M* supplemental $CaCL_2$. Probably the best hemostatic result we have ever seen with this model. Subject only lost 0.5 L blood (some of this was alginate mixed in with the blood), and was above the target MAP for the latter half of the 3 h observation period. That's never happened before. Not sure if this was just a fluke "N of one" finding, or if there was really something going on here. I would like to repeat this exact same sequence this Friday. Perhaps the supplemental 1 *M* $CaCl_2$ has been detrimental to subject outcome.

VII. PLAN

Repeat this treatment (alginate + Factor II/Factor XIII, without the 1 *M* calcium for the alginate) on Fri, July 31st, 2015.

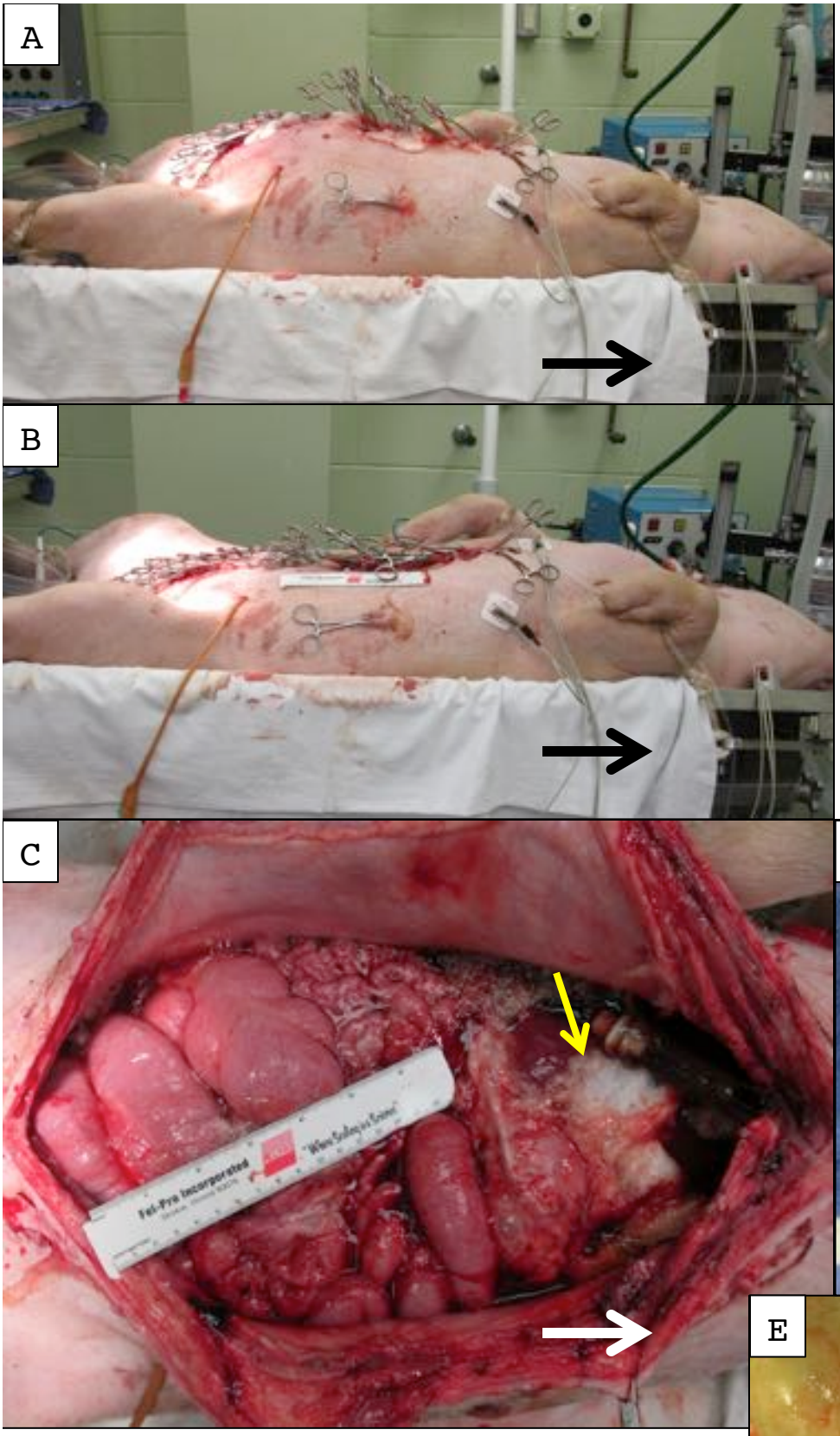


Figure 1, swine 299.
(A) Lateral view of swine at 3 min after injection completed; IAP ~25 mm Hg with distension and subcut emphysema.
(B) At end of 3 h observation period; IAP = 0, abdomen back to scaphoid. Subject stable with HR = 80 BPM, O2 Sat = 92%, EtCO2 = 27mm Hg, MAP >100 mm Hg, T = 33.8˚C. Black arrow = cephalad.
(C) Abdomen reopened at 3 h; subject alive & well. Only trace amount of foam visible (yellow arrow). Very little free blood and clot visible.
(D) Total amount of free blood removed, all on lap pads (2 L size bucket).
(E) Total amount of clots removed, in palm of hand.

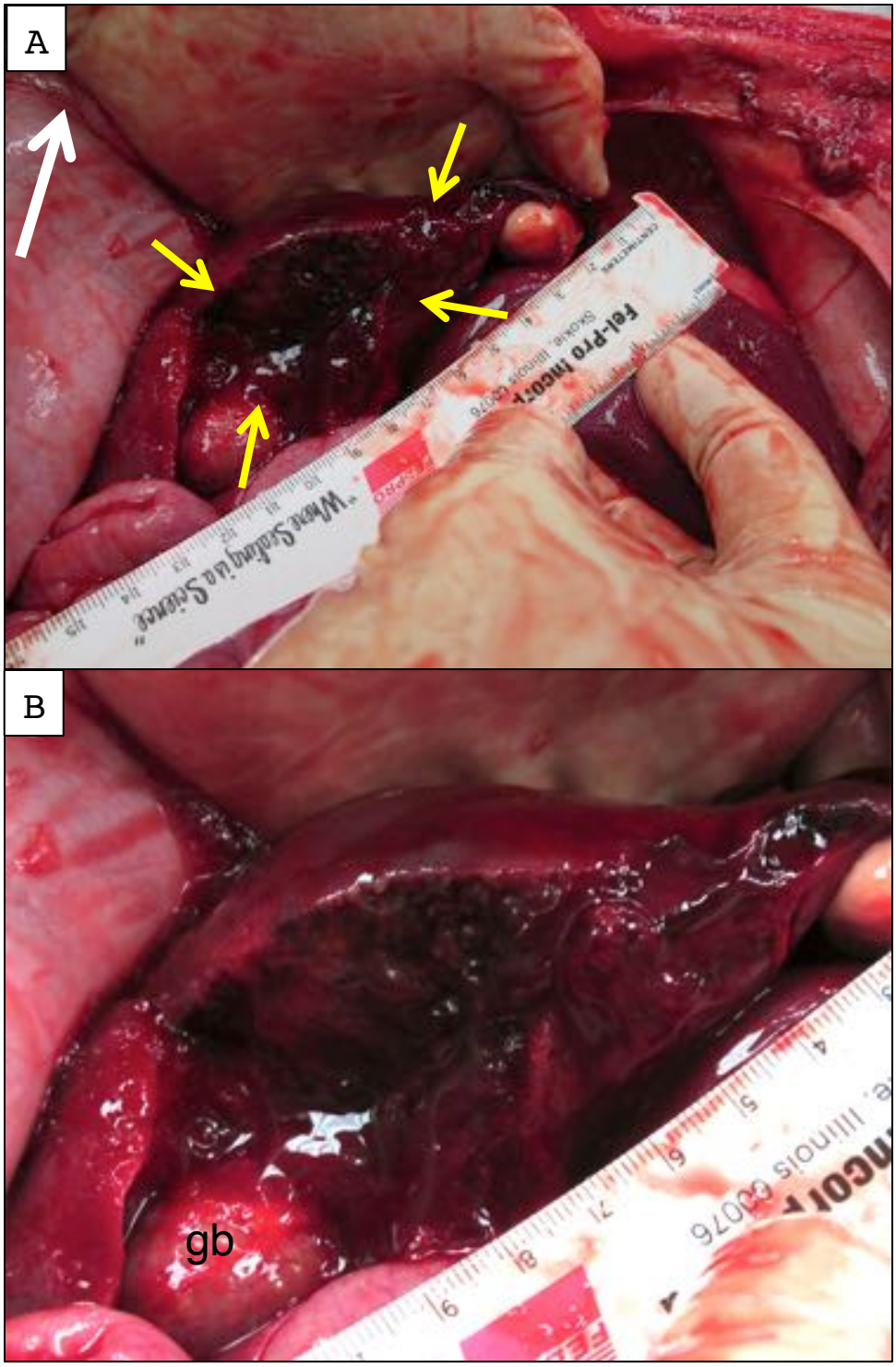
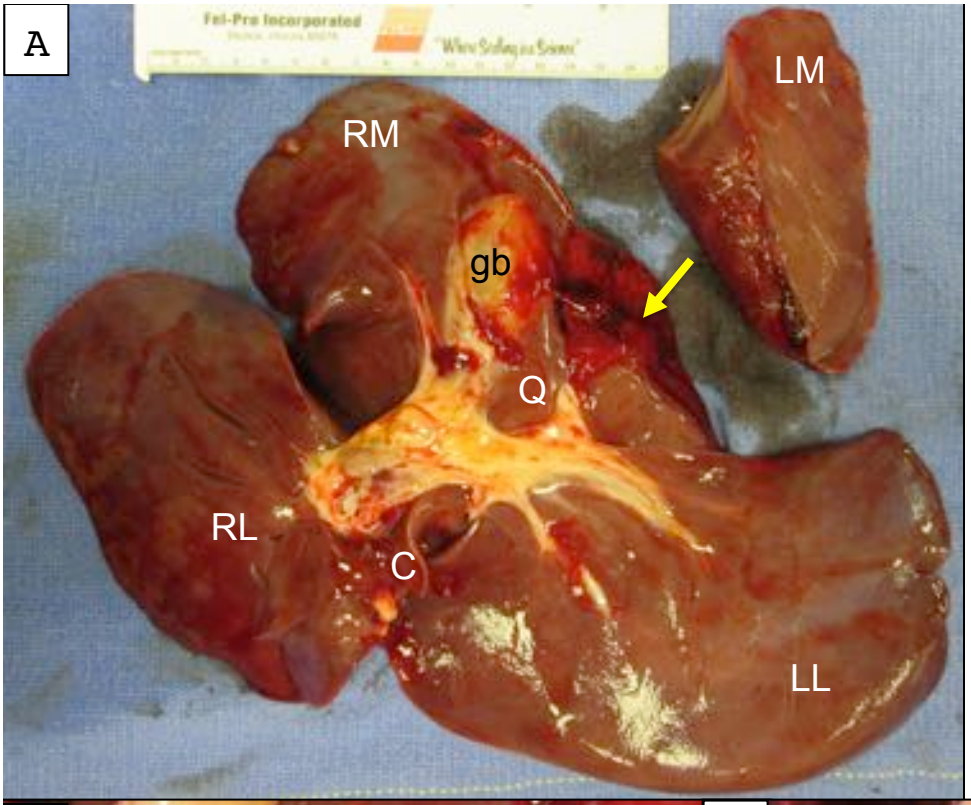
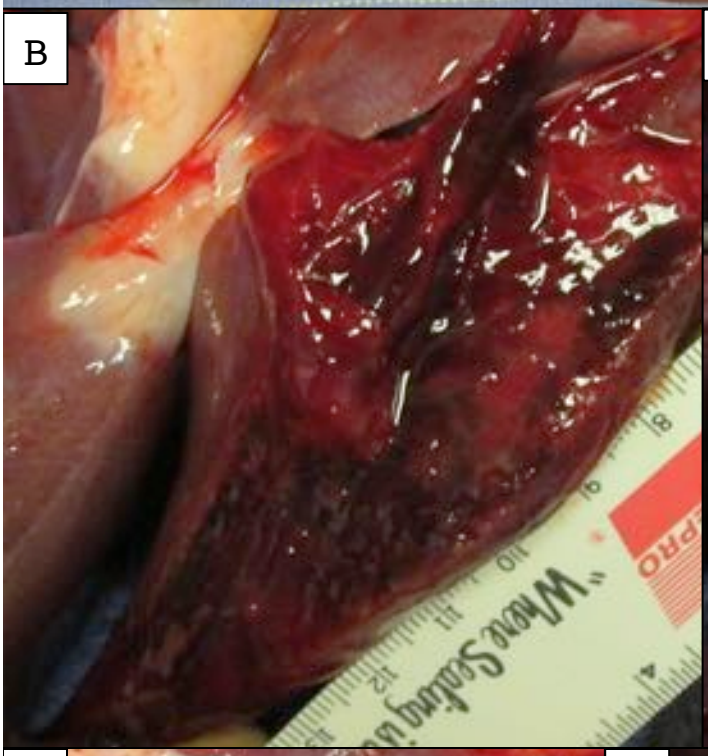
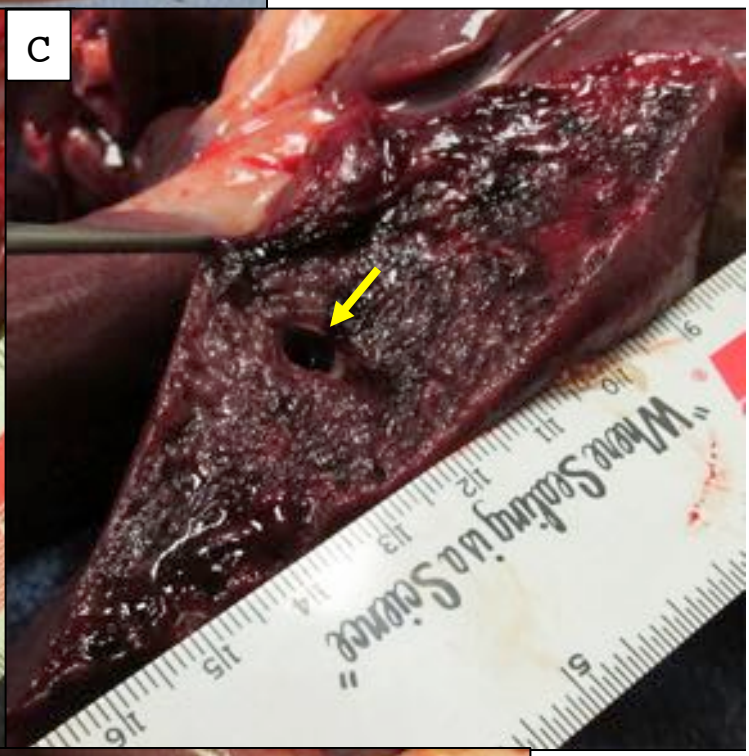
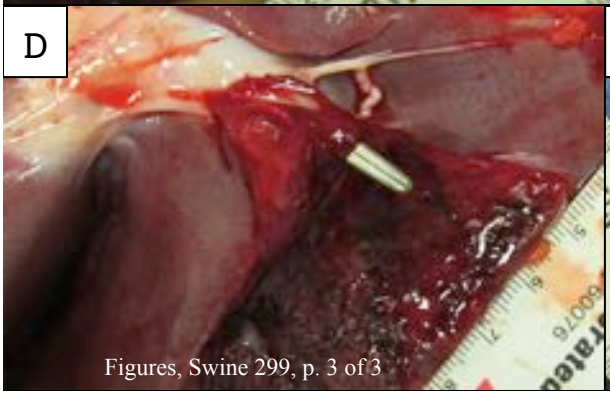


Figure 2, swine 299. Evaluation at 3 h after injury; subject alive and well.
(A) Injury site at base of LM lobe (yellow arrows). No heavy clot present, but injury site was sealed with a thin layer of clot with thick viscous ("gooey") liquid. No active hemorrhage. Cephalad = white arrow.
(B) Similar view of panel A, with close up; gb = gallbladder.









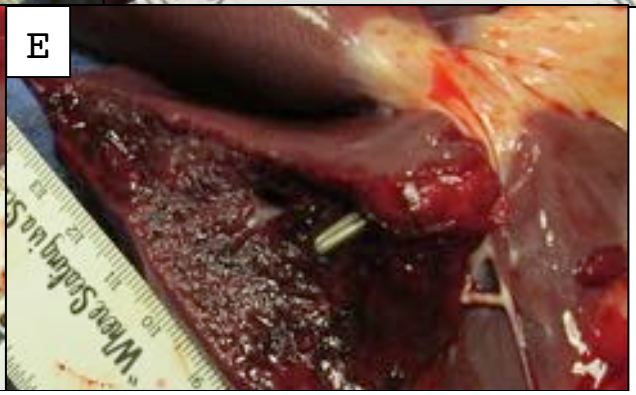


Figure 3, swine 299. Liver *ex vivo*, (A) Inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection/injury site. Anterior is at top of image. (B) Close-up of viscous clot covering injury site. (C) Similar to panel B, but clot has been wiped away. Arrow = hepatic vein orifice. (D & E) Demonstration of two separate transected portal veins that had been supplying the LM lobe.

I. OVERVIEW
Date: July 31, 2015
Swine no: 300
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam and Factors II/XIII
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz

II. PRE-INJURY PHASE

Start time: 07:53 AM
Swine sex: Male
Date swine received from UNL Mead: 07/24/2015
Pre-procedure wt: 33.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor (nonfunctional on subject #300)
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 81
- MAP:114
- Temp: 38.9
- EtCO2: Monitor malfunctioned for this parameter

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:20 AM
Spleen wt: 276.5 gm
LR (22°C) infused after splenectomy: 900 mL at 50 mL/min (MAP dropped when infusion started, changed flow rate from 150mL/min to 50mL/min)

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 276.5 + 30 + 67.1 (sponges) = 373.6 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 50(ear vein/jugular drip)= 950.0 mL

Pre-injury VS
- HR: 94

- MAP: 99
- Temp: 37.9
- EtCO2 : N/A
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:48 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; **no** $CaCl_2$.

Clotting factors: Factors II & XIII; **no** Fibrinogen.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 186.0g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 9.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:50AM (within 2 minutes of injury)

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 9:00 AM

10 min post-injury VS

- HR: 93
- MAP: 53
- Temp: 37.5
- EtCO2:
- IAP: 8

Blood draw no. 3: (30 min post-injury): 9:20 AM

30 min post-injury VS

- HR: 91
- MAP: 69
- Temp: 37.0

- EtCO2: N/A
- IAP: 2

Blood draw no. 4: (60 min post-injury): 9:50AM

60 min VS
- HR: 88
- MAP: 65
- Temp: 35.4
- EtCO2: N/A
- IAP: 1

Blood draw no. 5: (90 min post-injury): 10:20AM

90 min post-injury VS
- HR: 84
- MAP: 67
- Temp: 35.1
- EtCO2: N/A
- IAP: 0

Blood draw no. 6: (120 min post-injury): 10:50AM

120 min post-injury VS
- HR: 80
- MAP: 74
- Temp: 34.6
- EtCO2: N/A
- IAP: 0

Blood draw no. 7: (150 min post-injury): 11:20AM

150 min post-injury VS
- HR: 76
- MAP: 68
- Temp: 27.4
- EtCO2: N/A
- IAP: 0

Blood draw no. 8: (180 min post-injury): 11:50AM

180 min post-injury VS
- HR: 75
- MAP: 72
- Temp: 33.6
- EtCO2: N/A
- IAP: 0

Survival at 180 min? Yes

Target MAP attained ? No
Time of death: 11:50 AM
Cause of death: Exsanguination from transection of supradiaphragmatic IVC (euthanasia)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 226.1 mL (suction) + 300.2mL (lap pads + foam residue) + 60.6mL (clots)+ 210 mL (phlebotomies) = 796.9 mL
- IV fluid given: LR (37°C): 1750 mL (infused) + 25 mL (ear vein) + 25 mL (jugular drip) = 1,800 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen scaphoid (see Figs) with IAP zero. Small patch of foam, soft without structural integrity (see Figs). Small amount of blood mixed in with alginate; some of this could be suctioned, the rest had to be mopped out with lap pads. Thick mass of clot at injury site (Figs); no active hemorrhage. Small amount (60 mL) free clot. No surface changes noted to any internal organs.
Volume foam recovered: scant (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1 large, to LM lobe.
Portal vein injury: 2 branches, to LM lobe
Other: none
Ex vivo liver wt: 101.1g (resected LM lobe) + 917.1 (remaining liver) = 1018.2 g
Tissue harvested: Liver for Instron experiments.

VI. COMMENTS

Noncompressible injury treated with alginate foam alone (no 1 *M* $CaCl_2$) and FII/FXIII, N = 2 of this group. Again, easy survival with less blood loss (<500 mL, if the phlebotomies are not counted and some of the loss in the suction bucket and on the lap sponges is attributed to the alginate) than we typically see. Although this subject's MAP did not recover to above target like subject #299, this still was an impressive result. Note that in this subject and in the previous (#299) subject, injection of the alginate still produced an immediate increase in IAP (mid-20's) even though there was no stiff foam inside the abdomen. This is presumably secondary to the butane gas, which leaked out over the 3 h observation period (by 30-45 min, the IAP had gone back to zero). So it seems that this short period of increased IAP may have been enough time for the tamponade effect to do its trick. Of course we still will need 5-6 more repeats to generate statistics before being comfortable that this is a real result. If it is, then the other group we will need to generate is the alginate minus calcium without any clotting factors.

A mid-term planning question is whether to complete the set of four groups that we had been working on up until this week (1 = no treatment; 2 = calcium alginate foam alone; 3 = calcium alginate foam + rFII + rFXIII; 4 = calcium alginate foam + pdFI-FS) and try and get that published, and then publish another paper where we study alginate minus calcium ± clotting factors. I am torn between the two options, because on one hand we desperately need to publish in order to increase chances at continuation funding, but on the other hand it would be nicer to have a more complete story on the alginate before we publish. For now we'll accrue data on the first four groups, according to the schedule I emailed out earlier this month.

VII. PLAN

Next subjects will be on Tue and Fri of this coming week, Aug 4th and 7th, using calcium alginate foam + FII/FXIII and calcium alginate foam + FS, respectively.

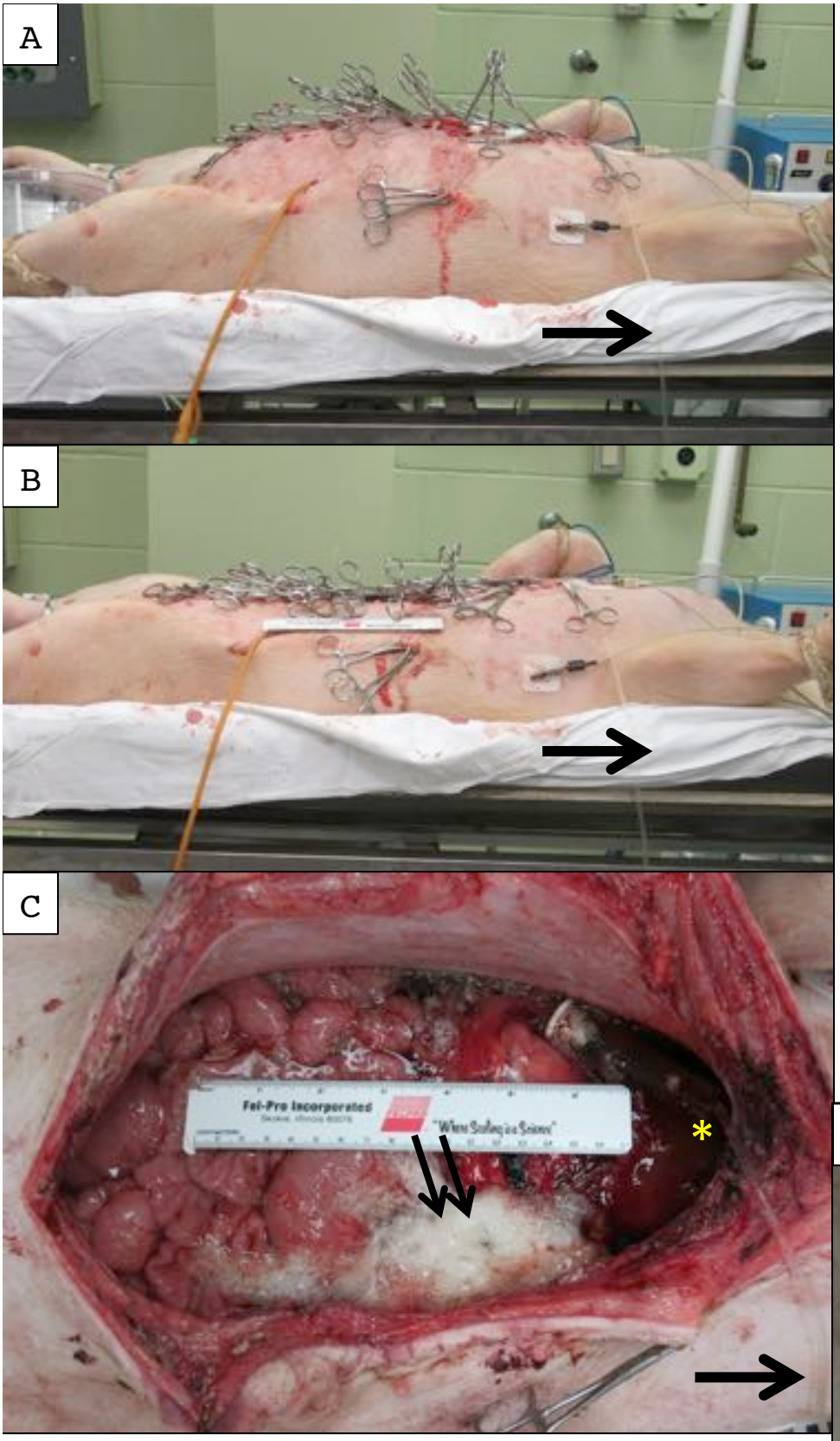


Figure 1, swine 300.
(A) Lateral view of swine at 3 min after injection completed; IAP ~25 mm Hg with distension and subcut emphysema.
(B) At end of 3 h observation period; IAP = 0, abdomen back to scaphoid. Subject stable with HR = 76 BPM, O2 Sat = 94%, EtCO2 = N/A, MAP = 72 mm Hg, T = N/A. Black arrow = cephalad.
(C) Abdomen reopened at 3 h; subject alive & well. Only trace amount of foam visible (double arrow). Very little free blood and clot visible. Asterisk = injury site.
(D) Total amount of foam removed from abdomen, held in hand.

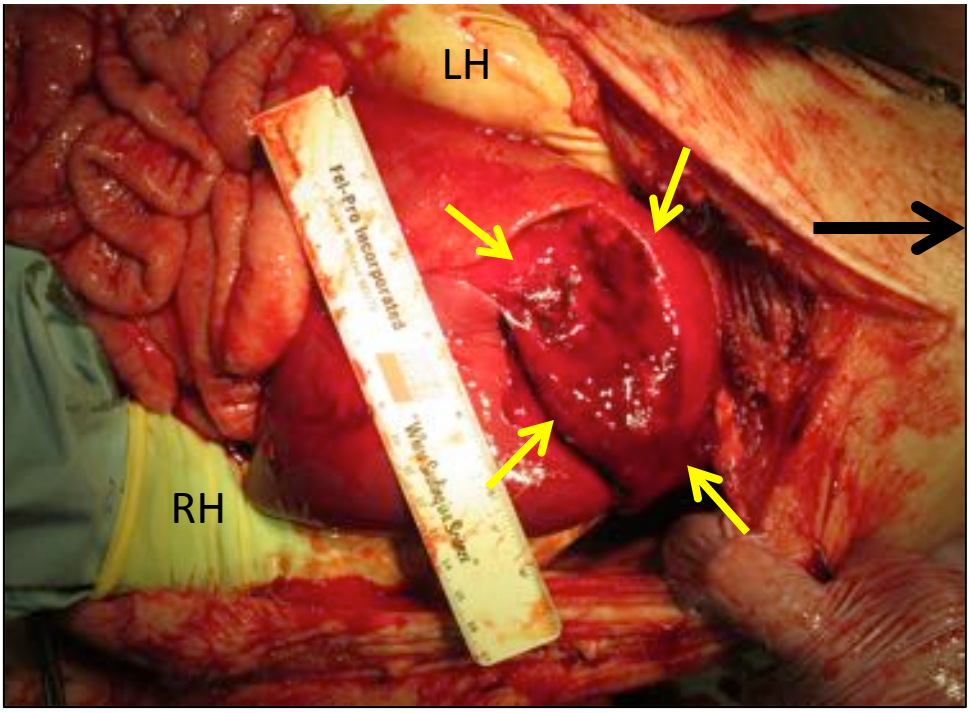


Figure 2, swine 300. Injury site at 3 h after injury; subject alive and well. Liver *in situ* is being elevated out of abdomen with surgeon's right & left hands (RH & LH). Injury site at base of LM lobe (yellow arrows) covered with heavy clot. No active hemorrhage. Cephalad = black arrow.

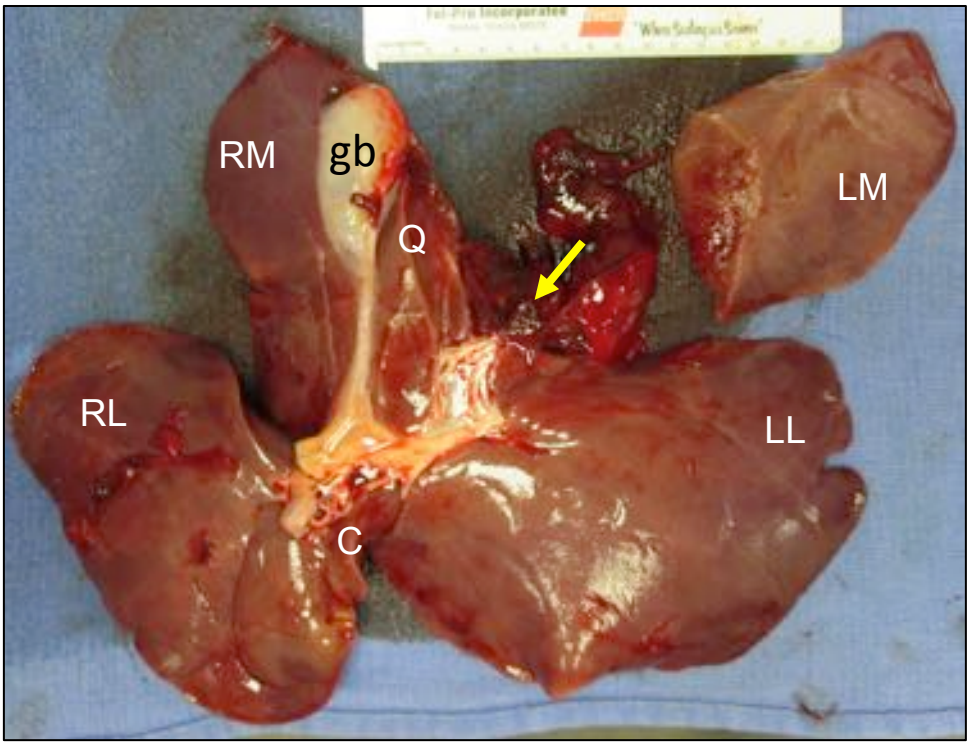


Figure 3, swine 300. Liver *ex vivo*, (A) Inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrow = resection/injury site. Anterior is at top of image.

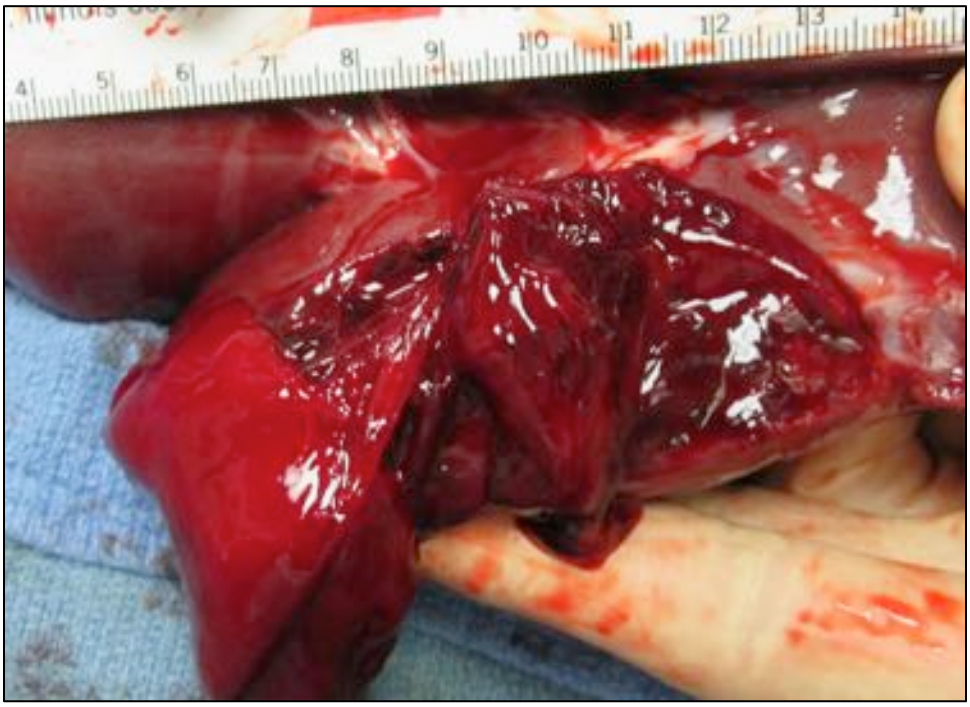


Figure 4, swine 300. Close-up of clot covering injury site on base of LM lobe.

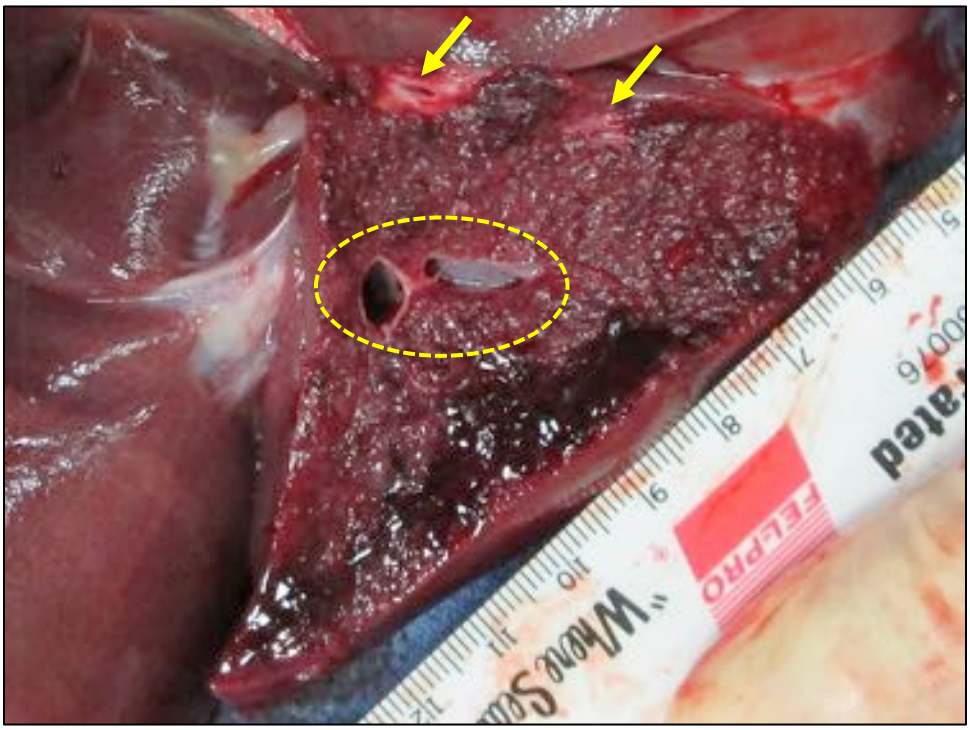


Figure 5, swine 300. Similar to Fig. 4, but clot has been wiped away. Arrows = transected branches of portal vein. Dashed yellow circle = transected hepatic vein (appears as two, but single large vein cut right as it forked into two).

I. OVERVIEW
Date: August 04, 2015
Swine no: 301
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate foam (no $CaCl_2$) and Factors II/XIII
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz, Johnson

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II. PRE-INJURY PHASE

Start time: 07:50 AM
Swine sex: Male
Date swine received from UNL Mead: 07/24/2015
Pre-procedure wt: 35.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor (stand alone device, not interfaced to Bionet monitor)
2. EKG clips
3. Right carotid artery angiocath (20g), cutdown; for BP monitor
4. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
5. Transabdominal cystotomy for 16 Fr Foley catheter
6. Rectal temp probe
7. Pulse oximetry
8. Heating pad below subject
9. Intraabdominal pressure monitor

Initial VS
- HR: 97
- MAP:104
- Temp: 38.6
- EtCO2: 36

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:15 AM
Spleen wt: 295.4 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 295.4 + 30 + 9.9 (sponges) = 335.3 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 100 (jugular drip)= 1,000.0 mL

Pre-injury VS
- HR: 77
- MAP: 115
- Temp: 38.2

- EtCO2 : 43
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:30 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; **no** $CaCl_2$. We were set up to inject $CaCl_2$ today, but the pump malfunctioned, so only the alginate, clotting factors, and butane were delivered.

Clotting factors: Factors II & XIII; **no** Fibrinogen.

Technique: with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen.

Total mass injected: 306.8g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 95

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 9.9 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:31AM (within 90 s of injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:40 AM

10 min post-injury VS

- HR: 155
- MAP: 50
- Temp: 37.9
- EtCO2: 42
- IAP: 17

Blood draw no. 3: (30 min post-injury): 9:00 AM

30 min post-injury VS

- HR: 130
- MAP: 40
- Temp: 37.7

- EtCO2: 37
- IAP: 7

Blood draw no. 4: (60 min post-injury): 9:30AM

60 min VS
- HR: 134
- MAP: 42
- Temp: 37.3
- EtCO2: 36
- IAP: 6

Blood draw no. 5: (90 min post-injury): 10:00AM

90 min post-injury VS
- HR: 120
- MAP: 53
- Temp: 36.9
- EtCO2: 42
- IAP: 5

Blood draw no. 6: (120 min post-injury): 10:30AM

120 min post-injury VS
- HR: 115
- MAP: 54
- Temp: 36.4
- EtCO2: 39
- IAP: 4

Blood draw no. 7: (150 min post-injury): 11:00AM

150 min post-injury VS
- HR: 107
- MAP: 58
- Temp: 35.9
- EtCO2: 37
- IAP: 5

Blood draw no. 8: (180 min post-injury): 11:30AM

180 min post-injury VS
- HR: 104
- MAP: 55
- Temp: 35.6
- EtCO2: 41
- IAP: 5

Survival at 180 min? Yes

Target MAP attained ? No
Time of death: 11:40 AM
Cause of death: Exsanguination from transection of supradiaphragmatic IVC (euthanasia)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 320.3 mL (suction) + 610.4 mL (lap pads+ foam residue) + 268.1mL (clots)+ 210 mL (phlebotomies) = 1,408.8 mL
- IV fluid given: LR (37°C): 1,800 mL (infused) + 0 mL (ear vein) + 0 mL (jugular drip) = 1,800 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen minimally distended (see Figs); not tense (IAP 4 mm Hg). Upon re-opening abdomen, small pocket of butane still present, which had been keeping the IAP at 4. Small amounts of unclotted blood mixed with alginate ("jelly"), along with a few clots were seen (see Figs). No surface changes to any of the internal organs. Injury site was covered with a large mass of clot and jelly-like substance (see Figs).
Volume foam recovered: minimal, not measured (see Figs)
Heart: not examined
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 branch, to LM lobe
Other: none
Ex vivo liver wt: 81.0g (resected LM lobe) + 879.4 (remaining liver) = 960.4 g
Tissue harvested: liver for Instron experiments, liver biopsy from injury site for histology

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VI. COMMENTS

Noncompressible injury treated with alginate but no $CaCl_2$ (syringe pump malfunctioned today; we had planned to use 1 M $CaCl_2$), combined with FII/FXIII but no fibrinogen (N = 3 for this Rx). Survived 180 minutes, with ~1 L blood loss that can be attributed to blood loss from the injury. Alginate + clotting factors seem to be hemostatically efficacious without the high calcium concentration.

VII. PLAN

Continue as planned on Fri Aug 7 with noncompress injury, treat with calcium alginate + pdFI-FS.

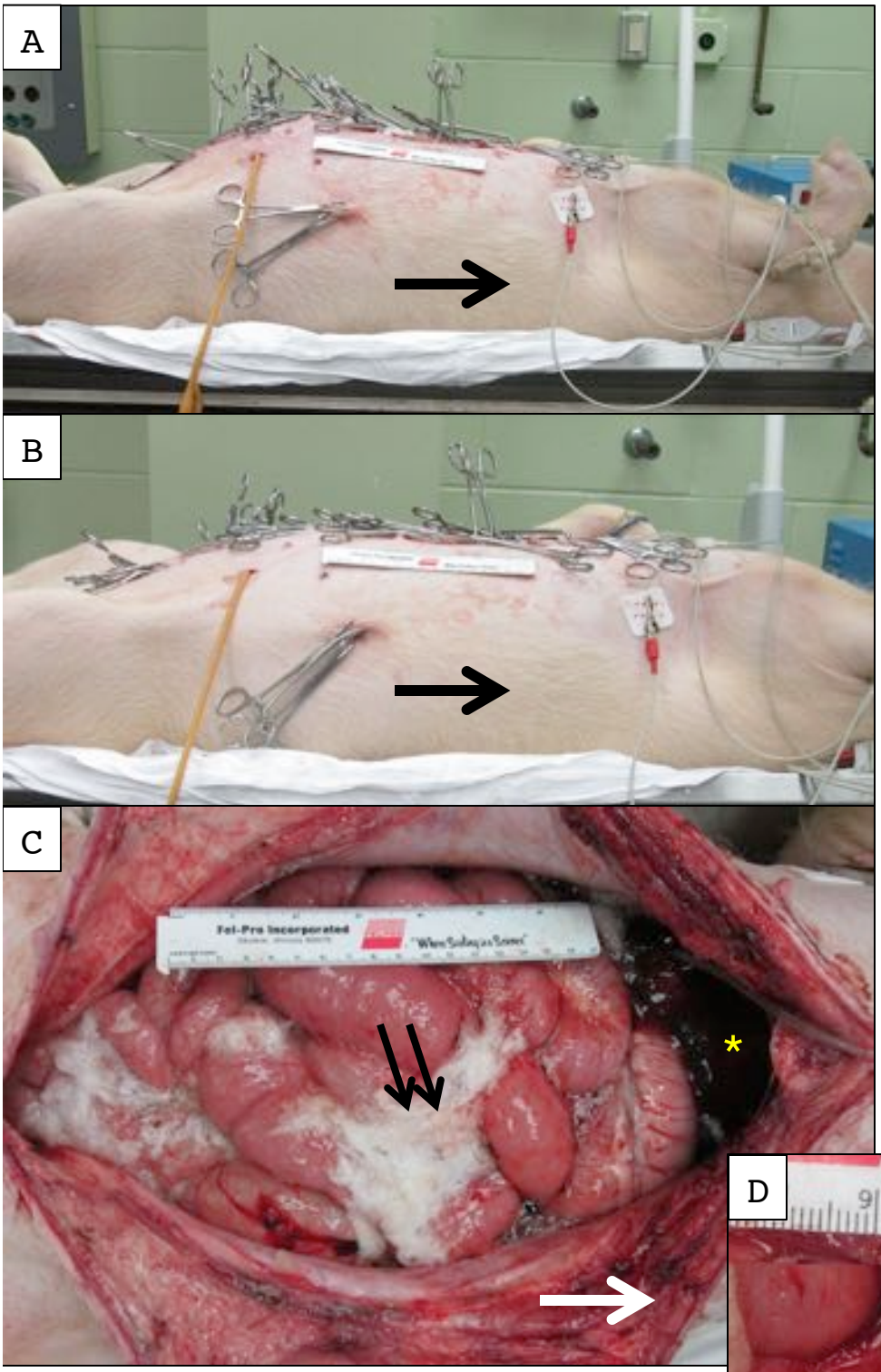

Figure 1, swine 301.
(A) Lateral view of swine at 3 min after injection completed; IAP ~25 mm Hg with distension and subcut emphysema.
(B) At end of 3 h observation period; IAP = 4 mm Hg, abdomen less distended. Subject stable with HR = 104 BPM, O2 Sat = 99%, EtCO2 = 41 mm Hg, MAP = 55 mm Hg, T = 35.6˚C. Black arrow = cephalad.
(C) Abdomen reopened at 3 h; subject alive, MAP in 50's. Only trace amount of foam visible (double arrow). Very little free blood and clot visible. Asterisk = injury site.
(D) Close-up of jelly-like mixture of blood and alginate in left paracolic gutter.

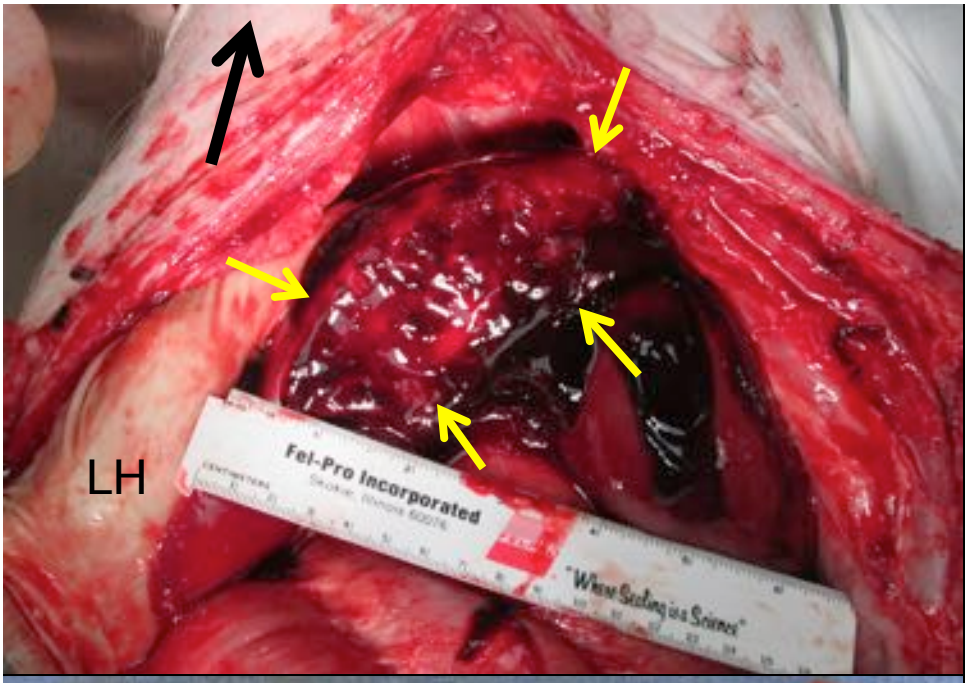

Figure 2, swine 301. Injury site at 3 h after injury; subject still alive. Liver *in situ* is being elevated out of abdomen with surgeon's right hand (RH). Injury site at base of LM lobe (yellow arrows) covered with clot and jelly-like substance. No active hemorrhage. Cephalad = black arrow.

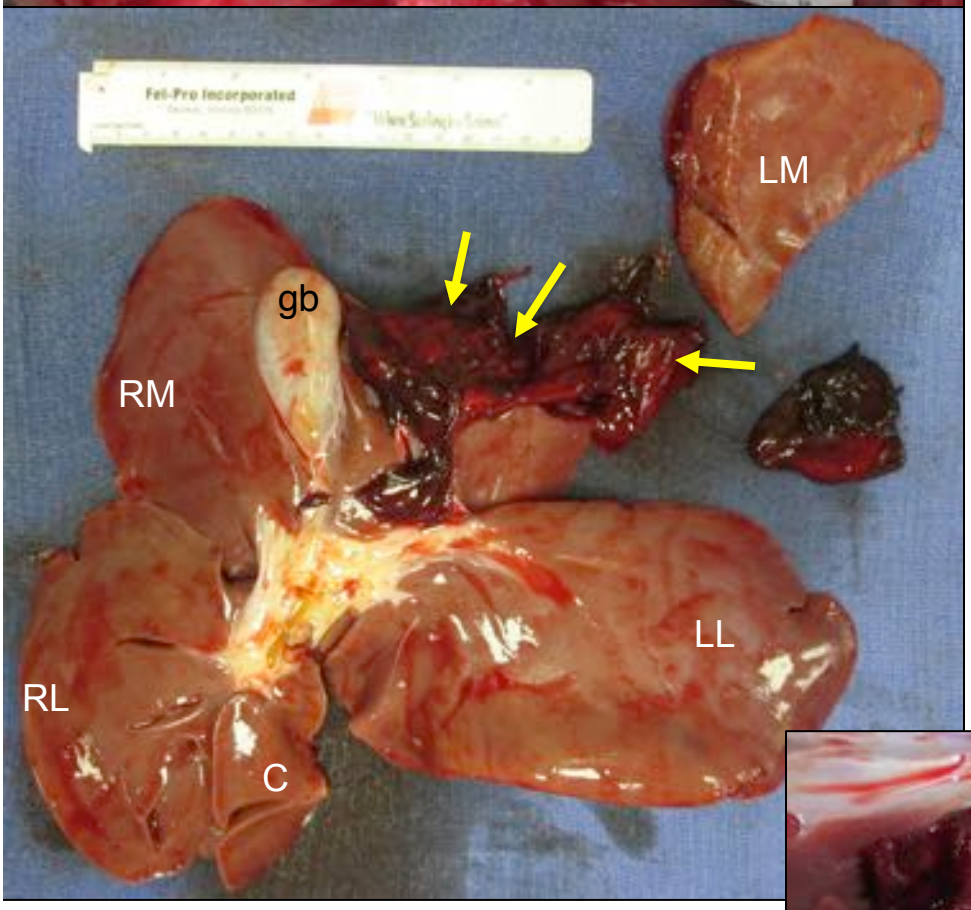

Figure 3, swine 301. Liver *ex vivo*, Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, with large amount of clot. Anterior is at top of image.

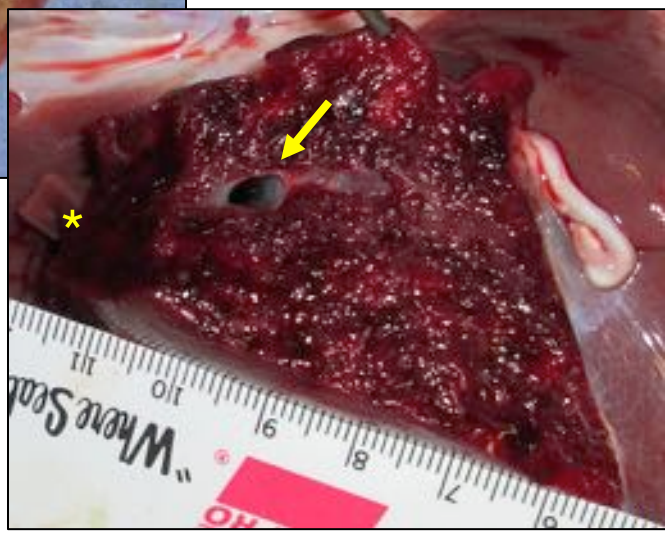

Figure 4, swine 301. Close-up of injury site on base of LM lobe, after clot/debris wiped away. Arrow = transected hepatic vein. Asterisk = site of specimen harvest from tissue/clot interface, for histology.

I. OVERVIEW
Date: August 07, 2015
Swine no: 302
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate Foam + FS
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz, Johnson

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II. PRE-INJURY PHASE

Start time: 07:45 AM
Swine sex: Male
Date swine received from UNL Mead: 07/24/2015
Pre-procedure wt: 42.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 119
- MAP:127
- Temp: 38.3
- EtCO2: 43

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:10 AM
Spleen wt: 287.9 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 287.9 + 30 + 0 = 317.9 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 50(ear vein/jugular drip)= 950.0 mL

Pre-injury VS
- HR: 124
- MAP: 116

- Temp: 37.7
- EtCO2 : 44
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:47 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; plus 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: FS (pdFI, rFII, rFXIII

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. Peak IAP recorded was 25 mm Hg.

Total mass injected: 463.9g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 11.8 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:51AM (~ 4' after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:55 AM

10 min post-injury VS

- HR: 122
- MAP: 47
- Temp: 37.7
- EtCO2: 39
- IAP: 5

Blood draw no. 3: (30 min post-injury): 9:20 AM

30 min post-injury VS

- HR: 117
- MAP: 64

- Temp: 37.3
- EtCO2: 47
- IAP: 2

Blood draw no. 4: (60 min post-injury): 9:45AM

60 min VS
- HR: 110
- MAP: 61
- Temp: 36.8
- EtCO2: 44
- IAP: 0

Blood draw no. 5: (90 min post-injury): 10:20AM

90 min post-injury VS
- HR: 102
- MAP: 64
- Temp: 36.3
- EtCO2: 45
- IAP: 0

Blood draw no. 6: (120 min post-injury): 10:50AM

120 min post-injury VS
- HR: 97
- MAP: 67
- Temp: 35.9
- EtCO2: 44
- IAP: 0

Blood draw no. 7: (150 min post-injury): 11:20AM

150 min post-injury VS
- HR: 102
- MAP: 43
- Temp: 35.5
- EtCO2: 42
- IAP: 0

Blood draw no. 8: (180 min post-injury): 11:50AM

180 min post-injury VS
- HR: 126
- MAP: 35
- Temp: 35.2
- EtCO2: 36
- IAP: 0

Survival at 180 min? Yes
Target MAP attained ? No
Time of death: 11:50 AM
Cause of death: Exsanguination
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 1,299.8 mL (suction) + 182.8 mL (sponge/lap) + 102.2 mL (clots) + 631.5mL (foam) + 210 mL (phlebotomies) = 2,426.3 mL
- IV fluid given: LR (37°C): 2,100mL (infused) + 25mL (ear vein) + 25mL (jugular drip) = 2,150mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen mildly distended but not tense (IAP ~ 0 mm Hg); see Figs. Upon re-opening abdomen, foam by itself in the superficial region, stained with blood underneath. Moderate amounts of unclotted blood and a few clots were seen (see Figs). Generalized discoloration of intestines (see Figs). Injury site was covered with a large mass of foam but this was not adherent. Fresh blood was present at injury site, but subject MAP was very low (~20) during necropsy, so not sure how brisk bleeding was. Injury site itself covered with clot without foam.
Clot-injury site adhesion score = 0
Volume foam recovered: 631.5g (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 101.9g (resected LM lobe) + 964.4 (remaining liver) = 1,066.3 g
Tissue harvested: Liver for Instron experiments, liver from injury site with clot for histology, pancreas and rectum for primary cell culture.

VI. COMMENTS

Noncompressible injury treated with calcium alginate foam + FS. Injector worked fine, no issues with calcium delivery. Foam consistency appeared similar to previous runs of Ca alg foam. Survived 180 minutes with ~2.2 L blood loss (noticeably more than previous three runs of alginate minus calcium); marginal blood pressure (MAP ~30 at end). Lump of clot without foam covering to injury site, there may have been active bleeding at 3 h.

One of the subjective impressions I have had with using calcium alginate foam is the relatively large volume of nonclotted blood in the abdominal cavity. While there invariably has been clot right at the injury site, there has not been much clot away from the injury site, just liquid blood… perhaps there is some sort of clotting inhibition going on (e.g., from the excess free calcium, which always is present, as evidenced by the toxic effects to the organ surfaces).

We do not have the numbers yet to perform statistics, but I would venture to guess that the calcium-free alginate will produce lower blood loss numbers and better MAP than the calcium alginate foam, with the former not causing injury to the organ surfaces. Whether this would translate into increased survival might take higher subject numbers and/or increased injury severity (e.g., bilobar resection).

VII. PLAN

This week we will operate on protocol #00760 on Tue & Thu (8/11 and 8/13), using calcium alginate + FS on both days. On Friday we are scheduled to do a hepatic resection treated with PCL gauze + FS (the new NEDED Phase 2 protocol, #01005), but I am waiting on the IACUC approval letter, so Friday's procedure is pending.

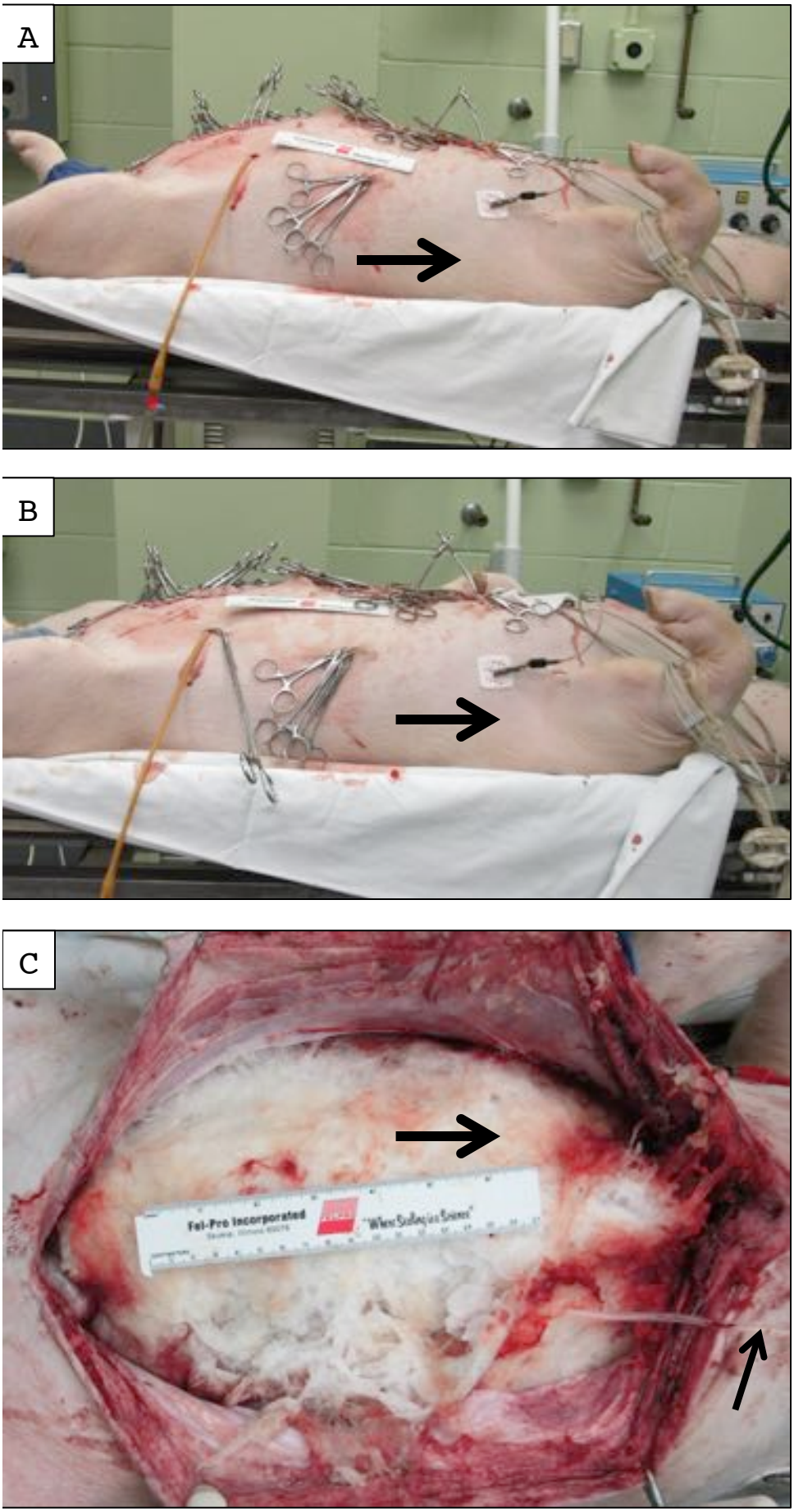

Figure 1, swine 302.
(A) Lateral view of swine at 3 min after injection completed; IAP ~25 mm Hg with distension and subcut emphysema. Arrow = cephalad
(B) At end of 3 h observation period; IAP = 0 mm Hg, abdomen less distended. Subject hypotensive with HR = 126 BPM, O2 Sat = 94%, EtCO2 = 36 mm Hg, MAP = 35 mm Hg, T = 35.0˚C.
(C) Abdomen reopened at 3 h; subject alive, MAP in 20's. Foam covering all viscera. Blood only visible at periphery. Large arrow = cephalad; small arrow = line for IAP monitor.

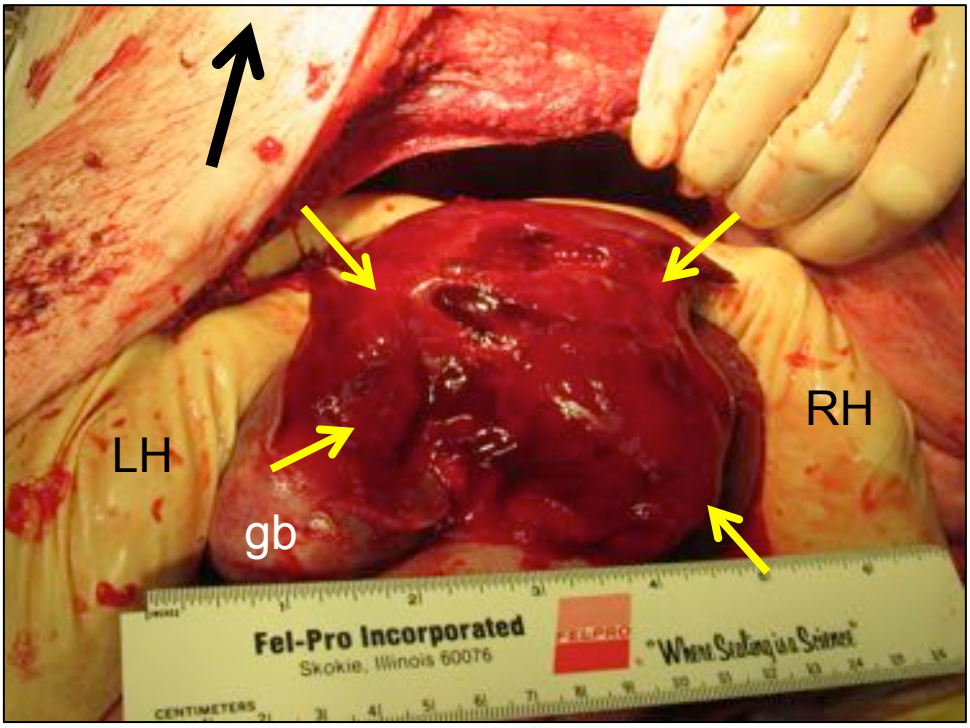

Figure 3, swine 302. Injury site at 3 h after injury; subject marginally alive. Liver *in situ* is being elevated out of abdomen with surgeon's left and right hands (LH & RH). Injury site at base of LM lobe (yellow arrows) covered with clot, no foam was adherent. No active hemorrhage, but very little blood pressure left. Cephalad = black arrow; gb = gallbladder.

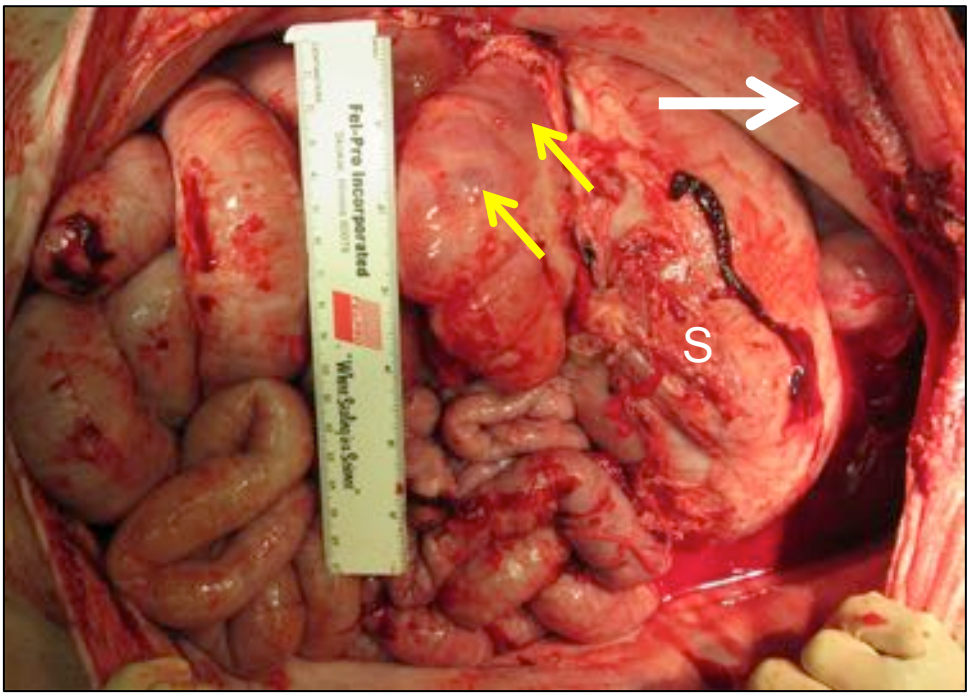

Figure 4, swine 302. Appearance of intestines after foam removed, prior to euthanasia. MAP ~20. Intestines diffusely discolored, with focal areas of purplish coloration (yellow arrows). White arrow = cephalad; S = stomach.

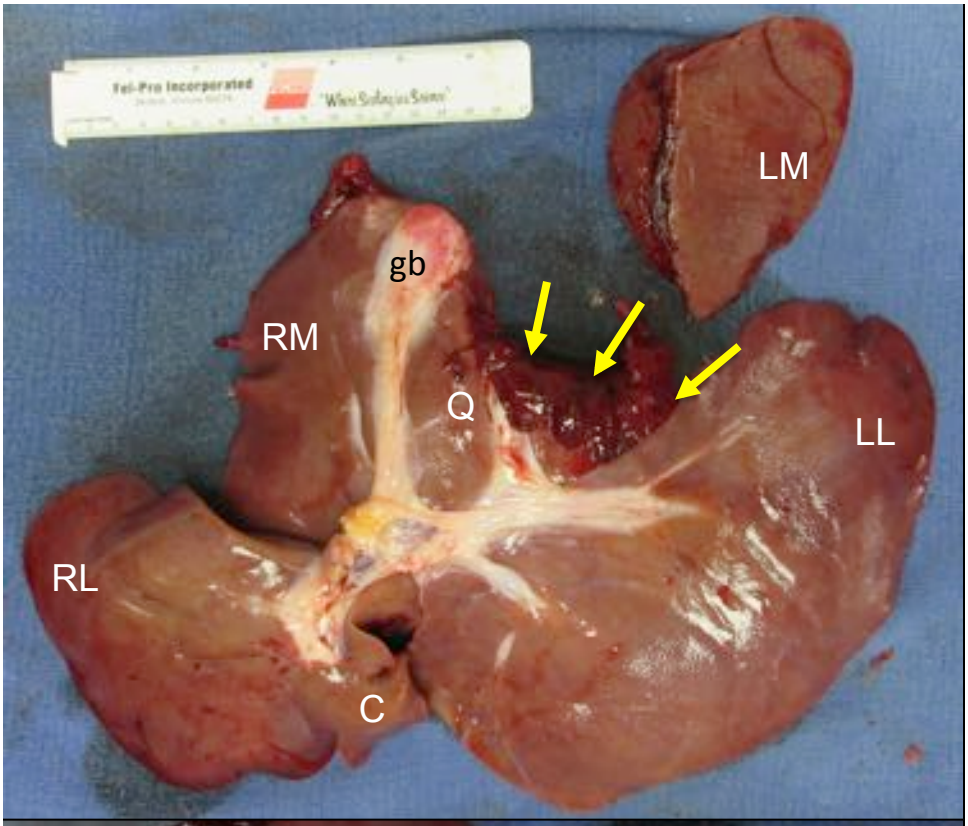

Figure 5, swine 302. Liver *ex vivo*, Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, with large amount of clot. Anterior is at top of image.

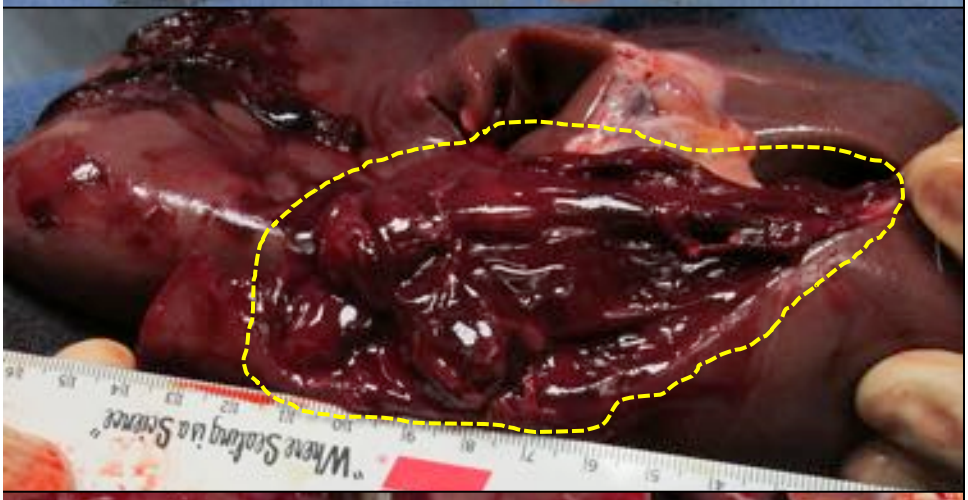

Figure 6, swine 302. Close-up oblique view of clot overlying injury site (dashed line).

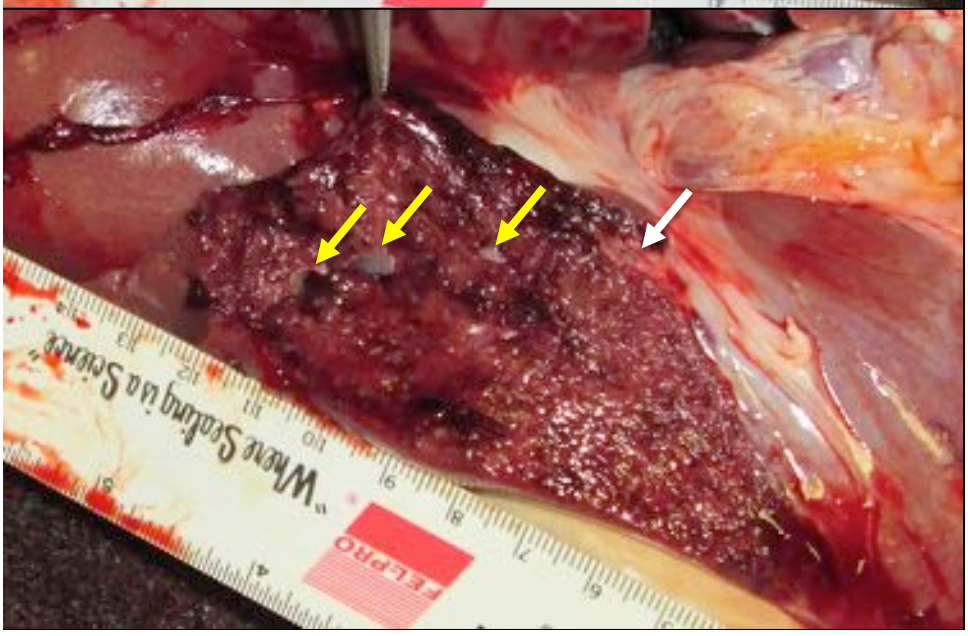

Figure 7, swine 302. Close-up of injury site on base of LM lobe, after clot wiped away. Yellow arrows = transected hepatic vein branches; white arrrow = portal vein branch.

I. OVERVIEW
Date: August 11, 2015
Swine no: 303
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate Foam + Factor II/XIII
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz, Johnson

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II. PRE-INJURY PHASE

Start time: 07:40 AM
Swine sex: Male
Date swine received from UNL Mead: 08/06/2015
Pre-procedure wt: 39.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 80
- MAP:111
- Temp: 38.3
- EtCO2: 43

Blood draw no. 1 (initial): 7:50 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:00 AM
Spleen wt: 303.2 gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 303.2 + 30 + 0 = 333.2 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 75 (ear vein/jugular drip) = 975 mL

Pre-injury VS
- HR: 88
- MAP: 100

- Temp: 37.2
- EtCO2 : 45
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:35 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam, 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: Factors II/XIII (had intended to give pdFI also, but FI would not go into solution—see Figs).

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 60 sec after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. IAP peaked around ~25 mm Hg. **Note**: we had difficulty with the calcium injection with this procedure 2˚ what turned out to be a clogged line, so very little calcium (perhaps) 20 mL) actually was injected into the subject.

Total mass injected: 599.8g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 11.1 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:38AM (~ 3' after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:45 AM

10 min post-injury VS

- HR: 92
- MAP: 40
- Temp: 37.0
- EtCO2: 30
- IAP: 17

Blood draw no. 3: (30 min post-injury): 9:05 AM

30 min post-injury VS

- HR: 186

- MAP: 31
- Temp: 36.8
- EtCO2: 34
- IAP: 11

Blood draw no. 4: (60 min post-injury): 9:40AM

60 min VS
- HR: 174
- MAP: 23
- Temp: 36.6
- EtCO2: 27
- IAP: 3

Blood draw no. 5: (90 min post-injury): 10:05AM

90 min post-injury VS
- HR: 174
- MAP: 26
- Temp: 36.3
- EtCO2: 33
- IAP: 2

Blood draw no. 6: (120 min post-injury): 10:35AM

120 min post-injury VS
- HR: 174
- MAP: 28
- Temp: 36.1
- EtCO2: 34
- IAP: 1

Blood draw no. 7: (150 min post-injury): 10:55AM (expired at this time point)

Survival at 180 min? No.
Target MAP attained ? No.
Time of death: 10:55 AM
Cause of death: exsanguination/hypotension
Interval from injury to death: 140 min

Post-treatment fluid data:
- Blood loss: 119.2 mL (suction) + 796.9 mL (sponge/lap) + 609.2 mL (clots) + (foam) + 180 mL (phlebotomies) = 1,705.3 mL
- IV fluid given: LR (37°C): 1,550 mL (infused) + 25 mL (ear vein) + 75 mL (jugular drip) = 1,650mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended but soft (IAP 0-1 mm Hg). Upon re-opening abdomen, very little foam present, but not stiff. Most of blood was mixed with alginate, similar to past three subjects that had not received 1M $CaCl_2$. No organ toxicity noted. No foam at injury site. Injury covered with clot; this was lightly adherent, and fell off during the liver explantation.
Clot-injury site adhesion score = 1
Volume foam recovered: nothing to measure (see Figs)
Heart: examined. No evidence of emboli or gas. IVC & cardiac chambers were distended with blood.
Number of hepatic veins lacerated: 2, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 90.7 g (resected LM lobe) + 783.4 (remaining liver) = 874.1 g
Tissue harvested: None

VI. COMMENTS

Noncompressible injury treated with alginate foam + II/XIII + $CaCl_2$, no fibrinogen. Calcium line malfunctioned, only a fraction of calcium delivered that should have been delivered; no stiff foam was generated. Perhaps this should be called a no-calcium control. Survived 140 min only with 1.5 L blood loss attributable to hemorrhage. Lump of clot covering injury site. Interestingly, very few if any of subjects that die after the noncompress injury do so in the 3rd hour. Most fatalities occur within 1st hour, and couple of have died in the 2nd hour. But not in the 3rd. Not sure what that means.

Not really sure how to group this subject because of above issues…

VII. PLAN

Continue with this series on Thu 8/13/15.

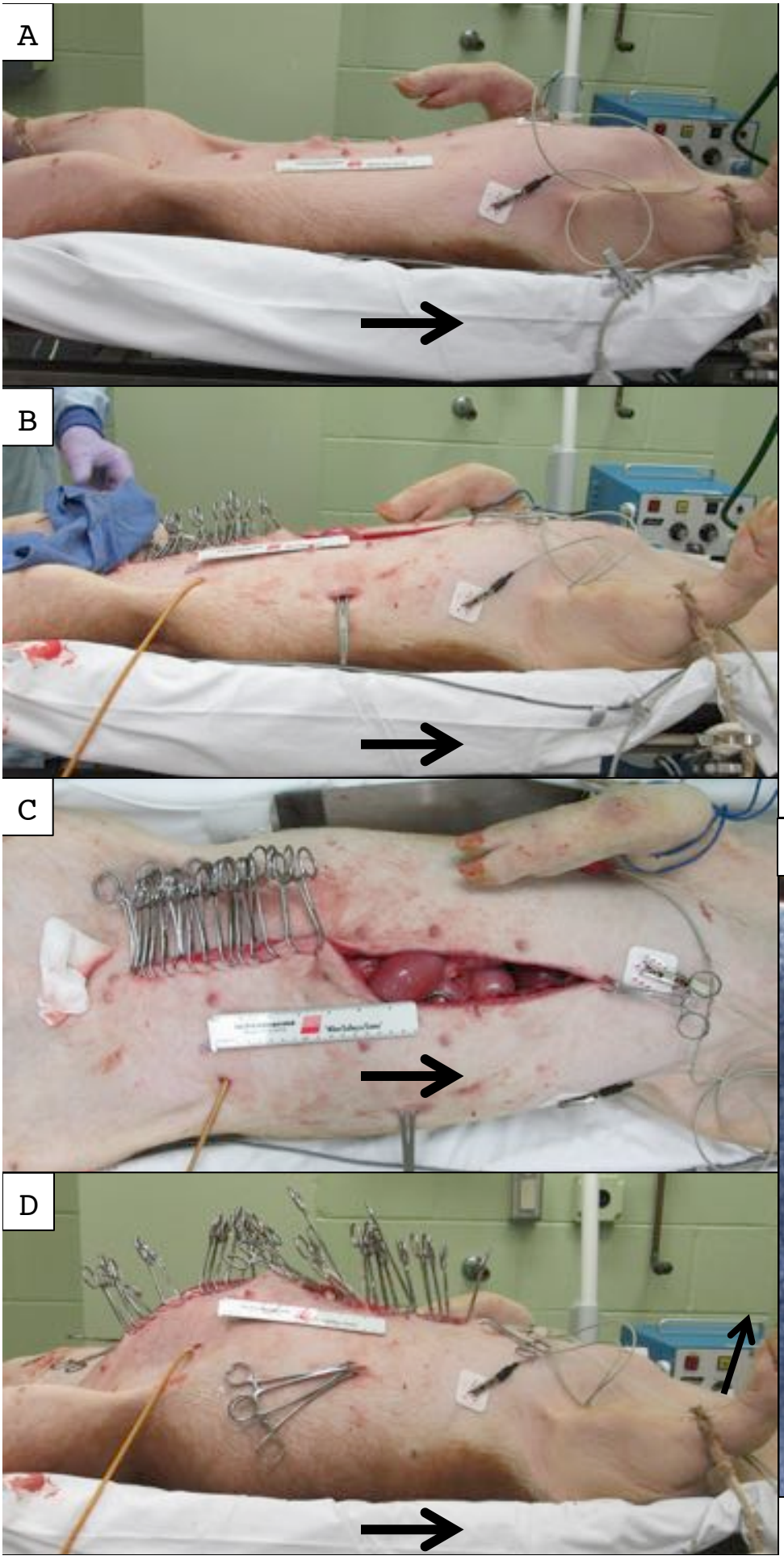


Figure 1, swine 303.
(A) Lateral view of swine just after induction of anesthesia, prior to any line placement or laparotomy. Black arrow = cephalad.
(B) Immediately prior to injury. Lower half of incision closed with towel clips.
(C) Same time as panel B, but overhead view.
(D) At 3 min after injection completed; IAP ~25 mm Hg with distension and subcut emphysema.
(E) pdFI in 50 mL conical tube, that did not go into solution.

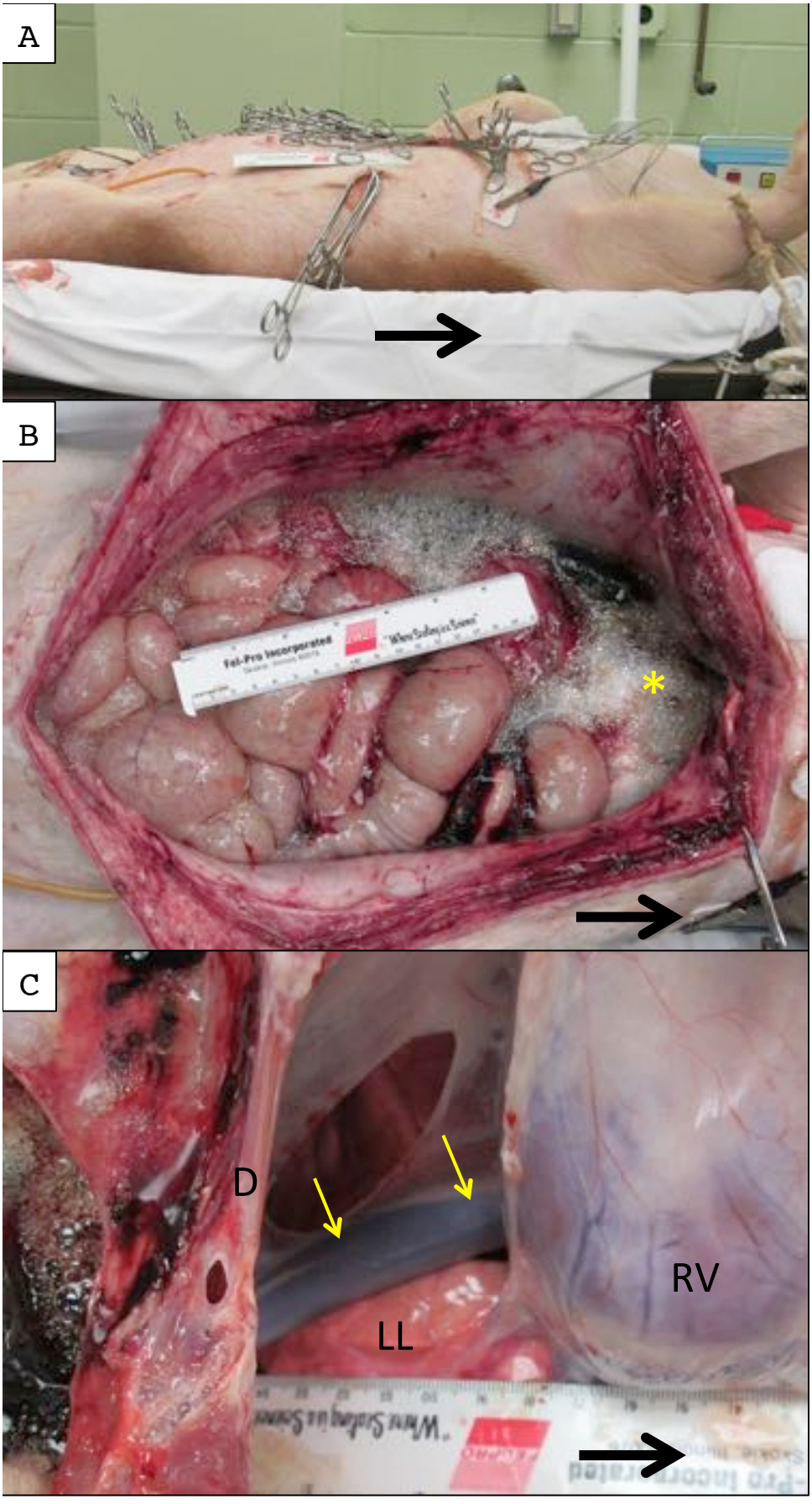


Fig. 2. Swine 303.
(A) Lateral view, immediately after expiration at 140 min post-injury. Black arrow = cephalad.
(B) Abdomen reopened immediately after expiration. Very little foam present, not stiff. No evidence organ toxicity. Asterisk = injury site, approximate.
(C) Inspection of chest cavity at necropsy. IVC (yellow arrows) was distended with blood. Cardiac chambers did not contain any emboli or gas. RV = right ventricle; LL = left lung; D = diaphragm.

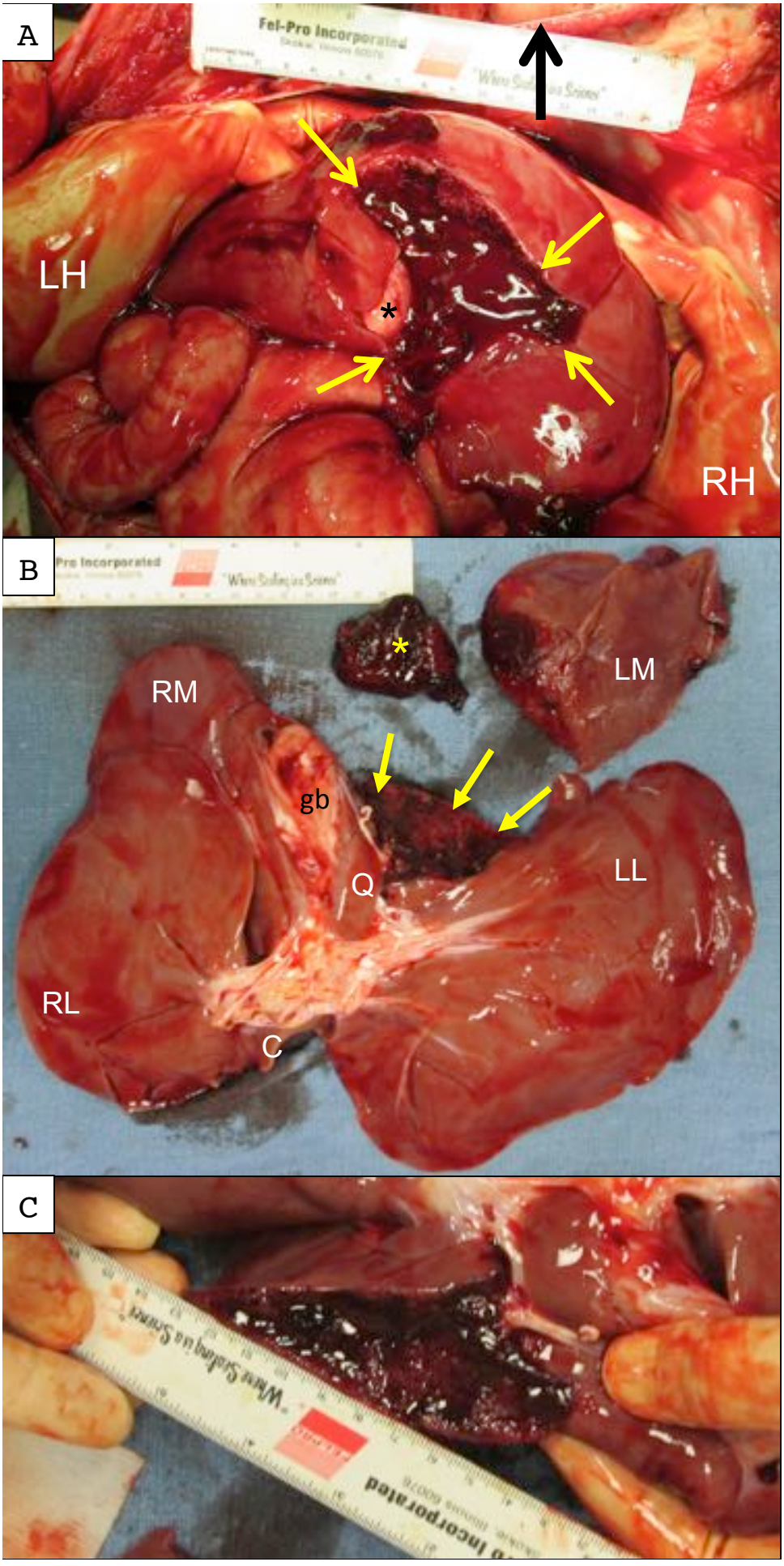


Figure 3, swine 303.
(A) Injury site at necropsy. Liver *in situ* is being elevated out of abdomen with surgeon's left and right hands (LH & RH). Injury site at base of LM lobe (yellow arrows) covered with clot, no foam was adherent. Cephalad = black arrow; asterisk = gallbladder.
(B) Liver *ex vivo*, Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe. *Asterisk = mass of clot that had been loosely adherent to the injury site. Anterior is at top of image.
(C) Close-up of injury site. Only a thin layer of clot remains.

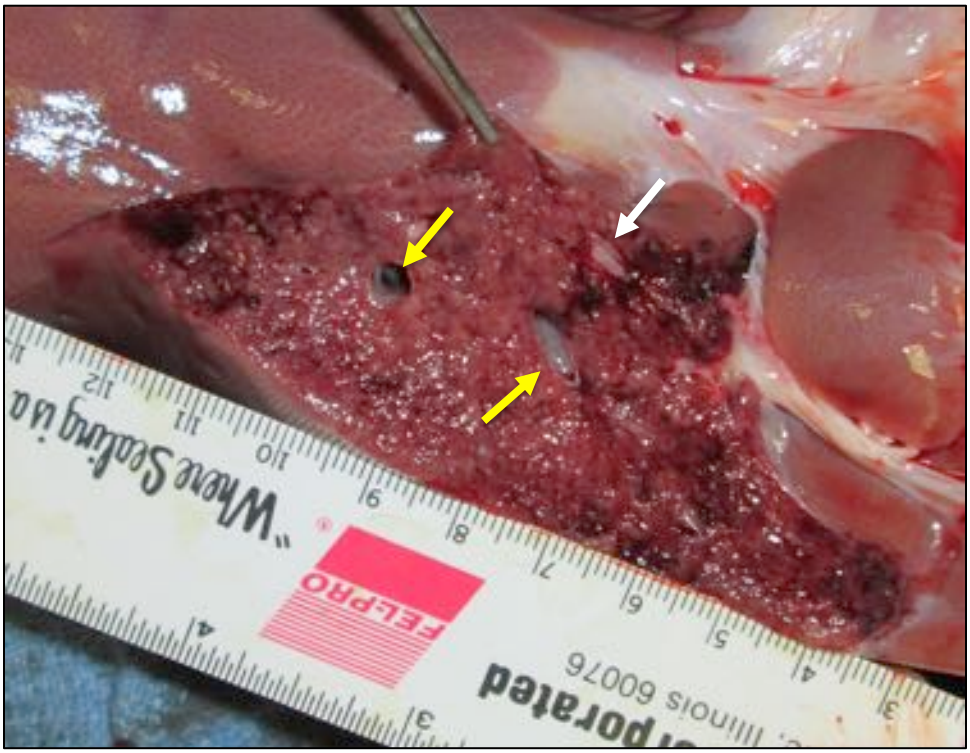


Figure 4, swine 303. Close-up of injury site on base of LM lobe, after clot wiped away. Yellow arrows = transected hepatic vein branches; white arrrow = portal vein branch.

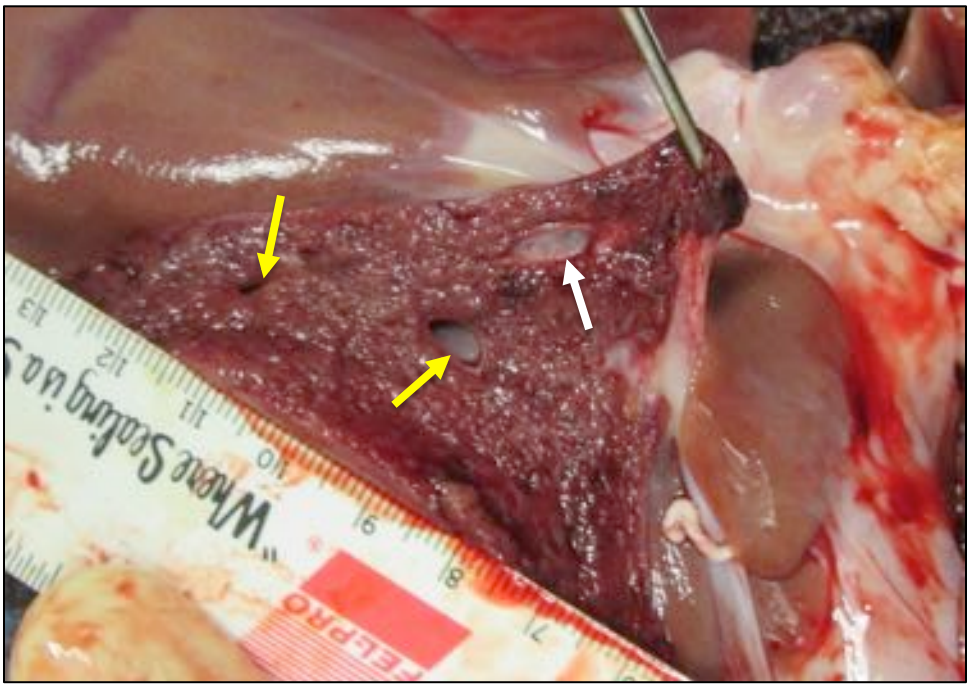


Figure 4, swine 303. Similar view as in Fig. 4, better demonstrating the PV branch.

I. OVERVIEW
Date: August 13, 2015
Swine no: 304
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: Alginate Foam + FS
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz, Johnson, Aravind (observing only)

II. PRE-INJURY PHASE

Start time: 07:45 AM
Swine sex: Male
Date swine received from UNL Mead: 8/06/2015
Pre-procedure wt: 37.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 83
- MAP:112
- Temp: 38.3
- EtCO2: 43

Blood draw no. 1 (initial): 8:15 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:23 AM
Spleen wt: 374.0 gm
LR (22°C) infused after splenectomy: 1100 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 374 + 30 + 11.8 = 415.8 mL
- LR (22°C) infused (spleen replacement + incidental): 1100 + 70(ear vein/jugular drip)= 1170.0 mL

Pre-injury VS
- HR: 74
- MAP: 110

- Temp: 37.4
- EtCO2 : 46
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:42 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the right upper quadrant.

Treatment formulation: Sodium alginate foam 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: Factors = pdFI, rFII, rFXIII (FS).

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Note: Injection of the foam + FS began ~150 sec after injury (delay beyond 1 min 2˚ problem with air pressure line), after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. IAP peaked around 35 mm Hg.

Total mass injected: 468.4g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:45AM (~ 3 min after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:50 AM

10 min post-injury VS

- HR: 72
- MAP: 44
- Temp: 37.3
- EtCO2: 46
- IAP: 21

Blood draw no. 3: (30 min post-injury): 9:10 AM

30 min post-injury VS

- HR: 82
- MAP: 53

- Temp: 37.3
- EtCO2: 39
- IAP: 1

Blood draw no. 4: (60 min post-injury): 9:40AM

60 min VS
- HR: 78
- MAP: 55
- Temp: 36.9
- EtCO2: 39
- IAP: 0

Blood draw no. 5: (90 min post-injury): 10:10AM

90 min post-injury VS
- HR: 77
- MAP: 59
- Temp: 36.4
- EtCO2: 37
- IAP: 0

Blood draw no. 6: (120 min post-injury): 10:40AM

120 min post-injury VS
- HR: 76
- MAP: 55
- Temp: 36.0
- EtCO2: 35
- IAP: 0

Blood draw no. 7: (150 min post-injury): 11:10AM

150 min post-injury VS
- HR: 78
- MAP: 54
- Temp: 35.7
- EtCO2: 34
- IAP: 0

Blood draw no. 8: (180 min post-injury): 11:40AM

180 min post-injury VS
- HR: 80
- MAP: 55
- Temp: 35.4
- EtCO2: 36
- IAP: 0

Survival at 180 min? Yes
Target MAP attained ? No
Time of death: 11:45 AM
Cause of death: Exsanguination from euthanasia (supradiaphragmatic IVC transection)
Interval from injury to death: 180 min

Post-treatment fluid data:

- Blood loss: 524.1 mL (suction) + 0 mL (sponge/lap) + 284.0 mL (clots) + 400.6mL (foam) + 210 mL (phlebotomies) = 1,418.7mL
- IV fluid given: LR (37°C): 1,925 mL (infused) + 25mL (ear vein) + 100 mL (jugular drip) = 2,050 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen minimally distended (see Figs) but soft (IAP = 0 mm Hg). Upon re-opening abdomen, some rush of gas present. Foam by itself in the superficial region (see Figs), then tinged with blood at interface underneath. Moderate amounts of unclotted blood and a some clots present (see Figs). Some ischemic areas over colon were noted (see Figs). Injury site was covered with a large mass of clot (see Figs), but no foam. Foam was present overlying the injury site, but foam itself was not adherent No evidence of ongoing hemorrhage.
Clot-injury adhesion score: 2
Volume foam recovered: 400.6g (see Figs)
Heart: not examined.
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 115.3g (resected LM lobe) + 879.1 (remaining liver) = 994.4 g
Tissue harvested: (1) Liver from injury site, (2) clot from injury site for histology.

VI. COMMENTS

Noncompressible injury treated with alginate foam + FS + 1 M $CaCl_2$. Survived 180 minutes with 0.8 L blood loss attributable to injury. Clot adherent to injury site, with no active bleeding at 3 h. Moderate intestinal toxicity noted.

Ayman raised the possibility that the serum calcium might be undergoing gradual increase during the 3 h observation period in subject's treated with calcium alginate foam. Subject hypercalcemia can cause all kinds of acute and chronic toxicities. We will measure the serum calcium with the labs in the next pig treated with calcium alginate to determine whether this might be an issue.

Potentially we are giving (0.084 L) x (1.14 M) = 96 mmol $CaCl_2$, or 96 mEq elemental calcium. I would assume that most of this calcium is absorbed by the alginate foaming reaction. But for you clinical folks, 96 mEq of free calcium is the equivalent of 7 ampules of $CaCl_2$ (!), in a subject that weighs ~35 kg. That is a <u>huge</u> amount of calcium to administer, and likely would be fatal if given over a short period of time. We will see if these theoretical numbers produce any change in serum calcium level in the actual experimental environment.

VII. PLAN

Next subject in this series will be on Aug 25th. In the interim, subjects for the NEDED protocol will be done. Chem 20 to be drawn with each phlebotomy to follow the serum calcium.

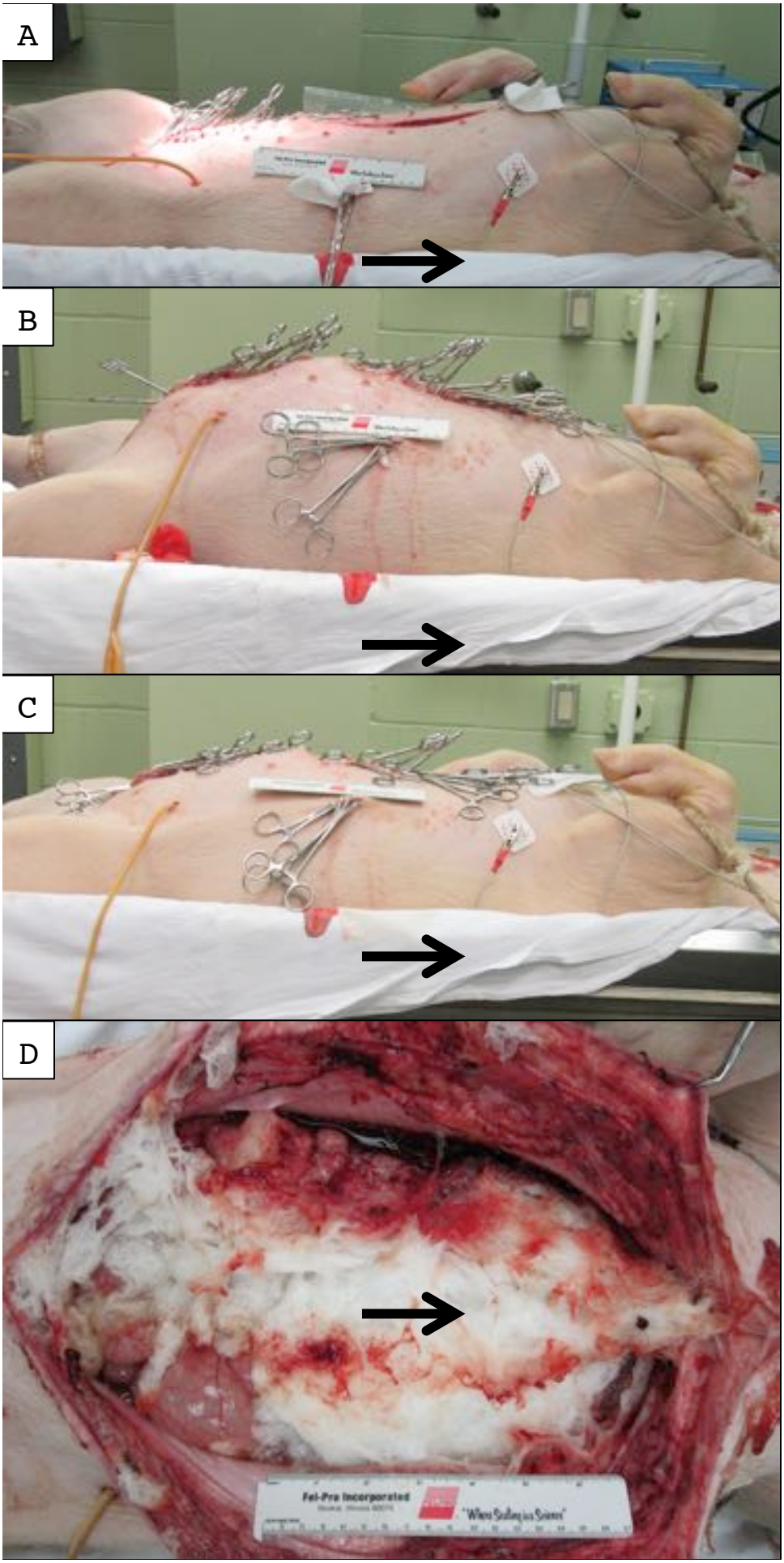


Figure 1, swine 304.
(A) Lateral view of swine, immediately prior to injury. Lower half of incision closed with towel clips. Black arrow = cephalad.
(B) At 3 min after injection completed; IAP ~35 mm Hg with distension and subcut emphysema.
(C) At 3 h after injury; subject alive with MAP = 55 mm Hg, HR = 80, T = 35.4˚C, EtCO2 = 36 mm Hg, O2 Sat = 99%, IAP = 0
(D)) Abdomen reopened immediately after 3 h observation period. Subject still alive. Foam covering intestines & injury site. Blood visible at periphery of foam.

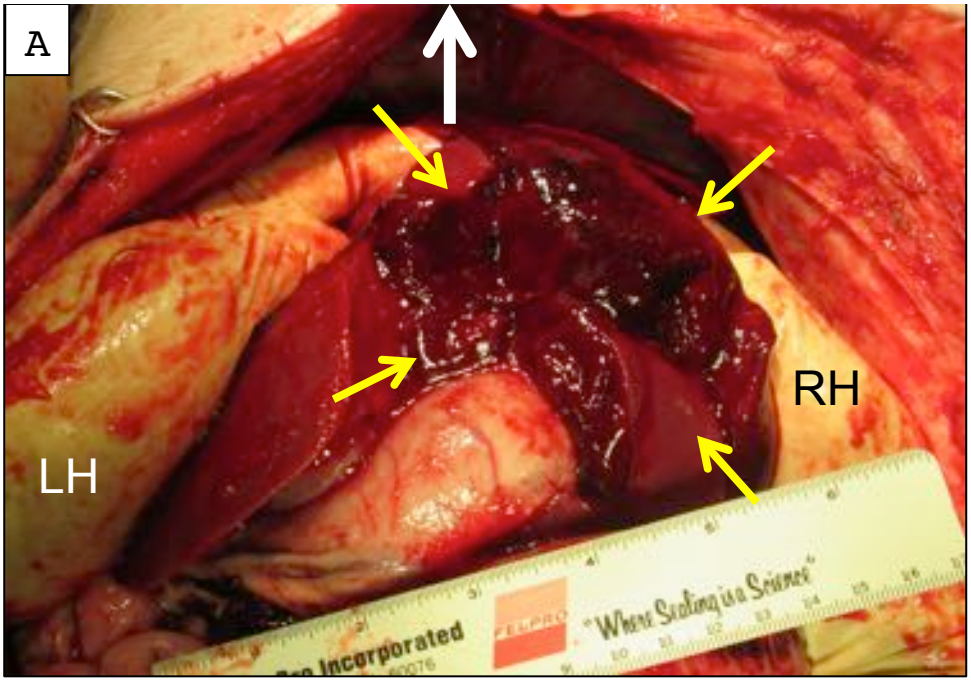


Figure 2, swine 304. Injury site at 3 h after injury; subject still alive. Liver *in situ* is being elevated out of abdomen with surgeon's left and right hands (LH & RH). Injury site at base of LM lobe (yellow arrows) covered with clot; no foam was adherent. Cephalad = white arrow.

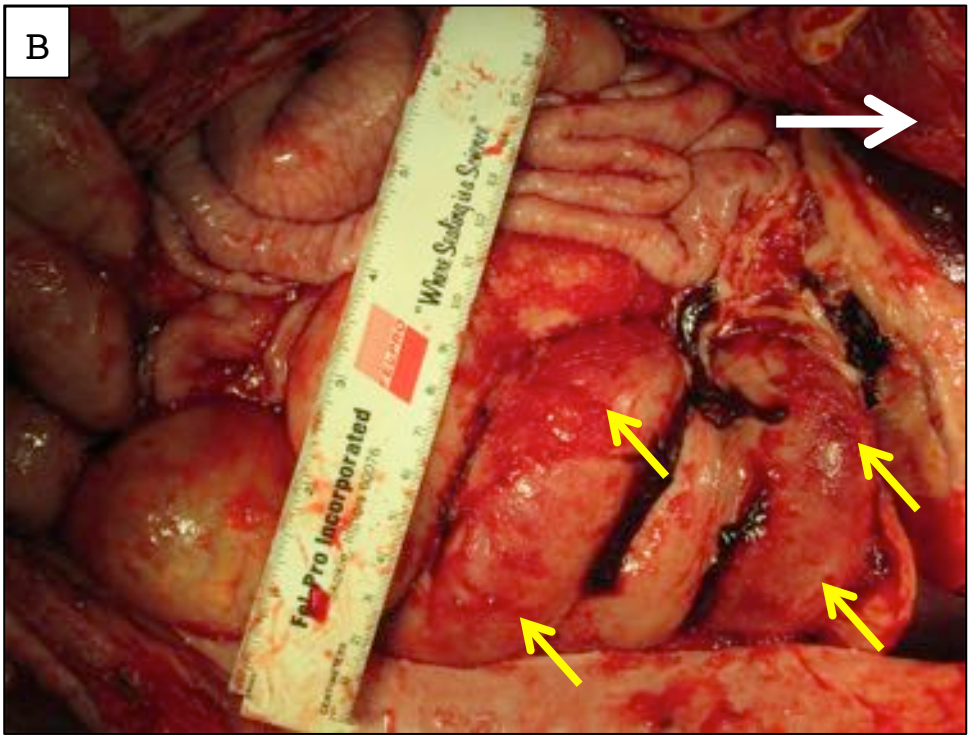


Figure 3, swine 304. Intestines after foam removal. Subject still alive. Areas of surface toxicity indicated with yellow arrows. Cephalad = white arrow.

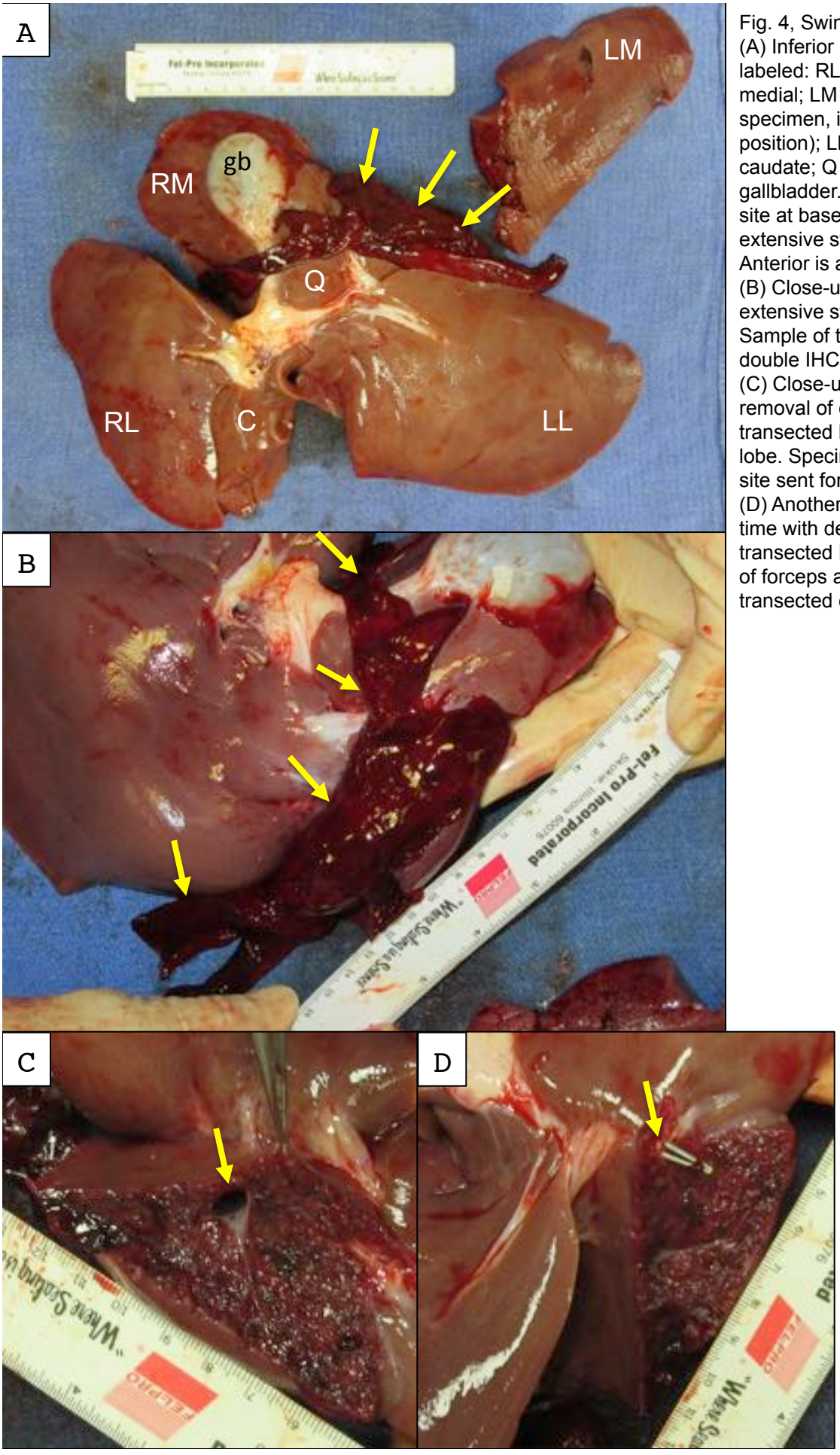


Fig. 4, Swine 304. Liver *ex vivo*.
(A) Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, covered with extensive stringy clot, but no foam. Anterior is at top of image.
(B) Close-up of injury site with extensive stringy clot (arrows). Sample of this clot was sent for FI double IHC.
(C) Close-up of injury site after removal of clot, with demonstration of transected hepatic vein (arrow) to LM lobe. Specimen from surface of injury site sent for IHC.
(D) Another view of injury site, this time with demonstration of transected PV to LM lobe (arrow; tips of forceps are emerging from transected end).

I. OVERVIEW
Date: August 25, 2015
Swine no: 307
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate foam without clotting factors
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-INJURY PHASE

Start time: 07:48 AM
Swine sex: Male
Date swine received from UNL Mead: 8/18/2015
Pre-procedure wt: 34.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 87
- MAP:126
- Temp: 38.2
- EtCO2:50

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:08AM
Spleen wt: 320.7 gm
LR (22°C) infused after splenectomy: 950 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 320.7 + 30 + 5.9 = 356.6 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 60 (ear vein/jugular drip)= 1,010.0 mL

Pre-injury VS
- HR: 83
- MAP: 122

- Temp: 37.3
- EtCO2 : 48
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:27 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base (at the level of the junction with the right medial lobe) with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the mid abdomen.

Treatment formulation: Sodium alginate foam 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: None.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. IAP maxed at 25 mm Hg during the injection.

Total mass injected: 374.9g alginate foam; only 20 mL of 1 M $CaCl_2$ (should have been ~80 mL).

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:45AM (~ 3 min after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:40 AM

10 min post-injury VS

- HR: 104
- MAP: 19
- Temp: 37.4
- EtCO2: 11
- IAP: 11

Survival at 180 min? No

Target MAP attained ? No

Time of death: 8:45AM

Cause of death: gas/foam embolism and exsanguination

Interval from injury to death: 18 min

Post-treatment fluid data:

- Blood loss: 523.7 mL (suction) + 0 mL (sponge/lap) + 620.6 mL (clots) + 0 mL (foam) + 60 mL (phlebotomies) = 1,204.3 mL
- IV fluid given: LR (37°C): 150 mL (infused) + 0 mL (ear vein) + 0 mL (jugular drip) = 150 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~ 11 mm Hg). Death occurred within 18 minutes post-injury. Upon re-opening abdomen, foam by itself was gelatinous and mostly in the superficial region; there was no stiffness to the foam. Large amounts of unclotted blood and clots were seen (see Figs). Injury site was not covered by anything except a very thin layer of fibrin. Subsequently chest was opened and explored to determine the cause of death. +++Foam and gas bubbles were seen in the right ventricle and vena cava (see Fig).

Volume foam recovered: Not measured
Heart: +++gas/foam in RV
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 114.9g (resected LM lobe) + 777.8 (remaining liver) = 892.7 g
Tissue harvested: none.

VI. COMMENTS

Noncompressible injury treated with calcium alginate foam without clotting factors. Survived 18 minutes; rapid death 2˚ to gas/foam embolism, but also hefty blood loss of 1,150 mL. We have not had a embolic death for a long time. My seat of pants impression is that foam is injecting too quickly, i.e., quicker than what I have experienced in the recent past. The abdomen fills up in what seems to be several seconds... that's a relatively vigorous, almost violent rate of abdominal filling. Makes me wonder if that is the cause of embolism. Also, foam stiffness was not adequate today, presumably from inadequate calcium… subject only received about 25% of the $CaCl_2$ that it should have received. Syringe pumps unable to keep up with rapid injection rate? I wonder if we would have better results if the injection could be slowed down so that it would occur during a 45-60 s interval.

VII. PLAN

Continue with this series (Ca alginate without clotting factors) on Thu Sep 27th.

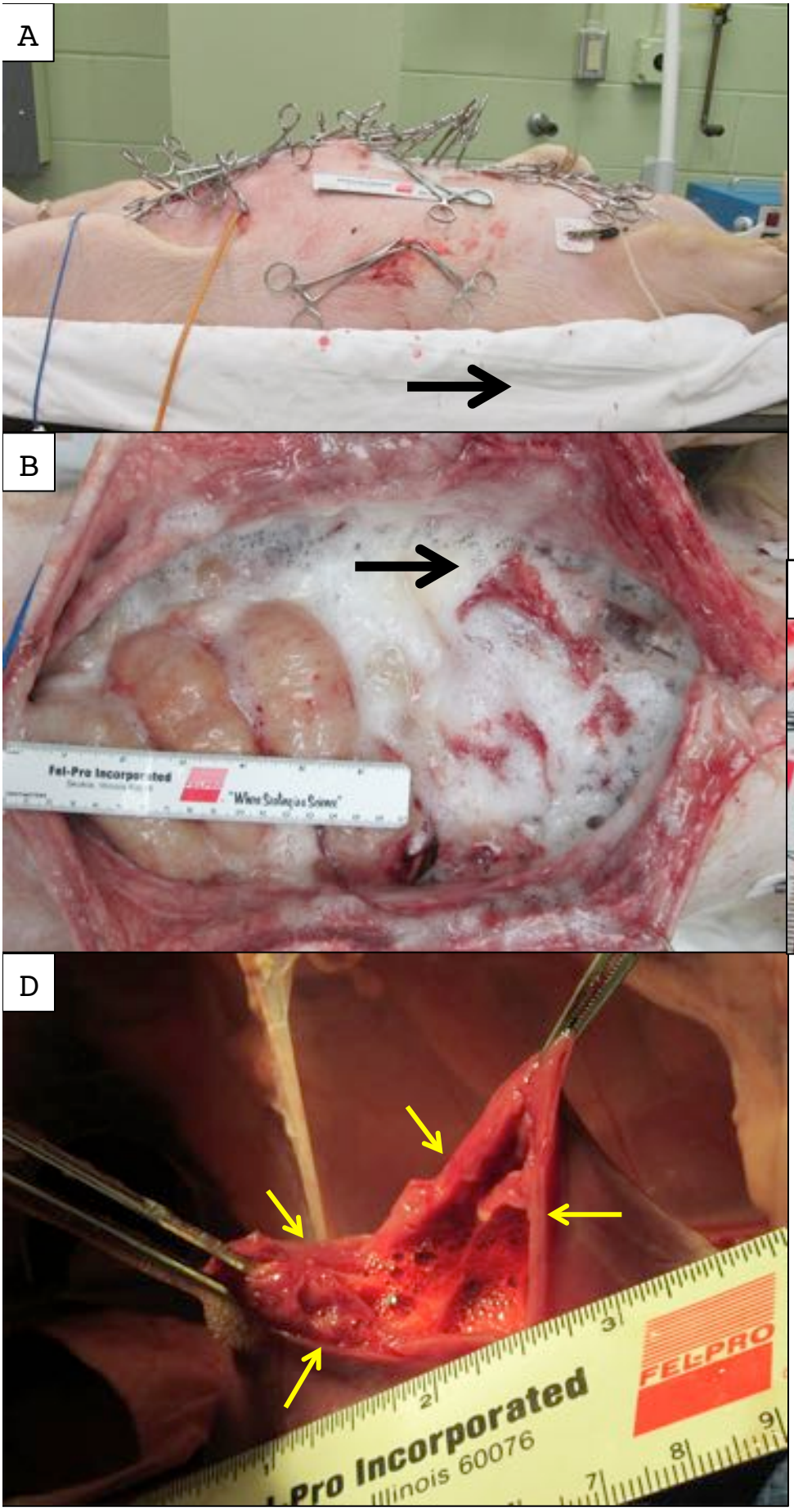


Figure 1, swine 307.
(A) Lateral view of swine, right after expiration at 18 min post-injury. Black arrow = cephalad.
(B) Abdomen re-opened after expiration. White foam with consistency of soap suds (no stiffness). No surface changes to viscera. Abdominal distension was secondary to butane carrier gas, and not foam.
(C) Close-up of foam removed from abdomen
(D) Thoracotomy and right ventriculotomy of heart at necropsy, demonstrating foam embolism (arrows) within the right ventricle.

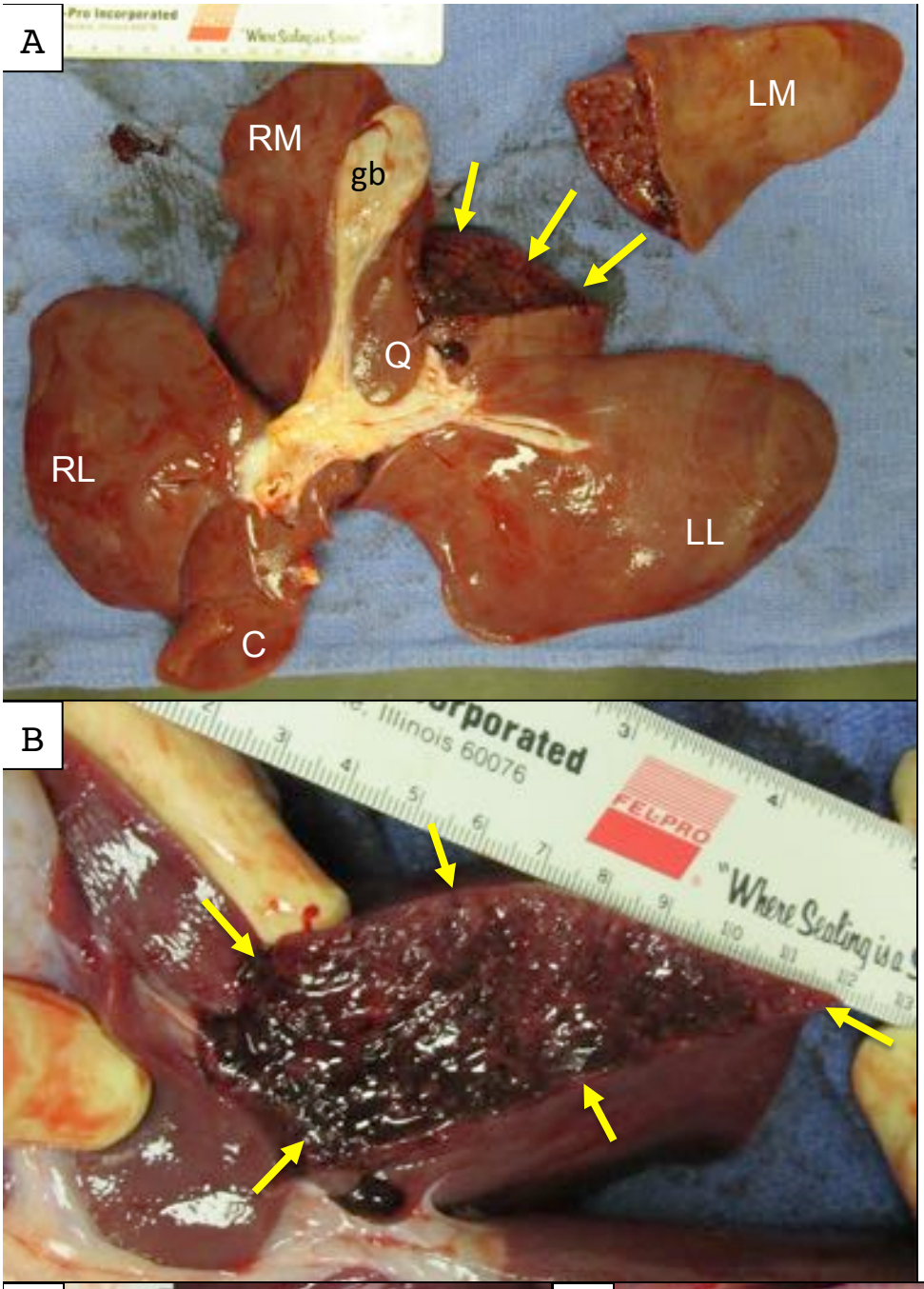


Fig. 2, Swine 307. Liver *ex vivo*.
(A) Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, covered with very thin layer of fibrin, but no foam. Anterior is at top of image.
(B) Close-up of injury site with very thin clot layer, without adherent foam (arrows).
(C) Close-up of injury site after removal of clot, with demonstration of transected hepatic vein (arrow) to LM lobe.
(D) Another view of injury site, this time with demonstration of transected PV to LM lobe (arrow; tips of forceps are emerging from transected end).

I. OVERVIEW
Date: August 27, 2015
Swine no: 308
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate foam alone, no clotting factors
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-INJURY PHASE

Start time: 08:10 AM
Swine sex: Male
Date swine received from UNL Mead: 8/18/2015
Pre-procedure wt: 35.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 118
- MAP:99
- Temp: 36.9
- EtCO2:not recorded/wrongly recorded due to equipment failure

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:35AM
Spleen wt: 219.6 gm
LR (22°C) infused after splenectomy: 650 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 219.6+ 30+ 0 = 249.6 mL
- LR (22°C) infused (spleen replacement + incidental): 650 + 35(ear vein/jugular drip)= 685.0 mL. Note: subject became hypotensive during 150 mL/min infusion of splenic replacement fluid, so infusion was temporarily stopped (1-2 min), and then restarted at 50 mL/min with no further incident.

Pre-injury VS

- HR: 81
- MAP: 106
- Temp: 35.9
- EtCO2 : 33
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:50 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors (at the level of the junction with the right middle lobe), producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the mid abdomen.

Treatment formulation: Sodium alginate foam 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: none.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. Injection was quite vigorous, and abdomen visually distended within several seconds. IAP momentarily reach 32 mm Hg during injection, then decreased to <10 over the next several min.

Total mass injected: 274.7g alginate foam.

Abdominal closure: “fully-closed” technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: ~3 min post-injury

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 9:00 AM

10 min post-injury VS

- HR: 107
- MAP: 32
- Temp: 35.7
- EtCO2: 14
- IAP: 08

Survival at 180 min? No
Target MAP attained ? No
Time of death: 9:05AM
Cause of death: gas/foam embolism
Interval from injury to death: 12 min

Post-treatment fluid data:

- Blood loss: 534.4 mL (suction) + 38.5 mL (sponge/lap) + 378.8 mL (clots) + 205.2 mL (foam) + 60 mL (phlebotomies) = 1,216.7mL (~950 mL from injury)
- IV fluid given: LR (37°C): 100 mL (infused) + 0 mL (ear vein) + 0mL (jugular drip) = 100 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

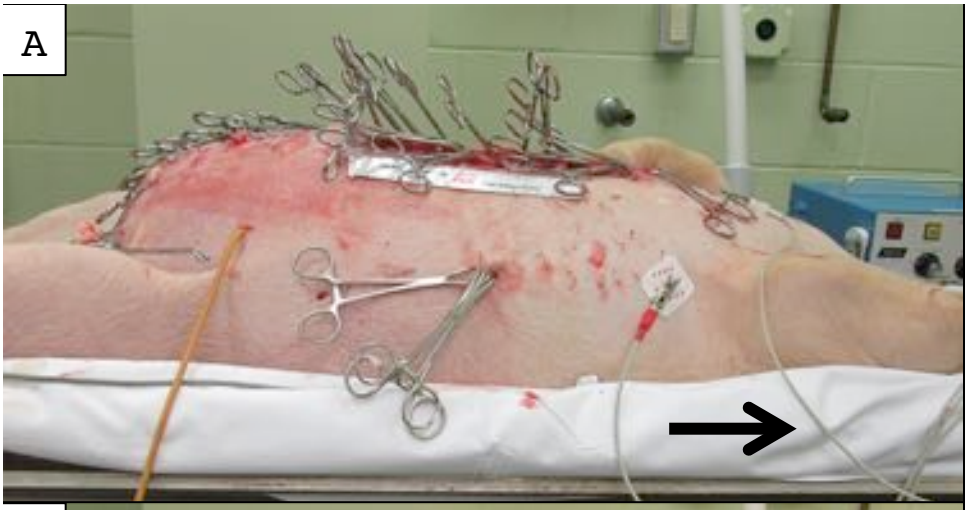
Findings upon abdominal/chest exploration: abdomen modestly distended; not very tense (IAP <10 mm Hg). Death occurred within 12 minutes post-injury. Upon re-opening abdomen, foam by itself was somewhat firm, not the best we have seen, but reasonable (see Figs), all in the superficial region of the abdomen. Moderate amounts of unclotted blood and clots were seen underneath. Injury site was not covered with any adherent clot or foam, except a very thin film of fibrin. Thoracotomy and ventriculotomy performed to determine the cause of death. Foamy gas bubbles were seen pouring out of the right ventricle (see Fig).
Volume foam recovered: ~500 mL
Heart: large amount gas/foam embolism in RV
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 76.1g (resected LM lobe) + 714.5 (remaining liver) = 790.6 g
Tissue harvested: none.

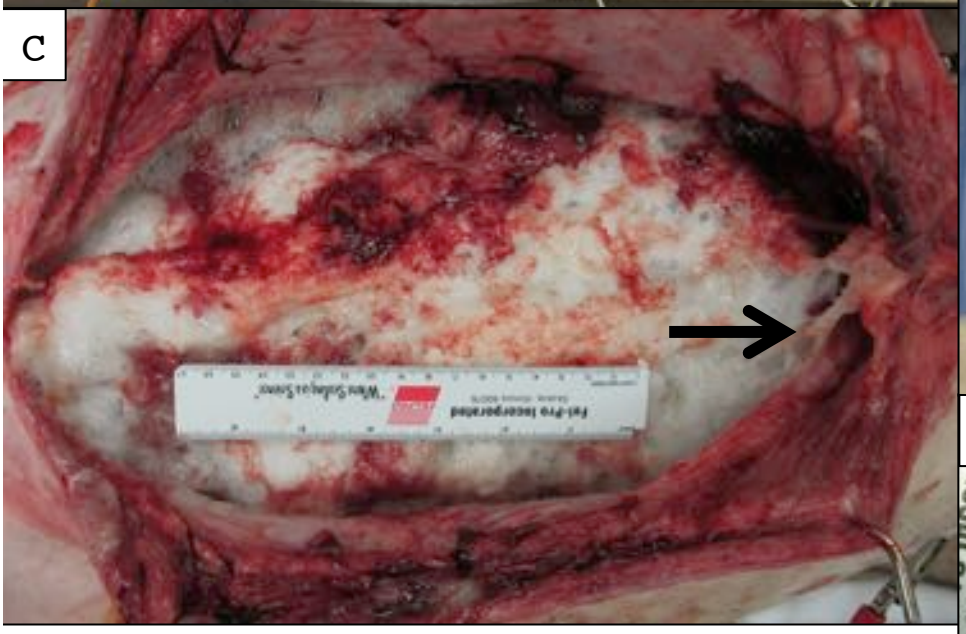
VI. COMMENTS

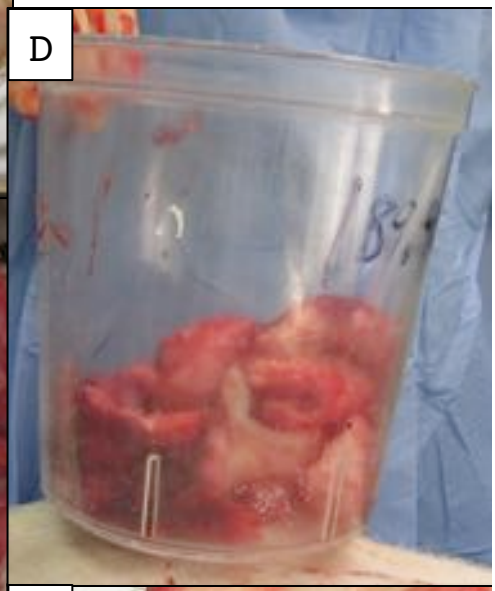
Noncompressible injury treated with calcium alginate foam without clotting factors. Foam was stiffer today, but not as stiff as we have seen previously. Dead at 12 minutes from gas/foam embolism. Last two subjects have died from gas/foam embolism… I doubt this is a coincidence. I am concerned that the rate of alginate injection is too fast, putting subject at risk for gas/foam embolism, and also not allowing calcium alginate reaction to produce stiff foam. Note that IAP drops rapidly as soon as injection stops… this suggests that foam is not adequately supporting IAP, that brief IAP elevation mostly due to injected butane. When we opened, there was only small (500 mL) volume of foam… not really enough to have for tamponade effect.

VII. PLAN

I think we need to put a hold on the foam experiments until we have a discussion regarding the foam delivery system, recent results, etc. While we have obtained some very interesting results recently, none of the foam subjects in the past month can be used as data points for our current study secondary to various technical issues.







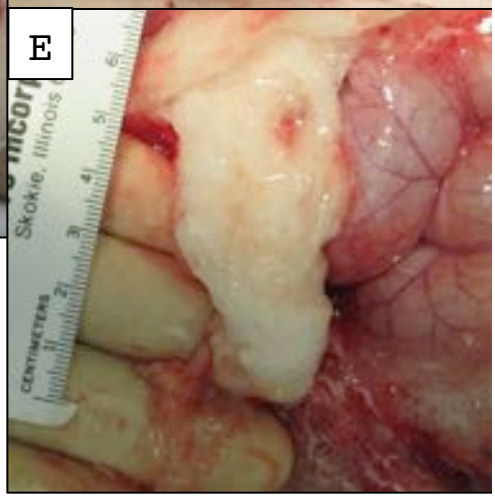


Figure 1, swine 308.
(A) Lateral view of swine, right after injection, 3 min post-injury. Black arrow = cephalad.
(B) Lateral view, at time of death (12 (12 min after injury).
(C) Overhead view of abdomen reopened after expiration.
(D) Intraabdominal foam retrieved in a 2 L canister (~500 mL foam).
(E) Close-up of foam. This iteration was stiffer than in subject #307.

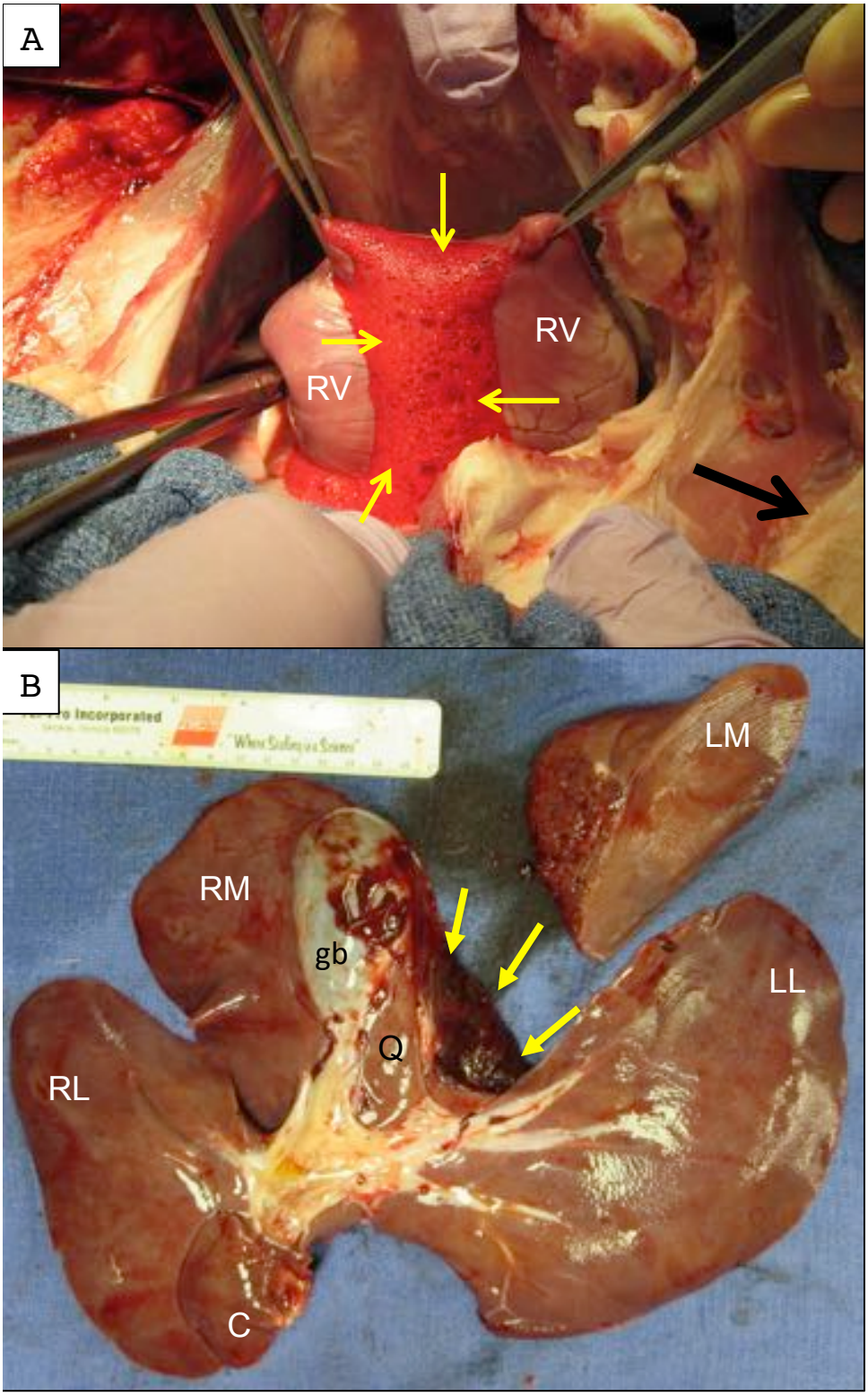


Fig. 2, Swine 308, necropsy.
(A) Thoracotomy and right ventriculotomy of heart, demonstrating gas/foam embolism (yellow arrows) pouring out of the right ventricle (RV). Black arrow = cephalad.
(B) Liver *ex vivo*, inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, covered with very thin layer of fibrin, but no foam. Anterior is at top of image.

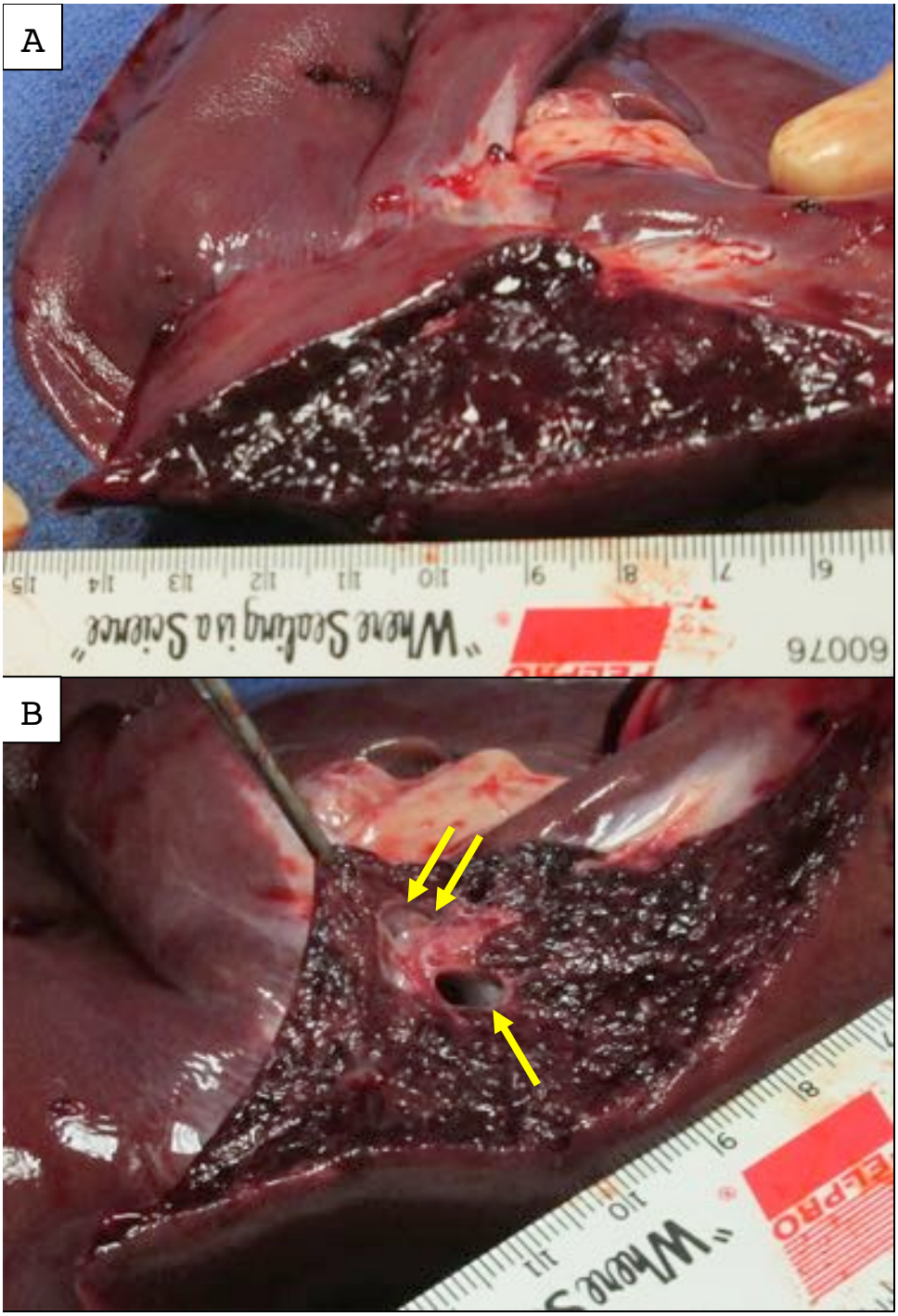


Fig. 3, swine 308. Liver *ex vivo*. (A) Close-up of injury site with very thin clot layer, without adherent foam. (B) Close-up of injury site after removal of thin clot layer, with demonstration of transected hepatic vein (arrow) to LM lobe and transected PV branch (double arrow).

I. OVERVIEW
Date: September 30, 2015
Swine no: 319
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: No Treatment
Personnel: Carlson, Hansen, Siford, Aravind

II. PRE-INJURY PHASE

Start time: 07:30 AM
Swine sex: Male
Date swine received from UNL Mead: 09/21/2015
Pre-procedure wt: 34.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 122
- MAP:73
- Temp: 38.3
- EtCO2: 53

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:10 AM
Spleen wt: 222.5 gm
LR (22°C) infused after splenectomy: 700 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 222.5 + 30 + 17.7 = 270.2 mL
- LR (22°C) infused (spleen replacement + incidental): 700 + 0(ear vein/jugular drip)= 700 mL

Pre-injury VS
- HR: 110
- MAP: 110
- Temp: 37.7

- EtCO2 : 48

III. INJURY & TREATMENT PHASE

Time of injury: 8:17 AM
Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at the junction with the RM lobe with 4 cm-blade scissors, producing a combined portal/hepatic venous injury. Ventral midline incision was closed with towel clips.
Treatment formulation: None
Clotting factors: None
Technique: (see Figs) The target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 90
Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 9.6 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 8:17AM (immediately after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:30 AM

10 min post-injury VS
- HR: 110
- MAP: 34
- Temp: 36.9
- EtCO2: 35

Blood draw no. 3: (30 min post-injury): 8:45 AM

30 min post-injury VS
- HR: 95
- MAP: 44
- Temp: 37
- EtCO2: 44

Blood draw no. 4: (60 min post-injury): 9:15AM

60 min VS
- HR: 85
- MAP: 30
- Temp: 36.5
- EtCO2: 41

Blood draw no. 5: (90 min post-injury): 09:45AM

90 min post-injury VS
- HR: 75
- MAP: 71
- Temp: 35.9
- EtCO2: 44

Blood draw no. 6: (120 min post-injury): 10:15 AM

120 min post-injury VS
- HR: 73
- MAP: 45
- Temp: 35.6
- EtCO2: 44

Blood draw no. 7: (150 min post-injury): 10:45AM

150 min post-injury VS
- HR: 78
- MAP: 54
- Temp: 35.7
- EtCO2: 34

Blood draw no. 8: (180 min post-injury): 11:15AM

180 min post-injury VS
- HR: 67
- MAP: 62
- Temp: 34.9
- EtCO2: 43

Survival at 180 min? Yes
Target MAP attained ? NO
Time of death: 11:30 AM
Cause of death: Exsanguination from intentional transection of supradiaphragmatic IVC (euthanasia)
Interval from injury to death: 193 min

Post-treatment fluid data:
- Blood loss: 450.1 mL(suction) + 94 mL(sponge/lap) + 150.1 mL(clots) + 0 mL(foam) + 210 mL (phlebotomies) = 904.2 mL
- IV fluid given: LR (37°C): 1850 mL (infused) + 50 mL (ear vein) + 0 mL (jugular drip) = 1,900 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen minimally distended, primarily from GI tract; stomach was fairly distended (see Figs). Modest amounts of clot and some unclotted blood present in the RUQ (see Figs). Injury site was covered with a large adherent clot.
Volume foam recovered: NA
Clot-injury adhesion score = 4 (out of 4)

Heart: Not examined
Number of hepatic veins lacerated: 1 small, from LM lobe
Portal vein injury: 1 medium, to LM lobe
Other: none
Ex vivo liver wt: 60.6 g (resected LM lobe) + 725.6 (remaining liver) = 786.2 g
Tissue harvested: none.

VI. COMMENTS

Noncompressible injury, no treatment control; initial profound hypotension, but then recovered and survived easily to 180 minutes with ~700 mL blood loss attributable to injury, and good recovery of MAP. LM lobe was smallish size in today's subject. Thick lump of clot covering and adherent to injury site, with no active bleeding at 3 h.

VII. PLAN

Continue with this series on 10/01/2015.

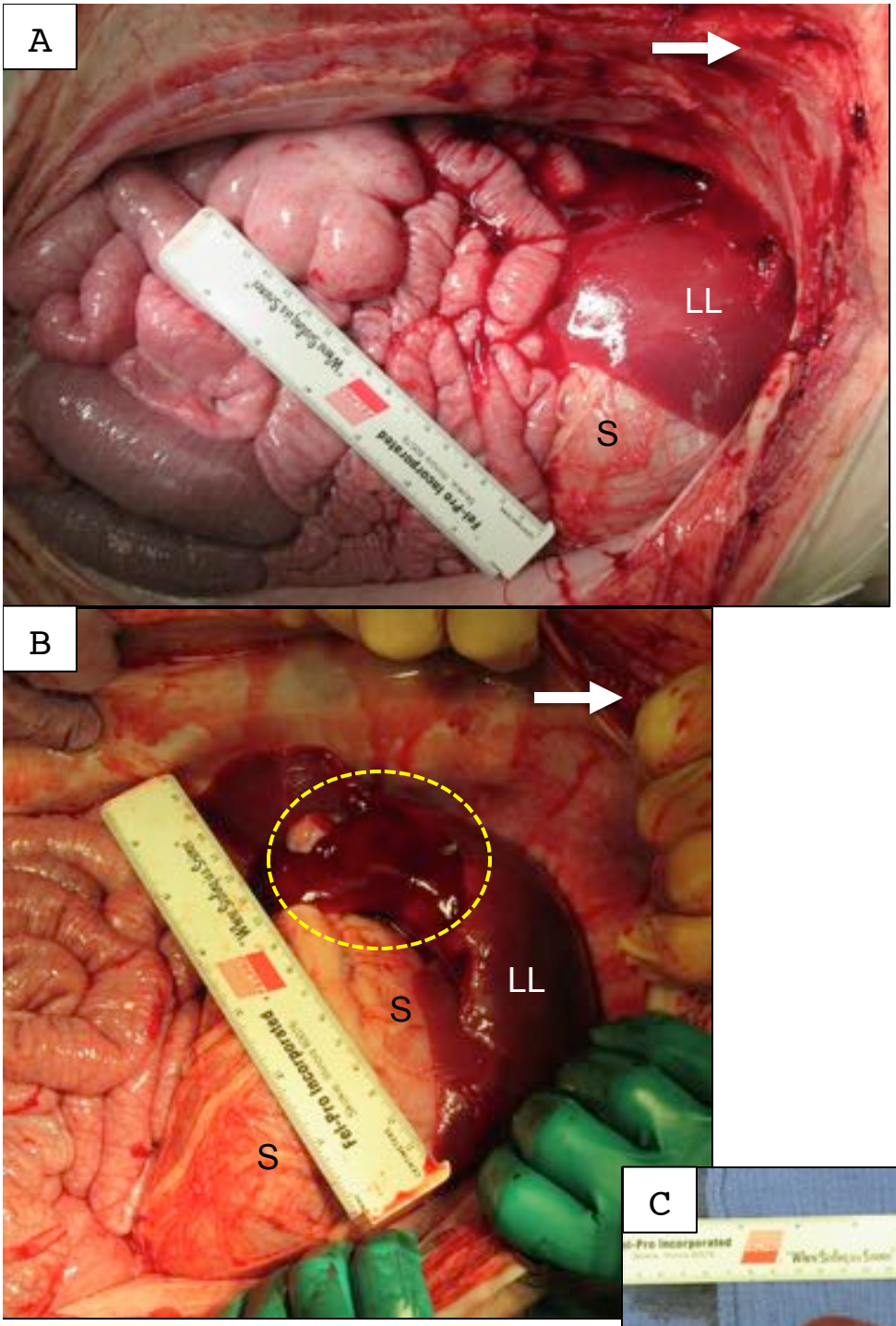


Fig 1, Swine 319.
(A) Immediately after 3 h observation. Animal alive & stable, MAP 50-60 mm Hg. Some clot in RUQ (asterisk), not excessive. Arrow = cephalad. S = stomach.
(B) Same view as panel A, after blood and loose clot were suctioned out. Injury site covered with adherent clot (dashed yellow oval).
(C) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with adherent clot (arrows).

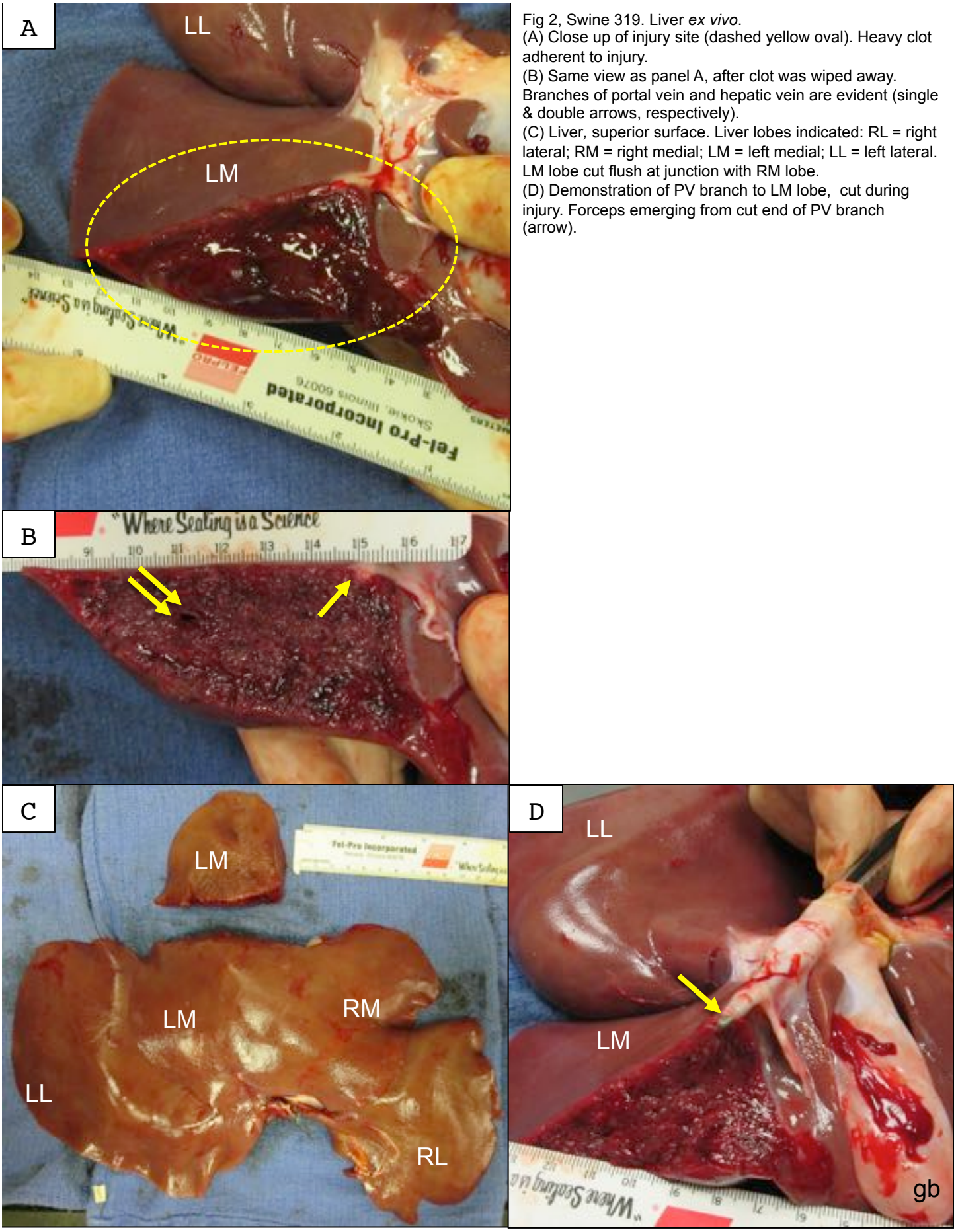


Fig 2, Swine 319. Liver *ex vivo*.
(A) Close up of injury site (dashed yellow oval). Heavy clot adherent to injury.
(B) Same view as panel A, after clot was wiped away. Branches of portal vein and hepatic vein are evident (single & double arrows, respectively).
(C) Liver, superior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial; LL = left lateral. LM lobe cut flush at junction with RM lobe.
(D) Demonstration of PV branch to LM lobe, cut during injury. Forceps emerging from cut end of PV branch (arrow).

I. OVERVIEW
Date: October 01, 2015
Swine no: 320
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: No Treatment
Personnel: Carlson, Hansen, Siford, Aravind

II. PRE-INJURY PHASE

Start time: 07:35 AM
Swine sex: Male
Date swine received from UNL Mead: 09/21/2015
Pre-procedure wt: 36.8 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 105
- MAP:109
- Temp: 37.1
- EtCO2: 41

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:07 AM
Spleen wt: 219.2 gm
LR (22°C) infused after splenectomy: 700 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 219.2 + 30 + 0 = 249.2 mL
- LR (22°C) infused (spleen replacement + incidental): 700 + 0 (ear vein/jugular drip)= 700 mL

Pre-injury VS
- HR: 91
- MAP: 130
- Temp: 37

- EtCO2 : 38

III. INJURY & TREATMENT PHASE

Time of injury: 8:15 AM
Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at the junction with the RM lobe with 4 cm-blade scissors, producing a combined portal/hepatic venous injury. Ventral midline incision was closed with towel clips.
Treatment formulation: None
Clotting factors: None
Technique: (see Figs) The target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the incision was rapidly closed with towel clips.
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.2 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.
Time resuscitation fluid began: 8:15AM (immediately after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:25 AM

10 min post-injury VS
- HR: 102
- MAP: 64
- Temp: 37
- EtCO2: 39

Blood draw no. 3: (30 min post-injury): 8:45 AM

30 min post-injury VS
- HR: 93
- MAP: 58
- Temp: 36.6
- EtCO2: 44

Blood draw no. 4: (60 min post-injury): 9:15AM

60 min VS
- HR: 85
- MAP: 65
- Temp: 36.3
- EtCO2: 40

Blood draw no. 5: (90 min post-injury): 09:45AM

90 min post-injury VS
- HR: 78
- MAP: 67
- Temp: 35.9
- EtCO2: 44

Blood draw no. 6: (120 min post-injury): 10:15 AM

120 min post-injury VS
- HR: 70
- MAP: 67
- Temp: 35.6
- EtCO2: 49

Blood draw no. 7: (150 min post-injury): 10:45AM

150 min post-injury VS
- HR: 78
- MAP: 54
- Temp: 35.7
- EtCO2: 34

Blood draw no. 8: (180 min post-injury): 11:15AM

180 min post-injury VS
- HR: 65
- MAP: 69
- Temp: 35
- EtCO2: 43

Survival at 180 min? Yes
Target MAP attained ? No
Time of death: 11:15 AM
Cause of death: Exsanguination from intentional transection of supradiaphragmatic IVC (euthanasia)
Interval from injury to death: 180 min

Post-treatment fluid data:
- Blood loss: 521.1 mL (suction) + 0 mL (sponge/lap) + 159 mL (clots) + 210 mL (phlebotomies) = 890.1 mL
- IV fluid given: LR (37°C): 1,900mL (jugular) + 0 mL (ear vein) = 1,900 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen minimally distended, primarily from GI tract. Modest amounts of clot and some unclotted blood present in the RUQ (see Figs). Injury site was covered with a very large adherent clot (see Figs).
Volume foam recovered: NA
Clot-injury adhesion score = 4 (out of 4)

Heart: Not examined
Number of hepatic veins lacerated: 1 large, to LM lobe.
Portal vein injury: 1 large branch, to LM lobe
Other: clots in arterial line (see Figs)
Ex vivo liver wt: 63.7 g (resected LM lobe) + 973.2 (remaining liver) = 1036.9 g
Tissue harvested: none

VI. COMMENTS

Noncompressible injury, no treatment control; survived easily to 180 minutes with ~680 mL blood loss attributable to injury, and good final MAP. Large lump of clot covering and adherent to injury site, with no active bleeding at 3 h. Subject had evidence of diffuse clotting, as noted by clot in arterial line (we have noted this before, but now are paying more attention to).

If the data continues to trend as indicated by these last two subjects, we may have to modify the model again to increase its severity, yet retain the ability to detect treatment effect.

With earlier iterations of the noncompressible model, we were able to obtain a ~50% 1 h mortality with a posterior-placed portohepatovenous injury (the left lateral lobe hemitransection), but unfortunately we could not detect a treatment effect with this model. So last year we transitioned to an anterior-placed injury (the left medial lobe transection), which initially appeared to yield a ~50% mortality with no treatment, and also appeared to detect a treatment effect with the calcium alginate foam + biologics.

The two survivals in subjects 319 & 320 seem to call the above observation into question, because no-treatment survival may be approaching survival with foam treatment. Before we make any firm conclusion, however, we should obtain an adequate number of animals in each treatment group for a formal statistical comparison. But we may determine that the model severity needs to be increased.

Since both recent clinical and TEG observations have indicated that the subjects are becoming *hypercoagulable* after injury, the next step may be to counter this hypercoagulable state—as opposed to making a larger injury. A larger injury may simply exsanguinate all subjects within the first 10-15 min without giving an opportunity to observe treatment effect. If we could make a moderate-sized injury that would be bleed continuously without clotting off from hypercoagulability, then we may have a better chance of seeing a treatment effect. The hypercoagulability may be countered by simply hemodiluting the subjects before injury, as we did before in hypothermic hemodiluted model.

VII. PLAN

Continue with these experiments in the first week of November. We will review data, discuss options, and generate a plan with an OR schedule.

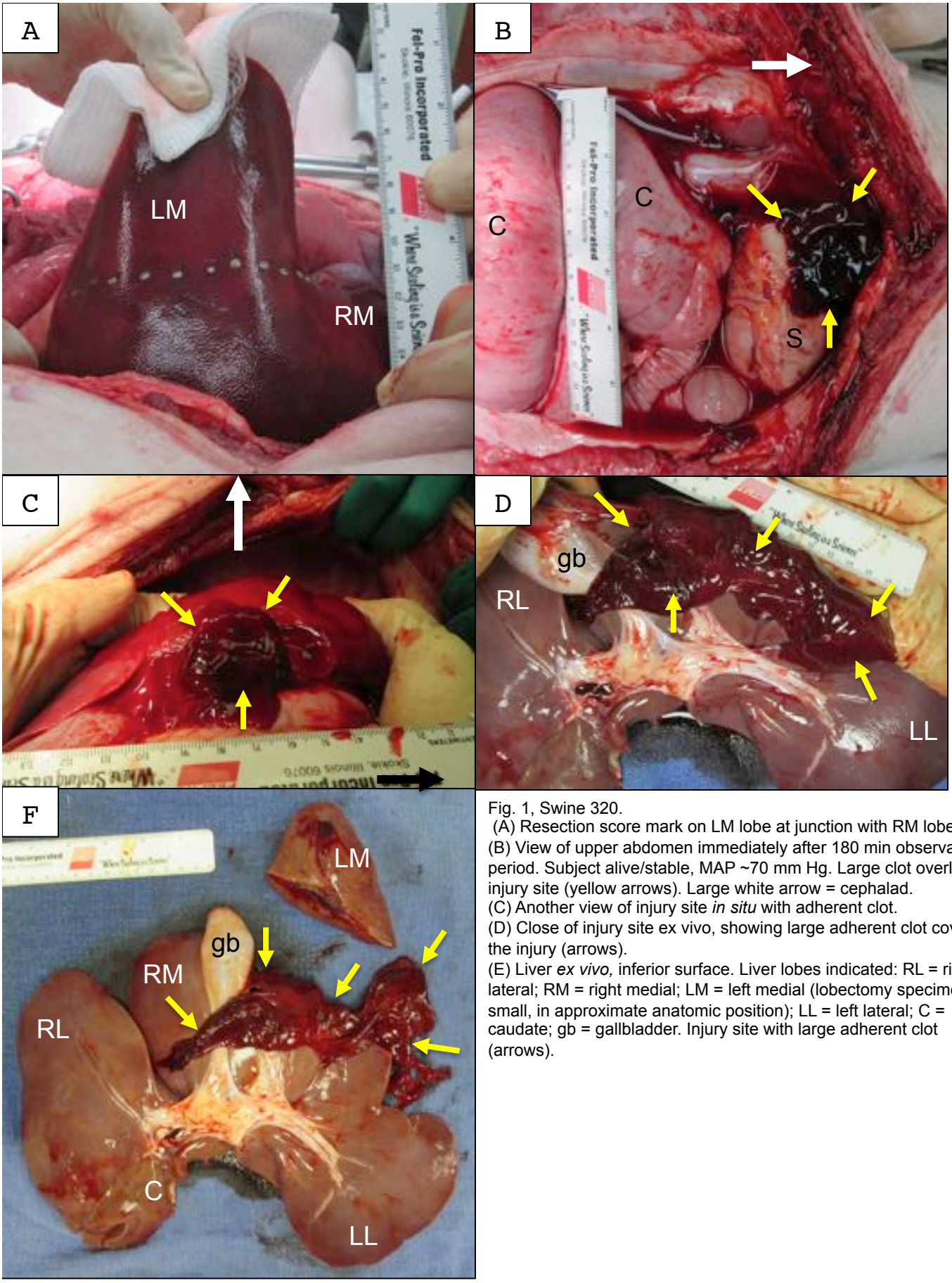


Fig. 1, Swine 320.
(A) Resection score mark on LM lobe at junction with RM lobe.
(B) View of upper abdomen immediately after 180 min observation period. Subject alive/stable, MAP ~70 mm Hg. Large clot overlying injury site (yellow arrows). Large white arrow = cephalad.
(C) Another view of injury site *in situ* with adherent clot.
(D) Close of injury site ex vivo, showing large adherent clot covering the injury (arrows).
(E) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, small, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with large adherent clot (arrows).

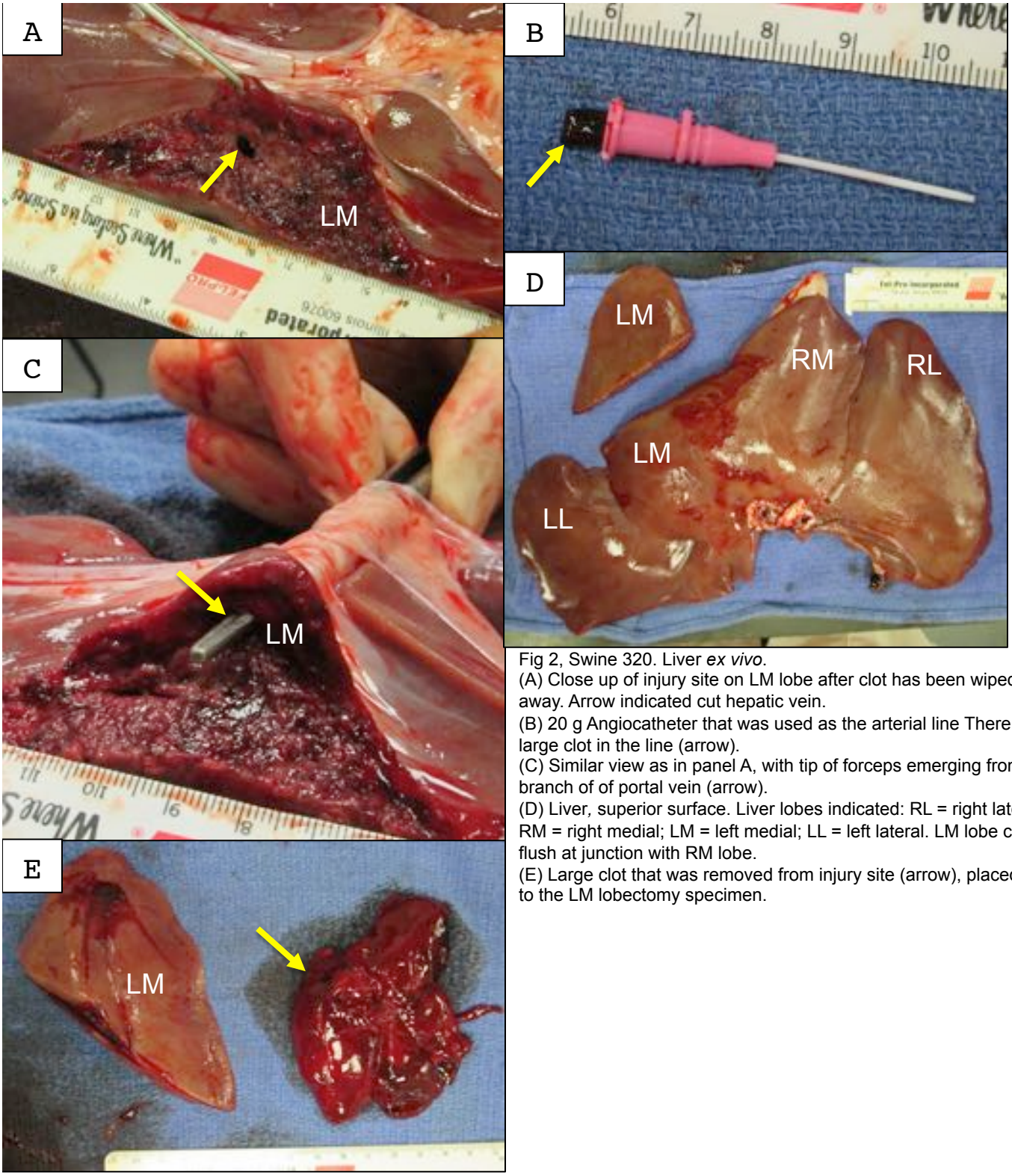


Fig 2, Swine 320. Liver *ex vivo*.
(A) Close up of injury site on LM lobe after clot has been wiped away. Arrow indicated cut hepatic vein.
(B) 20 g Angiocatheter that was used as the arterial line There was a large clot in the line (arrow).
(C) Similar view as in panel A, with tip of forceps emerging from cut branch of of portal vein (arrow).
(D) Liver, superior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial; LL = left lateral. LM lobe cut flush at junction with RM lobe.
(E) Large clot that was removed from injury site (arrow), placed next to the LM lobectomy specimen.

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Research Topic Area *	Hemorrhage Control & Resuscitation **Young Investigator Competition?** No
Abstract Category *	Oral Presentation
Title of Abstract *	Effect of crystalloid infusion rate in a porcine model of uncontrolled noncompressible intraabdominal hemorrhage
Abstract *	**May we publish your abstract?** Yes

(Abstract must contain background, methods, results, and conclusion section)

Background. To determine the effect of the rate of fluid administration on survival, vital signs, blood loss, and laboratory parameters in a porcine model of noncompressible hemorrhage. Methods. Twenty domestic swine (barrow, age 3 months, 32-36 kg) were anesthetized, had venous and arterial line placement, splenectomized through a midline incision, and then underwent hemitransection of the left lateral liver lobe (the noncompressible injury) without treatment. The incision was closed immediately after injury with towel clips. At 60 s after injury, warm Lactated Ringers solution was begun at either 150 or 20 mL/min IV (rapid and slow group, respectively, N = 10 per group); maximum volume was capped at 100 mL/kg. The rapid and slow group was monitored for 60 or 180 min, respectively, with continuous monitoring of vital signs and periodic lab draws. Results. Pre-injury parameters (body weight, vital signs, hematology panel, coagulation panel, blood gas analysis, and splenic weight) did not differ between the two groups. Survival after one hour in both the rapid and slow groups was 60%; no further death occurred in the slow group with observation out to 180 min. Notable differences between the rapid and slow groups for select endpoints are shown in Table 1. Necropsy demonstrated that an equivalent number of portal vein and hepatic vein branches had been transected in each group. There were no significant differences between groups for heart rate, temperature, total volume of LR infused, or liver weight. Conclusions. Although the two groups were not directly comparable (no formal randomization; longer observation time in the slow group), this study demonstrated that in a porcine model of noncompressible truncal hemorrhage, intravenous crystalloid resuscitation with a relatively slow infusion rate (20 mL/min) produced less blood loss and an improved laboratory profile (hemoglobin and protime), compared with resuscitation with a rapid infusion rate (150 mL/min). There was a nonsignificant trend of higher blood pressure in the slow group. While there was no effect on survival as reported, this endpoint might have been different if the rapid group had been followed for the same time period (180 min) as the slow group. This study supports the U.S. military's recent adoption of a "hypotensive resuscitation" protocol for warfighters injured in the field, which dictates that fluid resuscitation of an injured warfighter with hemorrhagic shock should be restricted until the subject arrives at a forward surgical unit. In other words, rapid/high-volume crystalloid resuscitation should be avoided in the early management of a subject with uncontrolled hemorrhage. Table 1. Comparison of select endpoints between the rapid and slow groups. Survival: 6/10 (Rapid) vs. 6/10 (Slow), p = 1.0; Final MAP (mm Hg): 28 ± 17 (Rapid) vs. 44 ± 22 (Slow), p = 0.2571; Blood Loss (mL): 2,852 ± 657 (Rapid) vs. 1,548 ± 371 (Slow), p = 0.0002; Protime, 15 min (s): 16.9 ± 5.1 (Rapid) vs. 11.3 ± 1.4 (Slow), p = 0.0011; Final Hb (g/dL): 3.4 ± 2.0 (Rapid) vs. 6.3 ± 2.4 (Slow); p = 0.0155

Learning Objectives *
(What should the attendee learn from this presentations? Enter at least 2, but no more than 3 learning objectives).

*__1.__ Concept of hypotensive resuscitation

*__2.__ Rate and volume may be important for crytalloid resuscitation

__3.__

Media Communications
(Provide an organizational point of contact for any media/public affairs questions related to your abstract/follow-on presentation)

Salutation: Dr.

First Name: Mark **Last Name:** Carlson

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Effect of Crystalloid Infusion Rate in a Porcine Model of Uncontrolled Noncompressible Intraabdominal Hemorrhage

U. R. Yanala, J. M. Johanning, I. I. Pipinos, G. Larsen,
W. H. Velander, M. A. Carlson

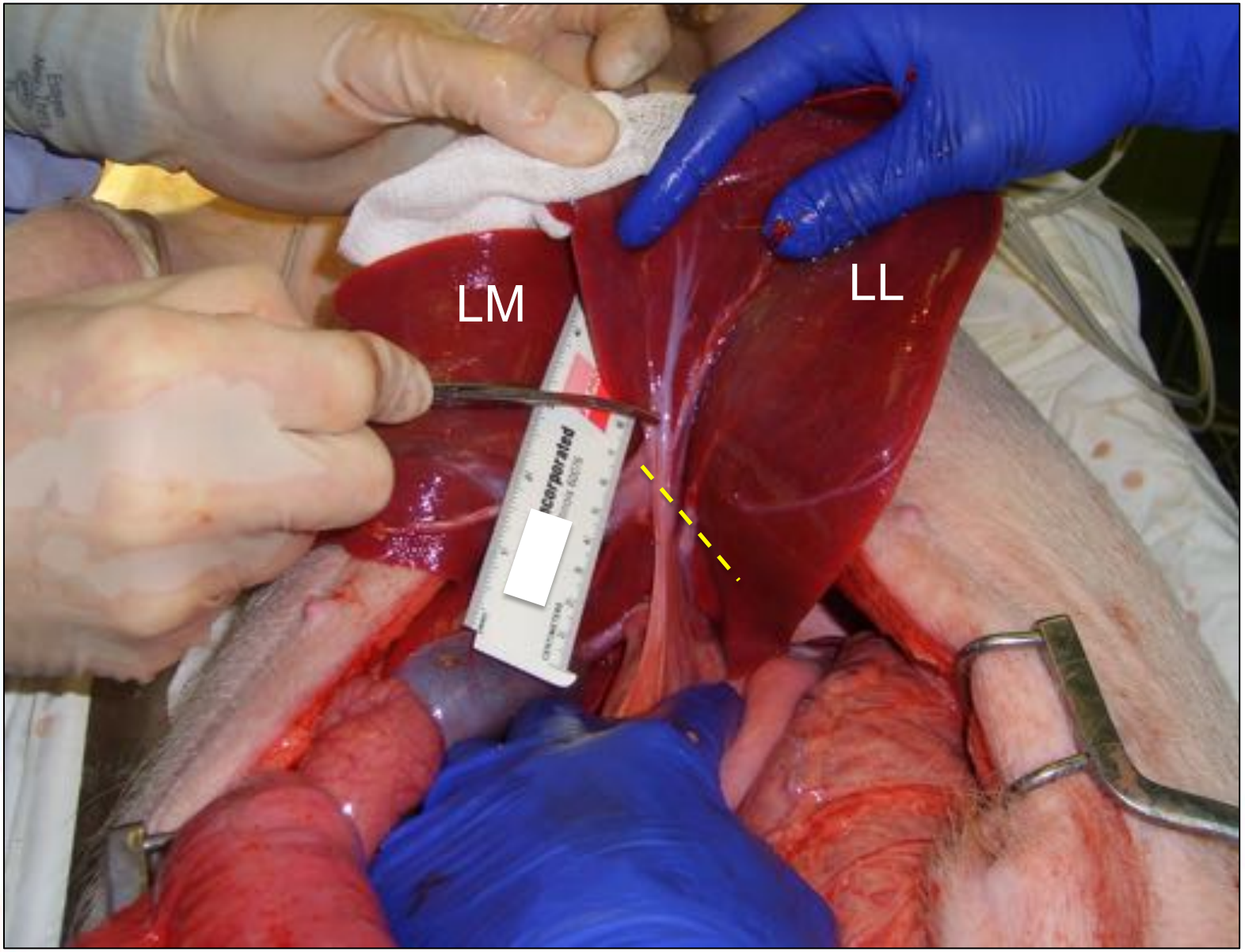
University of Nebraska Medical Center

CSA 2015, Chicago

Background

- Massive hemorrhage and traumatic brain injury each account for ~50% of early mortality on the modern battlefield.
- Intraabdominal hemorrhage is the leading cause of preventable death.
- Recent emphasis has been on “hypotensive resuscitation” as one modality to decrease mortality from hemorrhagic shock.

Injury Mechanism



- **AIM:** To compare the effect of crystalloid infusion rate in a model of uncontrolled intraabdominal hemorrhage.
- **Subjects:** Swine, 3 months old, (33–48 kg)
- **Target organ:** Liver
- **Injury model:** Hemitransection at the base of left lateral lobe (transection of 1 hepatic vein and 1-2 portal venous branches).

Injury Mechanism

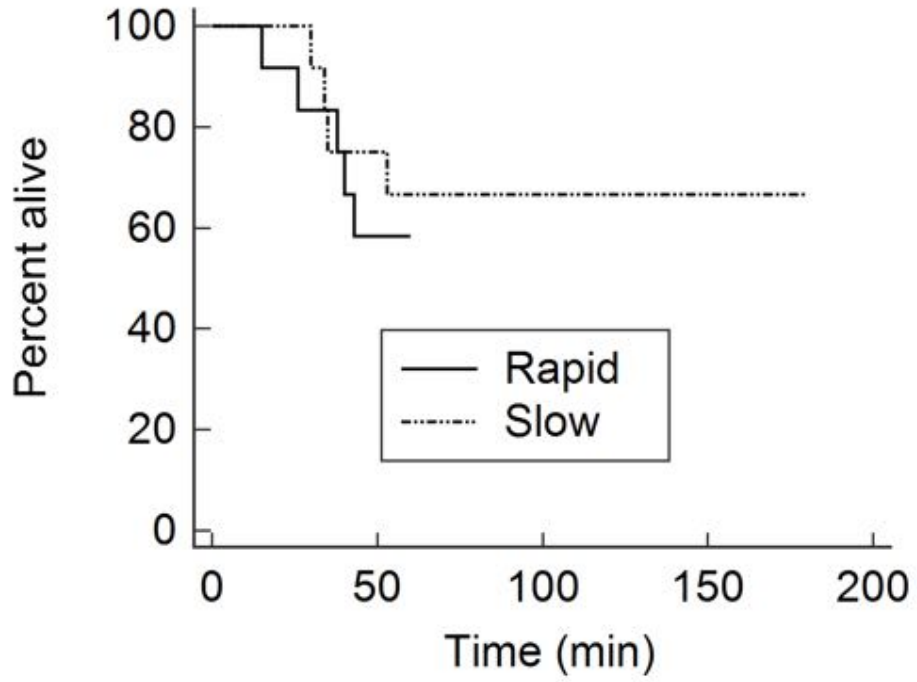
- Ventral midline incision
- Left lateral lobe of liver elevated
- Single scissors strike at base of left lateral lobe
- No topical/local treatment
- Incision closed rapidly with towel clips
- IV crystalloid resusc started 60 s post-injury

Resuscitation

- Lactated Ringers (LR), pre-warmed
- Two rates of infusion
    1. Rapid infusion (150 mL/min), “bolus”
    2. Slow infusion (20 mL/min)
- Same volume limit of LR for both groups (100 ml/kg, or 3.5 L for a 35 kg pig)

Results: Survival

- No difference in survival between the two groups
- Rapid infusion: 7/12 subjects survived
- Slow infusion: 8/12 subjects survived at 180 min



Results: Blood Loss & Fluids

Table 1. Blood loss, fluid input, liver weight, and lacerated veins.

	Blood loss (mL)		**Fluid input (mL)**		**Liver wt (g)**	**Veins Lacerated**	
Infusion Rate	Pre injury	Post injury	Pre injury	Post injury		Hepatic	Portal
Rapid	430 ± 151	2738 ± 693	1288 ± 458	3644 ± 523	852.9 ± 114.8	0.9 ± 0.3	1.3 ± 0.4
Slow	414 ± 48	1600 ± 360	1138 ± 181	3082 ± 1720	941.0 ± 127.3	1.0 ± 0.0	1.4 ± 0.5
P value*	0.297	**0.0001**	0.6861	0.8174	0.0833	0.4884	0.7075

All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Results: Vital Signs

Table 2. Vital sign data.

	MAP (mm Hg)			Heart rate (beats/min)			Temperature (°C)		
	0 min	15 min	60 min	Initial	15 min	60 min	Initial	15 min	60 min
Rapid	113 ± 14	55 ± 31	28 ± 16	111 ± 21	0.0885	113 ± 23	36.9 ± 0.6	36.5 ± 0.5	36.0 ± 0.6
Slow	105 ± 18	33 ± 17	39 ± 31	95 ± 19	110 ± 25	112 ± 23	36.3 ± 1.1	35.9 ± 1.0	35.1 ± 1.0
P value*	0.1489	0.141	0.5588	0.0885	0.7728	0.8471	0.1489	0.126	**0.0127**

MAP = Mean Arterial Pressure. All values are mean ± sd; time points are with respect to injury;
*Kruskal-Wallis test; significant tests are **bolded**.

Results: Hematology

Table 3. Hematologic testing results.

	Hemoglobin (g/dL)			Platelets (x 1,000/µL)		
Infusion Rate	0 min	15 min	60 min	Initial	15 min	60 min
Rapid	12.2 ± 0.9	4.6 ± 2.0	3.4 ± 2.3	306 ± 77	147 ± 66	119 ± 68
Slow	11.7 ± 0.9	8.4 ± 1.2	6.1 ± 2.0	315 ± 87	215 ± 55	173 ± 64
P value*	0.1939	**0.0002**	**0.0157**	0.729	**0.013**	**0.076**

All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Results: Coagulation

Table 4. Coagulation testing results.

	QFA (mg/dL)			Protime (s)			APTT (s)		
	0 min	15 min	60 min	Initial	15 min	60 min	Initial	15 min	60 min
Rapid	115 ± 13	41 ± 12	38 ± 11	11.5 ± 0.7	16.6 ± 4.7	17.0 ± 5.5	19.3 ± 1.9	22.6 ± 7.0	23.4 ± 8.1
Slow	109 ± 17	63 ± 9	53 ± 15	10.9 ± 0.6	11.5 ± 1.4	12.5 ± 2.4	18.3 ± 1.3	32.6 ± 7.9	33.8 ± 6.9
P value*	0.2253	**0.0004**	**0.0206**	0.106	**0.0005**	**0.0308**	0.1489	**0.0267**	**0.0339**

QFA = Quantitative Fibrinogen Assay; INR = International Normalized Ratio; APTT = Activated Partial Thromboplastin Time. All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Conclusions

- Slow infusion rate: improved outcome (blood loss, Hb, coagulation)
- Study not powered to detect a difference in survival
- Crystalloid infusion rate may be another consideration in the field resuscitation of a patient in hemorrhagic shock

Synthetic Resorbable vs. Cellulose Bandage for Minor Hemorrhage in a Porcine Model

U. R. Yanala, S. Noreiga, R. Spretz, J. Ragusa, L. Nunez, G. Larsen, M.A. Carlson.

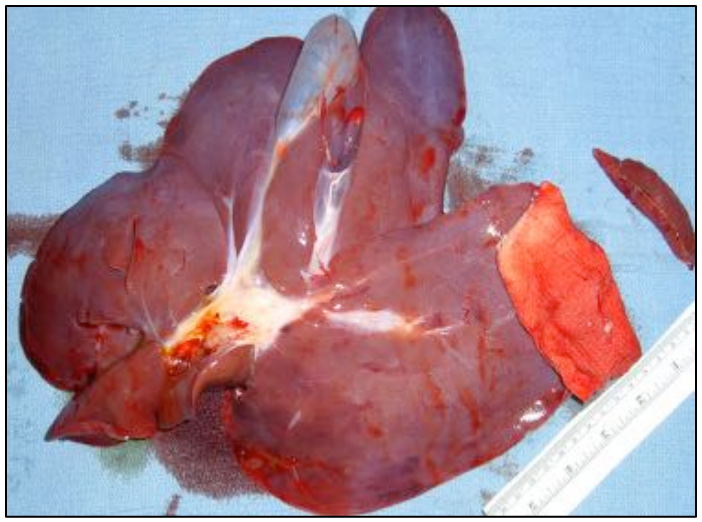
UNMC Nebraska Medicine

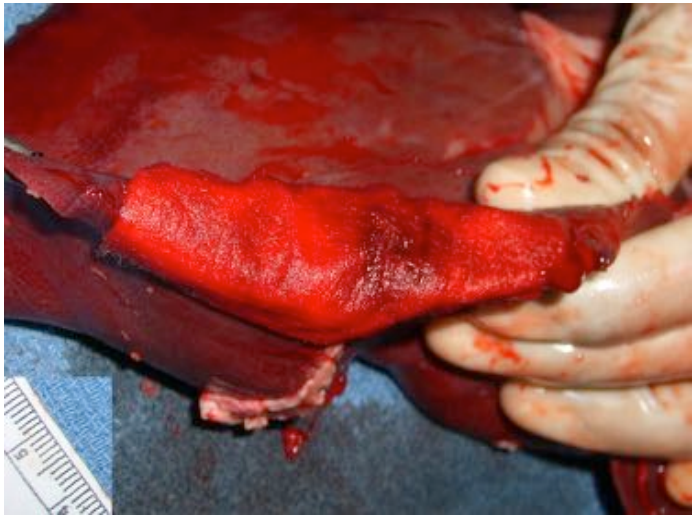
VA Nebraska-Western Iowa Health Care System

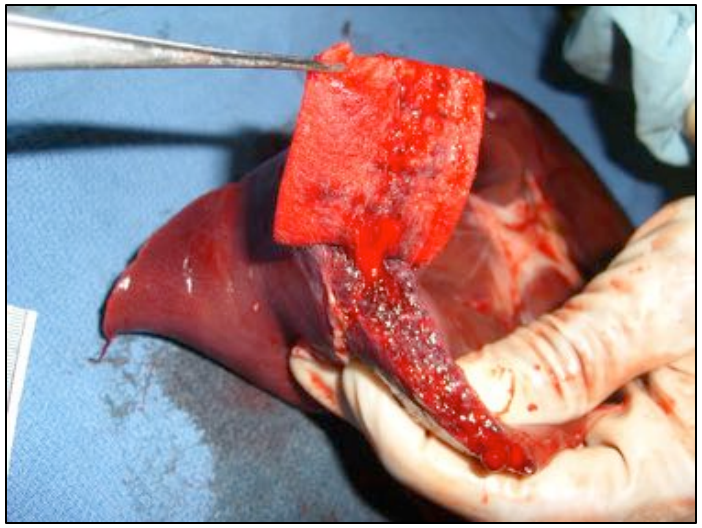
ASC 2015, Las Vegas.

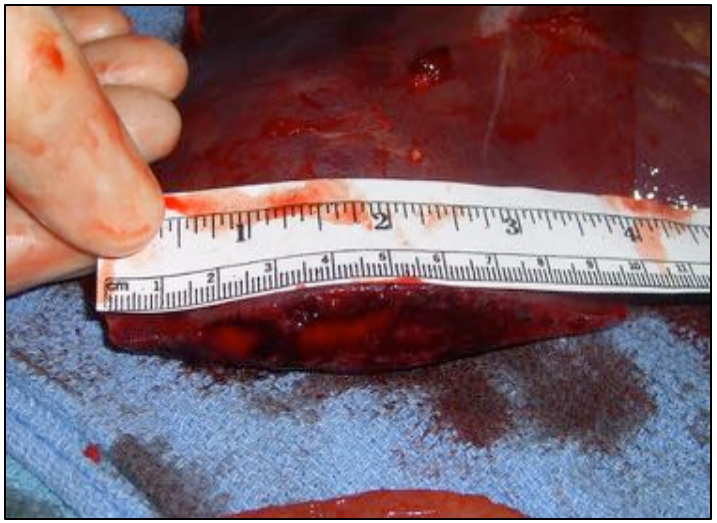
- **AIM:** To develop an economical alternative to currently existing topical resorbable hemostatic bandages.
- **SUBJECTS:** Swine, 3 months , 29-40 kg.
- **TEST BANDAGE:** Macro porous Polycaprolactone Mesh (PCL).
- **CONTROLS:** Oxidized Regenerated Cellulose (SURGICEL®, Ethicon®).
- **TARGET ORGAN:** Liver, left lateral lobe.

Methods:









Results:

Data (mean ± sd) from efficacy study at the 60 minute end-point.

Variable	PCL	ORC	Unpaired t-test
Resection mass (g)	7.6 ±1.9	6.7 ± 2.0	0.32
Blood Loss (mL)	93 ± 27	111 ± 55	0.38
LR Resuscitation (mL)	594 ± 425	1952 ± 1363	0.01*
MAP (mm Hg)	89 ± 8	93 ± 11	0.30
Hb (g/dL)	12.8 ± 1.2	12.9 ± 0.9	0.85
Platelets (1,000/μL)	266 ± 56	327 ± 94	0.11

PCL = Polycaprolactone; ORC = Oxidized Regenerated Cellulose; MAP = mean arterial pressure; Hb = hemoglobin; * = $p < 0.05$,

Conclusions:

- No significant differences in efficacy and toxicity between PCL & ORC bandages.
- PCL bandage could represent a lower cost alternative to ORC for the treatment of minor surgical bleeding.

Subject: 2016 ASC Abstract Submitted:ASC20161426

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Effect of Factor XIII in an ex vivo assay of hemostatic bandage adhesion

D. J. Zhou[1,2] , R. Spretz[1,3] , G. Larsen[3,4] , W. H. Velander[4] , M. A. Carlson[1,2] ; [1]University Of Nebraska Medical Center, Department Of Surgery, Omaha,NE,USA ; [2]VA Nebraska-Western Iowa Healthcare System, Omaha,NE,USA ; [3]LNK Chemsolutions, LLC, Lincoln,NE,USA ; [4]University Of Nebraska-Lincoln, Department Of Chemical & Biomolecular Engineering, Lincoln,NE,USA

Introduction:
Bandage adhesion to bleeding tissue in the setting of traumatic coagulopathy can be improved with fibrin glue (FG). The objective of this study was first to establish an ex vivo assay of bandage adhesion to liver and then to test our hypothesis that use of FG containing recombinant Factor XIII (rFXIII) would improve the adhesion strength (AS) of bandage glued to liver compared to FG without rFXIII.

Methods:
Customized FG (0.2 mL; 9 mg/mL plasma-derived fibrinogen, pdFI, + 106 U/mL r-thrombin + 0.36 mg/mL rFXIII) or commercial FG (0.2 mL Tisseel; Baxter), an FG that contains ~75 mg/mL pdFI and only trace pdFXIII, was applied to a 1×2 cm interface between custom electrospun polycaprolactone (PCL) mesh and a fresh porcine liver strip, and the interface was compressed with a 170 g weight for 5 min at 37°C (default setup). A T-peel adhesion test was performed with an Instron 5943 tensiometer with a 10 N load cell. Force vs. displacement data were used to calculate AS (N/cm), defined as average force during the peel divided by the interface width. AS data were compared with ANOVA ($\alpha<0.05$) and unpaired t-tests ($p<0.05$).

Results:
Using the default setup, AS of custom FG was ~2-fold greater by gluing the PCL mesh to the capsular surface of the liver vs. raw parenchyma (Fig. 1A), so use of the liver capsule was incorporated into the default setup. Neither capsular surface wetness (patted dry vs. pre-wet with PBS) nor prolonged compression time (5 vs. 10 min) affected AS (Fig. 1B). There appeared to be decreased AS with lower temperature during compression (25 vs. 37˚C), but this was not significant (Fig. 1B). Decreasing FG volume by 50% (0.05 vs. 0.1 mL/cm^2) resulted in a lower AS (Fig. 1B). Increasing FG volume beyond 0.1 mL/cm^2 was ineffective secondary to glue spillage during compression. Removing rFXIII from the default setup decreased AS by ~50%, but doubling the [rFXIII] did not increase AS (Fig. 1C). AS of customized FG vs. commercial FG was not different (Fig. 1C).

Conclusion:
An ex vivo adhesion test of synthetic resorbable mesh applied to porcine liver with customized FG was optimized with respect to liver surface qualities, adhesion compression time and temperature, and FG quantity. AS was augmented by rFXIII in the FG. The customized FG produced AS similar to that of commercial FG, despite the former having only ~1/8 the pdFI. The AS equivalence between these two FGs likely was a result of the added rFXIII to the customized FG, suggesting that efficacy testing of rFXIII addition to biologic hemostatic devices may be warranted.

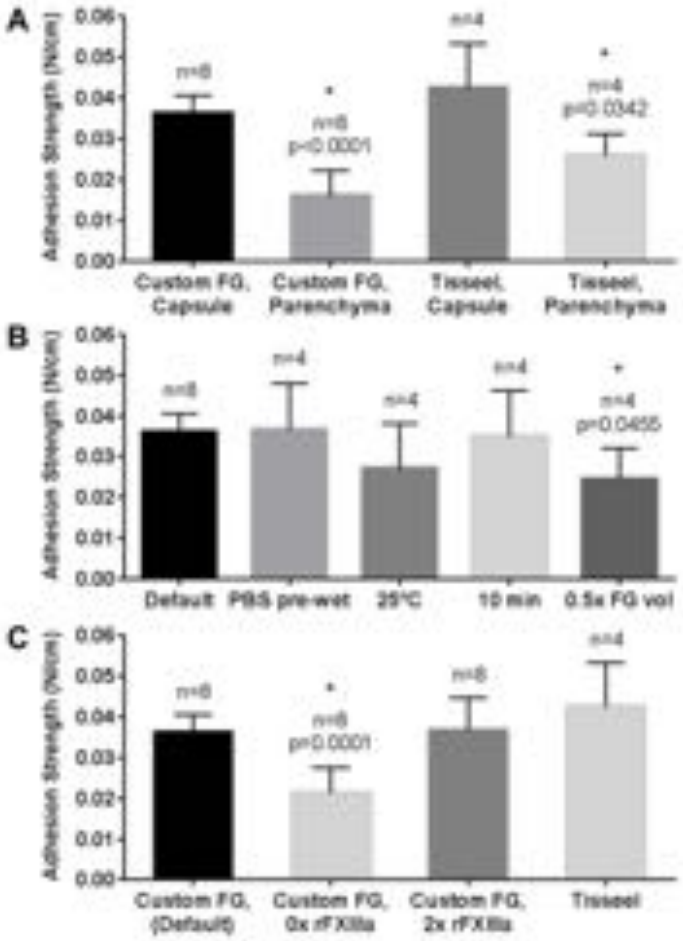


Fig. 1. AS data. *p < 0.05. Customized FG and Tisseel groups in A paired separately; groups in B compared pair-wise with Default; groups in C compared together via ANOVA.

Effect of Crystalloid Infusion Rate in a Porcine Model of Uncontrolled Noncompressible Intraabdominal Hemorrhage

U. R. Yanala, J. M. Johanning, I. I. Pipinos, G. Larsen,
W. H. Velander, M. A. Carlson

University of Nebraska Medical Center

CSA 2015, Chicago

Background

- Massive hemorrhage and traumatic brain injury each account for ~50% of early mortality on the modern battlefield.
- Intraabdominal hemorrhage is the leading cause of preventable death.
- Recent emphasis has been on “hypotensive resuscitation” as one modality to decrease mortality from hemorrhagic shock.

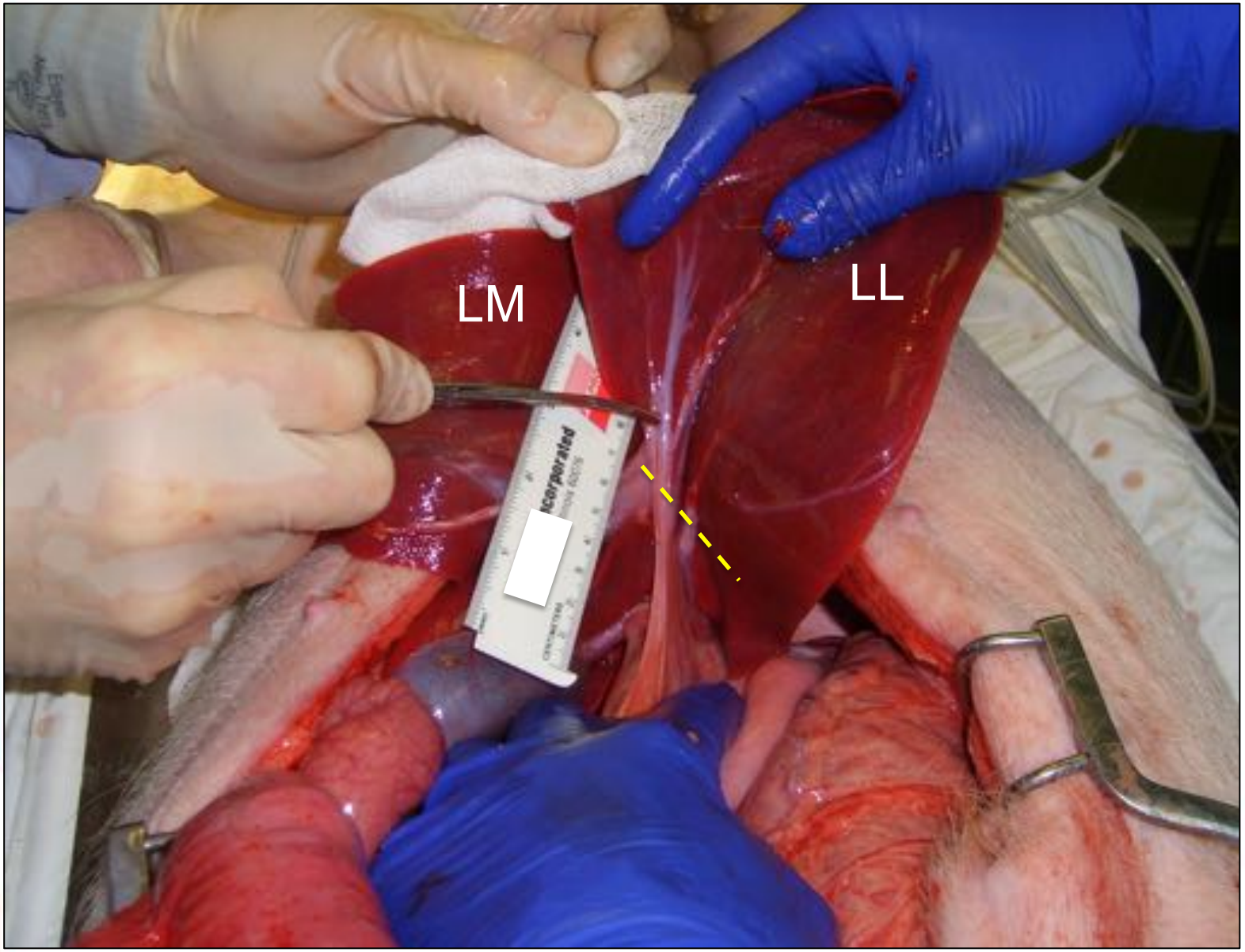
Current TCCC guidelines
(Tactical Combat Casualty Care)

- Blood is the fluid of choice for resuscitation of hemorrhagic shock.
- In the descending order of preference:
  1. Whole blood
  2. Plasma, RBC & Platelets (1:1:1 ratio)
  3. Plasma or RBC alone
  4. Hextend
  5. Crystalloids

Current TCCC guidelines

- In the absence of colloids, use crystalloids
- Crystalloids: 500 mL bolus infusions until one of the following is achieved.
  1. Palpable radial pulse
  2. Systolic blood pressure of 80-90 mm Hg
  3. Improved mental status.

Injury Mechanism



- **AIM:** To compare the effect of crystalloid infusion rate in a model of uncontrolled intraabdominal hemorrhage.
- **Subjects:** Swine, 3 months old, (33–48 kg)
- **Target organ:** Liver
- **Injury model:** Hemitransection at the base of left lateral lobe (transection of 1 hepatic vein and 1-2 portal venous branches).

Injury Model

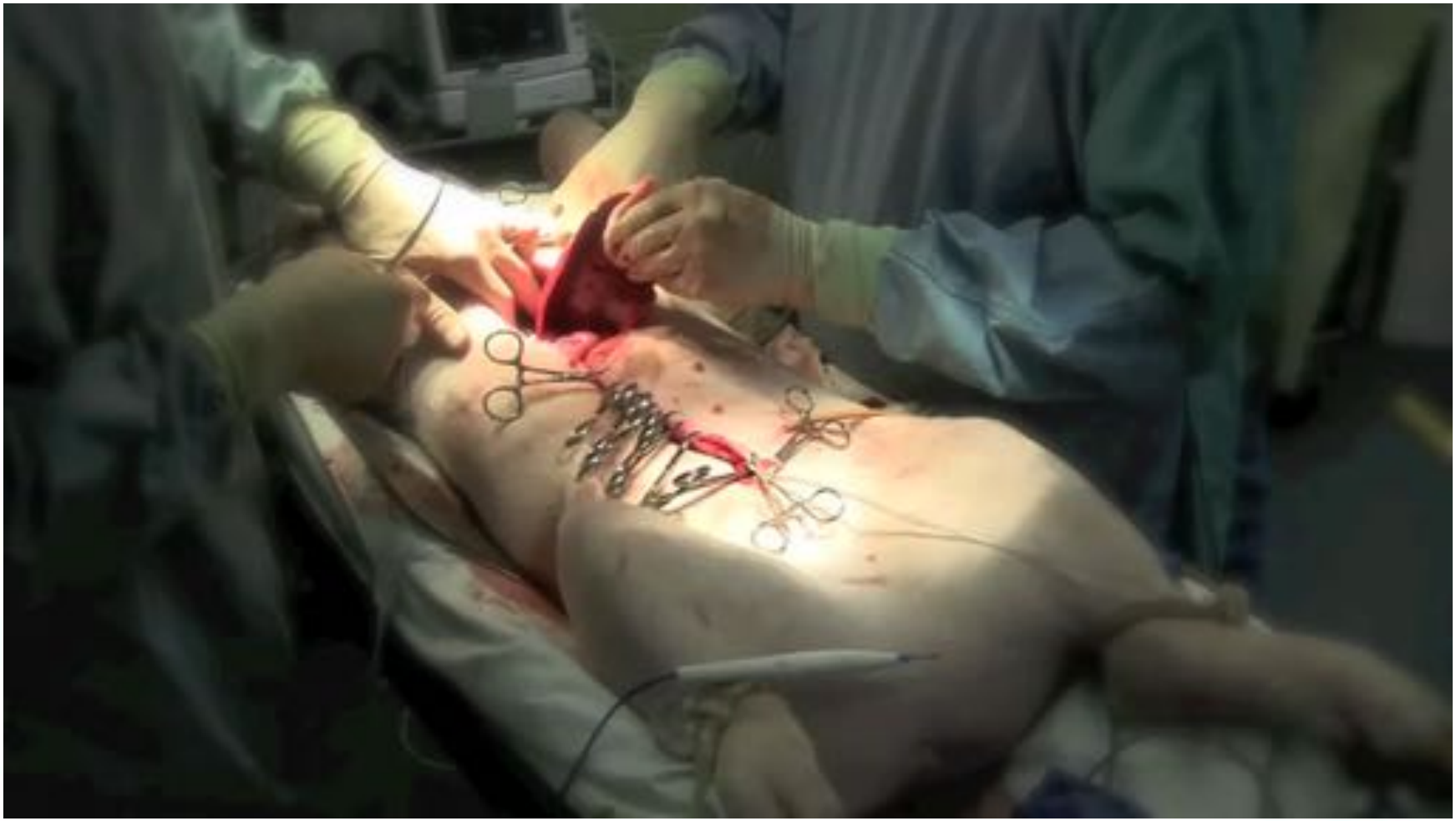
Injury model is intended to reflect a highly-fatal battle field injury that is:

- Truncal in location
- Inaccessible
- Uncontrollable
- Actively bleeding

Injury Mechanism

- Ventral midline incision
- Left lateral lobe of liver elevated
- Single scissors strike at base of left lateral lobe
- No topical/local treatment
- Incision closed rapidly with towel clips
- IV crystalloid resusc started 60 s post-injury

Injury Mechanism

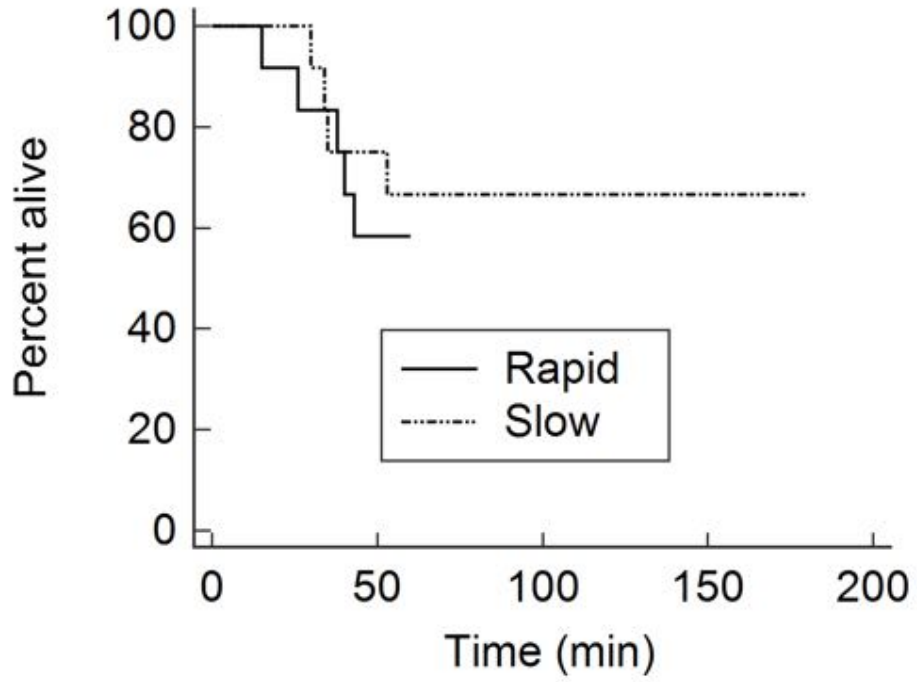


Resuscitation

- Lactated Ringers (LR), pre-warmed
- Two rates of infusion
    1. Rapid infusion (150 mL/min), “bolus”
    2. Slow infusion (20 mL/min)
- Same volume limit of LR for both groups (100 ml/kg, or 3.5 L for a 35 kg pig)

Results: Survival

- No difference in survival between the two groups
- Rapid infusion: 7/12 subjects survived
- Slow infusion: 8/12 subjects survived at 180 min



Results: Blood Loss & Fluids

Table 1. Blood loss, fluid input, liver weight, and lacerated veins.

	Blood loss (mL)		**Fluid input (mL)**		**Liver wt (g)**	**Veins Lacerated**	
Infusion Rate	Pre injury	Post injury	Pre injury	Post injury		Hepatic	Portal
Rapid	430 ± 151	2738 ± 693	1288 ± 458	3644 ± 523	852.9 ± 114.8	0.9 ± 0.3	1.3 ± 0.4
Slow	414 ± 48	1600 ± 360	1138 ± 181	3082 ± 1720	941.0 ± 127.3	1.0 ± 0.0	1.4 ± 0.5
P value*	0.297	**0.0001**	0.6861	0.8174	0.0833	0.4884	0.7075

All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Results: Vital Signs

Table 2. Vital sign data.

	MAP (mm Hg)			Heart rate (beats/min)			Temperature (°C)		
	0 min	15 min	60 min	Initial	15 min	60 min	Initial	15 min	60 min
Rapid	113 ± 14	55 ± 31	28 ± 16	111 ± 21	0.0885	113 ± 23	36.9 ± 0.6	36.5 ± 0.5	36.0 ± 0.6
Slow	105 ± 18	33 ± 17	39 ± 31	95 ± 19	110 ± 25	112 ± 23	36.3 ± 1.1	35.9 ± 1.0	35.1 ± 1.0
P value*	0.1489	0.141	0.5588	0.0885	0.7728	0.8471	0.1489	0.126	**0.0127**

MAP = Mean Arterial Pressure. All values are mean ± sd; time points are with respect to injury;
*Kruskal-Wallis test; significant tests are **bolded**.

Results: Hematology

Table 3. Hematologic testing results.

	Hemoglobin (g/dL)			Platelets (x 1,000/µL)		
Infusion Rate	0 min	15 min	60 min	Initial	15 min	60 min
Rapid	12.2 ± 0.9	4.6 ± 2.0	3.4 ± 2.3	306 ± 77	147 ± 66	119 ± 68
Slow	11.7 ± 0.9	8.4 ± 1.2	6.1 ± 2.0	315 ± 87	215 ± 55	173 ± 64
P value*	0.1939	**0.0002**	**0.0157**	0.729	**0.013**	**0.076**

All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Results: Coagulation

Table 4. Coagulation testing results.

	QFA (mg/dL)			Protime (s)			APTT (s)		
	0 min	15 min	60 min	Initial	15 min	60 min	Initial	15 min	60 min
Rapid	115 ± 13	41 ± 12	38 ± 11	11.5 ± 0.7	16.6 ± 4.7	17.0 ± 5.5	19.3 ± 1.9	22.6 ± 7.0	23.4 ± 8.1
Slow	109 ± 17	63 ± 9	53 ± 15	10.9 ± 0.6	11.5 ± 1.4	12.5 ± 2.4	18.3 ± 1.3	32.6 ± 7.9	33.8 ± 6.9
P value*	0.2253	**0.0004**	**0.0206**	0.106	**0.0005**	**0.0308**	0.1489	**0.0267**	**0.0339**

QFA = Quantitative Fibrinogen Assay; INR = International Normalized Ratio; APTT = Activated Partial Thromboplastin Time. All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Conclusions

- Slow infusion rate: improved outcome (blood loss, Hb, coagulation)
- Study not powered to detect a difference in survival
- Crystalloid infusion rate may be another consideration in the field resuscitation of a patient in hemorrhagic shock

University of Nebraska Medical Center

BREAKTHROUGHS FOR LIFE.®

Submitted abstract for the 2016 meeting of the Central Surgical Association (March 10-12, 2016 in Montreal, Quebec); total character count with spaces (including Table) = 2,976 (limit = 3,000)

TREATMENT OF NONCOMPRESSIBLE INTRAABDOMINAL HEMORRHAGE WITH RESORBABLE FOAM SUPPLEMENTED WITH CLOTTING FACTORS

Author list: Johnson, Yanala, Larsen, Fatemi, Johanning, Pipinos, Velander, Carlson

Purpose
To perform a preliminary analysis of the effect of a resorbable foam ± biologics (clotting factors) on survival and other endpoints in a porcine model of noncompressible hemorrhage.

Methods
Non-randomized anesthetized domestic swine (barrow, age 3 months, 30-35 kg; N = 22) underwent neck line placement, were splenectomized through a midline incision with placement of an intraabdominal pressure monitor, and then underwent resection of the left medial liver lobe (15-20% of hepatic mass). The abdomen was closed with towel clips, and treatment was injected intraabdominally through a separate stab incision, in four groups: (1) calcium alginate foam (CAF) + fibrin sealant (FS = plasma-derived fibrinogen + recombinant thrombin (rFII) and Factor XIII (rFXIII)); (2) CAF + rFII + rFXIII; (3) CAF alone; or (4) no treatment. The engineered foam injector nozzle produced a dual-phase foam, with CAF constituting the inner bulk, coated with a thin-layer of biologic-based foam. Warm Lactated Ringer's solution was given IV post-injury at 10 mL/min for mean arterial pressure (MAP) <80% of pre-injury MAP. Subjects were monitored (vitals signs and labs) for 3 h or death, followed by necropsy with blood loss measurement.

Results
Pre-injury data (body mass, splenic mass, blood loss, vital signs, hematology and coagulation testing, arterial blood gases) were not different among treatment groups. Intraabdominal pressure in groups 1-3 rose to ≤35 mm Hg during treatment injection, but then decreased gradually to <10 mm Hg by 30 min. Results for 3 h survival and final Shock Index (SI) were different among groups (Table 1). There were no significant differences in lab testing at the final time point (3 h or imminent death). Blood loss, liver weights (main organ or resection specimen), and number of transected hepatic and portal veins at necropsy also were not different among the four groups.

Conclusions
This preliminary analysis of injectable foam treatments for noncompressible intraabdominal hemorrhage suggested that injured subjects treated with CAF + biologics had improved survival and SI endpoints. These differences were not associated with differences in hematologic, coagulation, blood gas, or other endpoints. Development of injectable resorbable calcium alginate foams supplemented with biologics will continue, with the goal of producing a pre-hospital treatment for noncompressible intraabdominal hemorrhage.

Table 1. Endpoint assays at the final time point (3 h or death).

Endpoint	CAF + FS	CAF + FII/FXIII	CAF only	No Rx	*p*-value*
Survival (*n*/total)	7/7	6/7	1/4	2/4	**0.031**
SI (MAP/HR)	3.4 ± 1.2	2.2 ± 2.0	7.6 ± 4.3	4.8 ± 3.8	**0.035**
MAP (mmHg)	43 ± 20	59 ± 22	20 ± 19	35 ± 27	0.069
Hb (g/dL)	8.4 ± 2.8	7.8 ± 2.3	7.7 ± 0.7	7.4 ± 2.7	0.920
Base excess (mEq/L)	-6.6 ± 7.3	-1.4 ± 4.6	-3.2 ± 3.6	-9.0 ± 6.8	0.290
EtCO2 (mm Hg)	31 ± 14	30 ± 10	14 ± 14	20 ± 12	0.096
Blood Loss (mL)	1,553 ± 505	1,193 ± 374	1,487 ± 189	1,382 ± 622	0.500

Data are mean ± sd. *ANOVA or Kruskal Wallis for continuous data, chi-square for categorical.

Prehospital management of torso hemorrhage

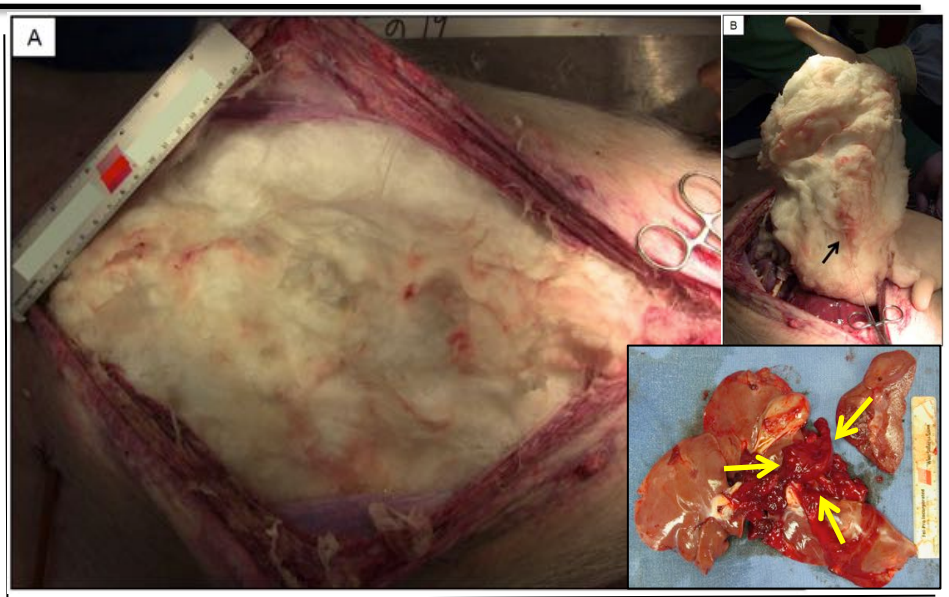
Focus: En Route Care (Lifesaving Interventions)

PI: Carlson, Mark A. **Org:** University of Nebraska Medical Center (UNMC)

NATIONAL STRATEGIC RESEARCH INSTITUTE at the University of Nebraska

Problem, Hypothesis and Military Relevance

- Noncompressible torso hemorrhage: 50% of battlefield mortality
- Current Rx: resuscitation + evacuation
- Hypothesis: expansile, biologically-active therapy injected intraabdominally will improve survival of severe noncompressible torso hemorrhage
- Military Relevance: reduction of early mortality from noncompressible torso hemorrhage with therapy given in field/en route



Proposed Solution

- Objective: develop therapy for noncompressible torso hemorrhage in a porcine model
- Proposed Rx: injectible expansile resorbable foam + human clotting factors (FI/FII/FXIII)
- Foam injected into peritoneal cavity, in field or en route; only minimal training required
- Expansile foam tamponades bleeding; clotting factors enhance foam adhesion; nontoxic, does not require later removal
- Outcome: field ready device to treat severe noncompressible torso hemorrhage

Timeline and Cost

Activities FY	16	17	18
Acute efficacy studies (pig model of noncompressible torso hemorrhage)			
Chronic toxicity evaluation (pig model of 30-day foam + factor intraabdominal implantation)			
Development of field delivery device (compact, simple, rapid)			
Estimated Budget ($K)	$250K	$150K	

Prehospital Management Of Torso Hemorrhage (Hemostatic Foam)

PI: Carlson, Mark A. (402-995-5371; macarlso@unmc.edu)

National Strategic Research Institute, 984238 Nebraska Medical Center, Omaha, NE 68198-4238

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402-555-5555
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Executive Summary. The problem to be studied in this proposal will be life-threatening intraabdominal hemorrhage for which there are no effective treatments; specifically, exsanguinating noncompressible hemorrhage from a penetrating-type injury to the abdomen. About half of early mortality after battlefield injury is secondary to uncontrolled hemorrhage; other early mortality mostly is secondary to traumatic brain injury. Battlefield injuries that result in exsanguination typically involve the trunk. In order to decrease early mortality from truncal injury, an effective therapy for uncontrolled hemorrhage from truncal injury is needed. This project will develop a injectable, expansile, resorbable foam, that also will contain multiple human clotting factors, for field/en route treatment of uncontrolled/noncompressible intraabdominal hemorrhage. This goal will be accomplished in an established preclinical (porcine) model of noncompressible intraabdominal hemorrhage.

Technical Approach

Project Goal: to optimize our current foam therapy (an injectable, expansile, resorbable foam supplemented with fibrinogen, thrombin, Factor VII, and/or Factor XIII) that has been efficacious in the treatment of noncompressible, exsanguinating liver injury.

Duration & Cost: 18 months; $400,000.

Description. Concurrent *in vitro* work will be done on all components of the foam therapy, along with efficacy testing in an established swine model of noncompressible hemorrhage (acute/nonsurvival), all at GLP level. Hemostasis will be achieved through combined effects of (1) tamponade from the expansile foam, (2) adhesion of the foam via a film of fibrin sealant, and (3) enhancement of the endogenous clotting cascade through the action of recombinant Factor XIII. In addition, a percutaneous/transabdominal delivery system for the foam will be developed, intended for field treatment of injured subjects with noncompressible truncal hemorrhage. A 30-day survival trial in swine will be performed at the end of the award period, in order to determine foam toxicity. Our preclinical studies will target the combination biologic product and medical device requirements recommended for the application pathway to clinical study, as published by the USFDA Office of Combination Products.

Preliminary Work in our laboratories to date includes *in vitro* and *in vivo* development and characterization of the following: (1) completely recombinant fibrin sealant; (2) plasma-derived fibrin sealant; (3) a variety of plasma-derived human proteins for use in hemostatic and acute wound applications, including fibronectin and gamma-gamma' fibrinogen; and (4) a variety of nanoengineered synthetic resorbable mesh materials for use as hemostatic bandages, both in combination with fibrin sealant or without. All of the manufacturing capabilities associated with these products have been developed by us and are mature. In addition, we have all of the requisite porcine hemostasis models (e.g., noncompressible intraabdominal hemorrhage, hemodiluted hypothermic hemorrhage, large vessel (aortofemoral) hemorrhage, elective hepatic resection) in place and working for the proposed research. We have numerous

articles in print/in press/in review/in preparation that cover all aspects of the above preliminary work.

Military Relevance. This project is directed at the focus area of En Route Care (Lifesaving Interventions) contained within the 2015 Air Force Surgeon General's Requirement GAP's: "Research to evaluate pre-hospital lifesaving interventions to include clinical outcomes and the use of novel pre-hospital resuscitation therapies/approaches." This technology of this project is intended to reduce prehospital mortality of severe noncompressible hemorrhage from torso injury with a injectable foam device intended for field/enroute use.

Capability, Experience, and Management

Key Personnel: the following group has collaborated since 2008 in the field of hemostasis, biologics, and biomaterials, and has all the necessary facilities, resources, and personnel presently assembled to perform the proposed work.

1. Mark A. Carlson, MD (PI). Professor of Surgery at UNMC; directs a porcine research lab at the Omaha VA Medical Center; experience with clinical surgery.
2. William H. Velander, PhD (Co-I). Professor in the Department of Chemical and Biomolecular Engineering at UNL; expertise in protein engineering and synthesis.
3. Gustavo Larsen (Co-I). Professor in the Department of Chemical and Biomolecular Engineering at UNL; expertise in design and fabrication of biocompatible materials.

DoD Experience. Preliminary work by the above investigators has been funded by a $5M DoD award ("Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound"; USAMRAA Contract No. W81XWH-11-1-0836), which will expire in September of 2016. The intent of this proposal is to optimize the technology developed through the above US Army award to obtain a field-ready device for treatment of noncompressible torso hemorrhage.

Uniqueness. Central to this successful research enterprise is our unique possession of a combination of cost-effective, customizable hemostatic technologies, including (1) resorbable synthetic foam and hemostatic fabric manufacturing; and (2) an abundance of recombinant coagulation proteins for enhanced surgical sealant formulation, that enables study in preclinical large animal models. Our coagulation protein biotechnology and swine hemorrhage models are fully developed and reliable due to investment in equipment, animal resources, and laboratory space by the United States Army. Our team is comprehensive and time-tested in its expertise and integrated efforts for solving these difficult but critically relevant hemorrhagic scenarios.

Cost Summary: Total Project $400,000

Activities FY	16	17	18
Acute efficacy studies (pig model of noncompressible torso hemorrhage)			
Chronic toxicity evaluation (pig model of 30-day foam + factor intraabdominal implantation)			
Development of field delivery device (compact, simple, rapid)			
Estimated Budget ($K)	$250K	$150K	

PREPROPOSAL

United States Special Operations Command
Broad Agency Announcement For Extramural Biomedical Research and Development
W81XWH-USSOCOM-BAA 15-1
Release Date: 15 April 2015

PI: Carlson, Mark A. (Surgery, UNMC)
Co-I: Velander, William H. (Chemical & Biomolecular Engineering, UNL)
Co-I: Larsen, Gustavo (Chemical & Biomolecular Engineering, UNL)

Title:
"Locoregional Treatment Of (1) Noncompressible Truncal Hemorrhage and (2) Coagulopathic Hemorrhage"

I. Problem to Be Studied [character count with spaces = 3,924]

The problem to be studied in this proposal will be life-threatening intraabdominal hemorrhage for which there are no effective treatments. Three topics related to exsanguinating intraabdominal hemorrhage will be addressed using our unique coagulation biotechnology and resorbable material engineering:

1. Exsanguinating noncompressible hemorrhage from a penetrating-type injury to the liver.
2. Exsanguinating hemorrhage from a hepatic injury in the setting of hypothermic hemodilutional coagulopathy (i.e., hemorrhage associated with the "coagulopathy of trauma").
3. Optimal fluid resuscitation in a warfighter with uncontrolled intraabdominal hemorrhage from a penetrating injury, who is >30 min away from a forward surgical unit and operative intervention.

About half of early mortality after battlefield injury is secondary to uncontrolled hemorrhage; other early mortality mostly is secondary to traumatic brain injury. Battlefield injuries that result in exsanguination typically involve the trunk; extremity injuries, on the other hand, usually can be controlled with established techniques (e.g., direct pressure or tourniquet. Once in a forward surgical unit, a subject with exsanguinating hemorrhage frequently will become hypothermic, hypocoagulable, and acidotic (sometimes known as the "lethal triad of trauma"), secondary to multiple factors including fluid replacement and clotting factor depletion. Intraoperative treatment of intraabdominal hemorrhage in such a patient who has developed this coagulopathy of trauma has been extremely difficult.

This proposal will be a continuation of our foundational research on exsanguinating hemorrhage and hemostasis, which currently is supported by DoD funds in the project "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound" (USAMRAA Contract No. W81XWH-11-1-0836). Thus, our efforts benefit from an established research team with the requisite experience for the proposed experimental approach. Most importantly, our team contains both the molecular level and device engineering design expertise needed to target efficacious therapies that can be manufactured with cost-effectiveness.

Work in our laboratories to date includes *in vitro* and *in vivo* development and characterization of the following: (1) completely recombinant fibrin sealant; (2) plasma-derived fibrin sealant; (3) a variety of plasma-derived human proteins for use in hemostatic and acute wound applications, including fibronectin and gamma-gamma' fibrinogen; and (4) a variety of nanoengineered synthetic resorbable mesh materials for use as hemostatic bandages, both in combination with fibrin sealant or without. All of the manufacturing capabilities associated with these products have been developed by us and are mature. In addition, we have all of the requisite porcine hemostasis models (e.g., noncompressible intraabdominal hemorrhage, hemodiluted hypothermic hemorrhage, large vessel (aortofemoral) hemorrhage, elective hepatic resection) in place and working for the proposed research. We have numerous articles in print/in press/in review/in preparation that cover all aspects of the above preliminary work.

Central to this successful research enterprise is our unique possession of a combination of cost-effective, customizable hemostatic technologies, including (1) resorbable synthetic foam and hemostatic fabric manufacturing; and (2) an abundance of recombinant coagulation proteins for enhanced surgical sealant formulation, that enables study in preclinical large animal models. As described above, our coagulation protein biotechnology and swine hemorrhage models are fully developed and reliable due to investment in equipment, animal resources, and laboratory space by the United States Army. Our team is comprehensive and time-tested in both its expertise and integrated efforts for solving these difficult but critically relevant hemorrhagic scenarios.

II. Theoretical Rationale, Scientific Methods, and Design [character count with spaces = 3,940]

Note: entire project is scheduled for 3 years; months cited below (1 to 36) refer to this time frame. Total cost (directs+ indirects) for entire project = $1.5M.

Aim 1: Foam Therapy For Noncompressible Hemorrhage

Goal: to advance our current foam therapy (an injectable, expansile, resorbable foam supplemented with fibrinogen, thrombin, Factor VII, and/or Factor XIII) that has been efficacious in the treatment of noncompressible, exsanguinating liver injury to the level of good laboratory practice (GLP).

Description: concurrent *in vitro* work will be done on all components of the foam therapy, along with efficacy testing in an established swine model of noncompressible hemorrhage (acute/nonsurvival), all at GLP level. Hemostasis will be achieved through combined effects of (1) tamponade from the expansile foam, (2) adhesion of the foam via a film of fibrin sealant, and (3) enhancement of the endogenous clotting cascade through the action of recombinant Factor XIII. In addition, a percutaneous/transabdominal delivery system for the foam will be developed, intended for field treatment of injured subjects with noncompressible truncal hemorrhage. A 30-day survival trial in swine will be performed at the end of the award period, in order to determine foam toxicity. For both Aim 1 and 2, our preclinical studies will target the combination biologic product and medical device requirements recommended for the application pathway to clinical study, as published by the USFDA Office of Combination Products.

Duration: 36 months (1-36).

Cost: 40% of total $1.5M budget = $600K

Milestones: *in vitro* optimization of current foam by month 12; nonsurvival efficacy testing by month 30; toxicity testing by month 36; completed data file for use in combined biologic and medical device FDA application by month 36.

Aim 2: Hypothermic Hemodilutional Hemorrhage

Goal: to develop a synthetic resorbable nanoengineered mesh with a high molecular surface area and coated with a thin layer of recombinant human fibrin sealant, for the treatment of exsanguinating hemorrhage from a liver injury in the setting of hypothermic hemodilutional coagulopathy.

Description: in the porcine model of hypothermic, hemodilutional hemorrhage, endogenous coagulation is poor/nonexistent; hence, bandages will not adhere to wounds, which is identical to the situation in a cold/coagulopathic human patient. Concurrent work on all components of the mesh-sealant composite test bandage will be performed, including *ex vivo* tensiometer testing to optimize the bandage-wound adhesion phenomena. Work then will progress to efficacy testing of the test bandage in an established swine model of hypothermic, hemodilutional intraabdominal hemorrhage (acute/nonsurvival). A 30-day survival trial in swine then will performed to determine bandage toxicity. Similar to Aim 1, the Aim 2 experiments will be at the GLP level.

Duration: 36 months (1-36).

Cost: 45% of total $1.5M budget = $675K

Milestones: in vitro bandage optimization by month 12; ex vivo optimization by month 24; nonsurvival efficacy testing by month 30; toxicity testing by month 36; completed data file for use in combined biologic and medical device FDA application by month 36.

Aim 3: Fluid Resuscitation

Goal: to determine the optimal fluid resuscitation regimen for uncontrolled exsanguinating intraabdominal hemorrhage in a subject who cannot undergo immediate laparotomy/intervention (e.g., a warfighter injured in the field). This Aim will utilize an established swine model of noncompressible hemorrhage to compare fluid resuscitation regimens that would vary by fluid type (crystalloid *vs*. Hetastarch *vs*. Albumin), infusion rate (rapid/unrestricted vs. slow/restricted), and total volume ("high" vs. "low").

Duration: 36 months (1-36).

Cost: 15% of total $1.5M budget = $225K

Milestones: fluid type comparison by month 12; infusion rate comparison by month 24; volume comparison by month 36.

III. Significance and/or Uniqueness of the Proposed Effort [character count with spaces = 3,327]

The significance of Aims 1 is the development of a therapy for noncompressible intraabdominal hemorrhage that can be administered in the prehospital setting; at present, no such therapy exists. In order to decrease early mortality from truncal injury, an effective therapy for uncontrolled hemorrhage from truncal injury is needed; this need is specifically stated in the FY15 USSOCOM BAA (p. 8, section 3.a). Aim 1 will develop a injectable, expansile, resorbable foam, that also will contain multiple human clotting factors, for field treatment of uncontrolled/noncompressible intraabdominal hemorrhage. This will be accomplished in an established preclinical (porcine) model of noncompressible intraabdominal hemorrhage.

The significance of Aim 2 is the development of a bandage therapy for treatment of hemorrhage in a cold, coagulopathic subject, a clinical scenario that is problematic during prolonged field care. At present, no effective local therapy for exists hypothermic, hemodiluted hemorrhage. Improved treatments for this clinical scenario is a priority in FY15 USSOCOM BAA (p. 8, section 2). Aim 2 will focus on the problem of coagulopathic hemorrhage in the hypothermic, hemodiluted subject, using a resorbable nanoengineered bandage that is coated with recombinant fibrin sealant for enhanced wound adhesion. This will be accomplished in an established preclinical (porcine) model of hypothermic hemodilutional hemorrhage.

A unique aspect of both Aims 1 and 2 is that a recombinant fibrin sealant containing fibrinogen, thrombin, and activated Factor XIII will be used in all hemostatic devices. The recombinant fibrin sealant forms a kinetically fast and dense thin film with extraordinary covalent cross-linking derived from a novel activated Factor XIII. Clots normally gain strong adherence to wounds via the transglutaminase activity of Factor XIII. This minimizes the amount of sealant, and therefore cost, needed to gain hemostasis. We uniquely possess an abundant supply of recombinant components in the fibrin sealant necessary to develop an optimized hemostatic device in a preclinical large animal model study. Together with our coagulation science, biochemical/material engineering, and surgical experience, we are uniquely enabled to work on the difficult hemorrhagic problems described in this preproposal.

The significance of Aim 3 is the intent to study and improve prehospital fluid resuscitation strategies. Field observations and some preclinical data have suggested that the patient or warfighter with hypovolemic shock in the prehospital setting will have a better chance of survival with restricted fluid resuscitation. In fact, the current TCCC Guidelines (October 28, 2014 version) recommend that if a subject is in shock and blood products are not available, then resuscitation fluid (hetastarch or crystalloid) should be given only in 500 mL boluses, with reassessment after each bolus, until improved mental status, a palpable radial pulse, or a systolic pressure of 80-90 mm Hg is obtained. Improvement of prehospital resuscitation strategies is a stated priority in FY15 USSOCOM BAA (p. 8, section 3.a). In Aim 3, various fluid resuscitations strategies will be compared in the preclinical (porcine) model of uncontrolled noncompressible intraabdominal hemorrhage utilized in Aim 1.

IV. Military Relevance and Impact [character count with spaces = 3,631]

The Research Area of Interest in the FY15 USSOCOM BAA 15-1 that is addressed by this Preproposal is "Damage Control Resuscitation" (p. 8, section 3.a), including "treatment strategies that address the following elements: hypotensive resuscitation, optimal fluid(s), uncomplicated shock, non-compressible hemorrhaging…"

The military relevance and impact of Aim 1 will be in the locoregional treatment of noncompressible truncal hemorrhage. Currently, no effective clinical therapy exists for this problem other than rapid evacuation to a forward surgical unit. Noncompressible hemorrhage is a stated priority in the FY15 USSOCOM BAA. An effective therapy for noncompressible truncal hemorrhage should improve early battlefield survival, because about half of early battlefield deaths in modern warfare have been attributed to uncontrolled hemorrhage. The cause of hemorrhage from many if not most of these fatalities has been truncal injury. Importantly, our approach is unique in its forward-looking engineering and coagulation biotechnology that enables creation of hemostatic solutions which will be cost-effective.

The military relevance and impact of Aim 2 is in the locoregional treatment of hemorrhage in the cold coagulopathic subject (i.e., a subject suffering from the coagulopathy of trauma, or the "lethal triad" of trauma), which is a problem that can develop during prolonged field care. Improved therapy for this clinical scenario is a stated priority in FY15 USSOCOM BAA (ibid.; also p. 8, section 2, "Prolonged Field Care). An effective therapy for hemorrhage in the cold, coagulopathic subject would be welcome tool for the trauma surgeon faced with this scenario, either in a military forward surgical unit or a civilian trauma center.

The military relevance and impact of Aim 3 is in the prehospital fluid resuscitation of the patient in hypovolemic shock. This scenario is of particular relevance to the injured warfighter, who may be injured on the battlefield in a hostile environment without rapid access to blood products or an operating room. In this circumstance it would be vital to know the optimal fluid resuscitation regimen. Improved prehospital fluid resuscitation strategies is a stated priority in FY15 USSOCOM BAA. While clinical and preclinical data have guided TCCC Guidelines to some extent in this area, more preclinical data is needed to inform fluid resuscitation strategy in the critically injured subject with uncontrolled hemorrhage who does not have immediate access to definitive therapy.

It is important to note that all of the proposed research described in this preproposal, along with any resultant hemostatic technology or devices, would have crossover applications to (1) non-combat military trauma and (2) the civilian sector. This would include hemorrhage incurred during military training or other non-combat activities, and also for management of hemorrhage from blunt injury in any setting. Of note, a market with great potential revenue from sales of hemostatic products based on the proposed research would be elective surgery, either within the military or in the civilian sector. Hemostatic devices developed from the results of the proposed research could be used in many elective open and minimally invasive surgical procedures, including thyroidectomy, hepatic resection, pulmonary resection, aortic aneurysm repair, carotid endarterectomy, lower extremity arterial bypass, splenorrhaphy, cholecystectomy, low anterior resection of the rectum, and any of a myriad of surgical procedures in which an effective, fibrin sealant-supplemented hemostatic aid would be useful.

V. Brief Description of Animal and/or Human Use [character count with spaces = 2,876]

No human subjects will be utilized in the proposed work. Swine will be used for the hemorrhage modeling. The assembled research team has over six years' experience with ~300 pigs in both nonsurvival and survival models of hemorrhage in the PI's laboratory, and has a well-established and collaborative relationship with the local IACUC. All of the models to be utilized in this proposal have already been developed, and can be immediately utilized to generate data.

Domestic swine (3 months old, 35-40 kg) will first undergo splenectomy and then standardized hepatic injury while under general anesthesia. Hepatic injury will be applied by (1) lobectomy; (2) stellate laceration of the dome with a special clamp; or (3) an electric drill with a 1.25 in bit, all under an existing protocol. After application of a specific test treatment, nonsurvival subjects will be followed for up to 180 min, and then euthanized while under deep general anesthesia by exsanguination. Nonsurvival subjects in Aim 2 will be made coagulopathic by a exchange of 60% of their blood volume with cold hetastarch solution under an existing protocol. Survival subjects will be recovered, supported as necessary by existing protocols (including

buprenorphine for analgesia), followed for 30 days, and then euthanized by exsanguination (IVC transection) while under deep general anesthesia.

It will be necessary to use swine in this research, because there are no computer or other virtual models which can replicate the *in vivo* environment of severe traumatic hemorrhage, with or without coagulopathy. The number of swine necessary for this proposal will be determined with a power analysis, using blood loss, MAP, and 180 min survival as the endpoints in the nonsurvival studies, and quantitative histology as the endpoint in the survival studies, with alpha set at 0.05 and power set at 0.80.

The Omaha VA Medical Center is an AAALAC-accredited facility. All animal research performed at the Omaha VAMC is in accordance with recommendations contained in the *Guide for the Care and Use of Laboratory Animals* (8^{th} ed.) from the National Research Council and the National Institutes of Health, and is approved by the Omaha VAMC IACUC (and also the DoD ACURO if the research is funded by the DoD). The Omaha VAMC employs a veterinarian (Ellis Jensen, DVM) to supervise veterinary care in the VA's Animal Research Facility (ARF). Dr. Jensen (or a DVM designate) is available for consultation seven days a week. Dr. Jensen tours the ARF and inspects the condition of the animals several times per week. In addition to the attending DVM, the VA has staff in the ARF which feed and otherwise maintain the animal subjects seven days per week. Any animal that is losing weight, not taking appropriate oral intake, and/or has signs of a wound complication, will be referred to the DVM and the PI for evaluation.

VI. Plans and Strategy for Translation, Implementation, and/or Commercialization
[character count with spaces = 1,537]

For applications that are amenable to controlled clinical study (e.g., hemostatic aids intended for elective surgical procedures), clinical trials can be designed to compared their efficacy with currently-available comparator devices. Such trials would require separate and additional funding.

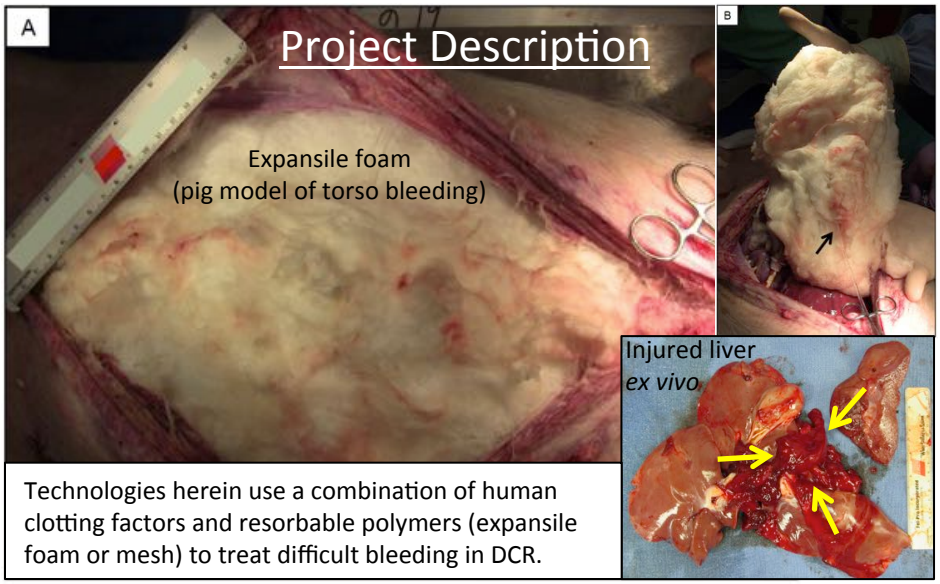
With regard to commercialization, one of the participating entities, LNK Chemsolutions LLC (a local engineering firm founded in 2000), will work to help bring the new hemostatic technologies to the market. The company has sold technology to, and contracted with Fortune 500 companies such as Kraft Foods North America and Philip Morris. It has several PCT and U.S. patents granted in the field of hemostatic technology and drug delivery, the trained personnel for designing commercial production equipment, and the pilot-scale engineering hardware to produce the proposed materials.

With regard to technology transfer, the Department of Defense will be able to partner with LNK Chemsolutions in the development and marketing of technologies that result from the proposed research. In addition, the company will also seek commercialization in the civilian market (with initial focus on straight licensing, or licensing with an exclusive fixed-term manufacturing deal) in partnership with entities having the market platform to deploy such products. Specifically, discussions are being held with entities such as Hemobras (Brazil's main plasma fractionator) and LFB (France, blood derivatives and antibodies producer) to pursue such opportunities.

Locoregional Treatment Of Noncompressible Truncal Hemorrhage and Coagulopathic Hemorrhage

(USSOCOM BAA 15-1)

Project Description



Technologies herein use a combination of human clotting factors and resorbable polymers (expansile foam or mesh) to treat difficult bleeding in DCR.

Technical Performance

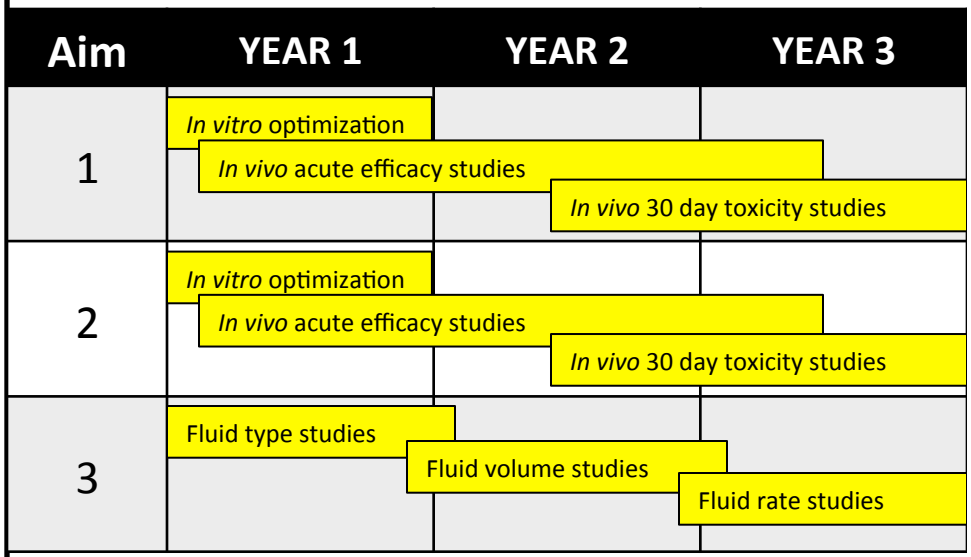
- Research Area: Damage Control Resuscitation (DCR)
- Aim 1: noncompressible hemorrhage (pigs), treated with expansile resorbable foam + clotting factors.
- Aim 2: cold coagulopathic hemorrhage (pigs), treated with resorbable nanoengineered mesh + clotting factors.
- Aim 3: optimization of nonblood fluid (type, amount, rate) for prehospital DCR, in pig model of torso bleeding.
- Dependencies: none. Experienced team with pig lab x 7 years, materials lab, and ++ access to human factors.
- Benefit to Special Operations Forces: stabilization of hemorrhagic subject in field/prolonged field care.

Cost ($K)

Aim	YEAR 1	YEAR 2	YEAR 3	Total
1	200	200	200	600
2	225	225	225	675
3	75	75	75	225
Totals	500	500	500	1,500

Organization: University of Nebraska
Principle Investigator (PI): Carlson, Mark A.
PI Phone Number: (402) 995-5371
PI E-mail Address: macarlso@unmc.edu

Schedule/Major Milestones



Deliverables: prototype with FDA filing at end Y3 for Aim 1 & 2; written report/recommendations for all Aims by end of Y3.

Preproposal For USAMRMC W81XWH-BAA-15-1
Version 2015-04-09

PI: Carlson, Mark A. (Surgery, UNMC)
Co-I: Velander, William H. (Chemical & Biomolecular Engineering, UNL)
Co-I: Larsen, Gustavo (Chemical & Biomolecular Engineering, UNL)

Title:
"Locoregional Treatment Of (1) Noncompressible Truncal Hemorrhage and (2) Coagulopathic Hemorrhage"

I. Problem to Be Studied [character count with spaces = 3,924]

The problem to be studied in this proposal will be life-threatening intraabdominal hemorrhage for which there are no effective treatments. Three topics related to exsanguinating intraabdominal hemorrhage will be addressed using our unique coagulation biotechnology and resorbable material engineering:

1. Exsanguinating noncompressible hemorrhage from a penetrating-type injury to the liver.
2. Exsanguinating hemorrhage from a hepatic injury in the setting of hypothermic hemodilutional coagulopathy (i.e., hemorrhage associated with the "coagulopathy of trauma").
3. Optimal fluid resuscitation in a warfighter with uncontrolled intraabdominal hemorrhage from a penetrating injury, who is >30 min away from a forward surgical unit and operative intervention.

About half of early mortality after battlefield injury is secondary to uncontrolled hemorrhage; other early mortality mostly is secondary to traumatic brain injury. Battlefield injuries that result in exsanguination typically involve the trunk; extremity injuries, on the other hand, usually can be controlled with established techniques (e.g., direct pressure or tourniquet. Once in a forward surgical unit, a subject with exsanguinating hemorrhage frequently will become hypothermic, hypocoagulable, and acidotic (sometimes known as the "lethal triad of trauma"), secondary to multiple factors including fluid replacement and clotting factor depletion. Intraoperative treatment of intraabdominal hemorrhage in such a patient who has developed this coagulopathy of trauma has been extremely difficult.

This proposal will be a continuation of our foundational research on exsanguinating hemorrhage and hemostasis, which currently is supported by DoD funds in the project "Technologies for Hemostasis and Stabilization of the Acute Traumatic Wound" (USAMRAA Contract No. W81XWH-11-1-0836). Thus, our efforts benefit from an established research team with the requisite experience for the proposed experimental approach. Most importantly, our team contains both the molecular level and device engineering design expertise needed to target efficacious therapies that can be manufactured with cost-effectiveness.

Work in our laboratories to date includes *in vitro* and *in vivo* development and characterization of the following: (1) completely recombinant fibrin sealant; (2) plasma-derived fibrin sealant; (3) a variety of plasma-derived human proteins for use in hemostatic and acute wound applications, including fibronectin and gamma-gamma' fibrinogen; and (4) a variety of nanoengineered synthetic resorbable mesh materials for use as hemostatic bandages, both in combination with fibrin sealant or without. All of the manufacturing capabilities associated with these products have been developed by us and are mature. In addition, we have all of the requisite porcine hemostasis models (e.g., noncompressible intraabdominal hemorrhage, hemodiluted hypothermic hemorrhage, large vessel (aortofemoral) hemorrhage, elective hepatic resection) in place and working for the proposed research. We have numerous articles in print/in press/in review/in preparation that cover all aspects of the above preliminary work.

Central to this successful research enterprise is our unique possession of a combination of cost-effective, customizable hemostatic technologies, including (1) resorbable synthetic foam and hemostatic fabric

manufacturing; and (2) an abundance of recombinant coagulation proteins for enhanced surgical sealant formulation, that enables study in preclinical large animal models. As described above, our coagulation protein biotechnology and swine hemorrhage models are fully developed and reliable due to investment in equipment, animal resources, and laboratory space by the United States Army. Our team is comprehensive and time-tested in both its expertise and integrated efforts for solving these difficult but critically relevant hemorrhagic scenarios.

II. Theoretical Rationale, Scientific Methods, and Design [character count with spaces = 3,844]

Note: entire project is scheduled for 4 years; months cited below (1 to 48) refer to this time frame. Total cost (directs+ indirects) for entire project = $5.0M.

Aim 1: Foam Therapy For Noncompressible Hemorrhage

Goal: to advance our current foam therapy (an injectable, expansile, resorbable foam supplemented with fibrinogen, thrombin, Factor VII, and/or Factor XIII) that has been efficacious in the treatment of noncompressible, exsanguinating liver injury to the level of good laboratory practice (GLP).

Description: concurrent *in vitro* work will be done on all components of the foam therapy, along with efficacy testing in an established swine model of noncompressible hemorrhage (acute/nonsurvival), all at GLP level. Hemostasis will be achieved through combined effects of (1) tamponade from the expansile foam, (2) adhesion of the foam via a film of fibrin sealant, and (3) enhancement of the endogenous clotting cascade through the action of recombinant Factor XIII. In addition, a percutaneous/transabdominal delivery system for the foam will be developed, intended for field treatment of injured subjects with noncompressible truncal hemorrhage. A 30-day survival trial in swine will be performed at the end of the award period, in order to determine foam toxicity. For both Aim 1 and 2, our preclinical studies will target the combination biologic product and medical device requirements recommended for the application pathway to clinical study, as published by the USFDA Office of Combination Products.

Duration: 48 months (1-48).

Cost: 50% of total $5.0M budget = $2.5M

Milestones: *in vitro* optimization of current foam by month 12; nonsurvival efficacy testing by month 36; toxicity testing by month 48; completed data file for use in combined biologic and medical device FDA application by month 48.

Aim 2: Hypothermic Hemodilutional Hemorrhage

Goal: to develop a synthetic resorbable nanoengineered mesh with a high molecular surface area and coated with a thin layer of recombinant human fibrin sealant, for the treatment of exsanguinating hemorrhage from a liver injury in the setting of hypothermic hemodilutional coagulopathy.

Description: in the porcine model of hypothermic, hemodilutional hemorrhage, endogenous coagulation is poor/nonexistent; hence, bandages will not adhere to wounds, which is identical to the situation in a cold/coagulopathic human patient. Concurrent work on all components of the mesh-sealant composite test bandage will be performed, including *ex vivo* tensiometer testing to optimize the bandage-wound adhesion phenomena. Work then will progress to efficacy testing of the test bandage in an established swine model of hypothermic, hemodilutional intraabdominal hemorrhage (acute/nonsurvival). A 30-day survival trial in swine then will performed to determine bandage toxicity. Similar to Aim 1, the Aim 2 experiments will be at the GLP level.

Duration: 48 months (1-48).

Cost: 40% of total $5.0M budget = $2.0M

Milestones: in vitro bandage optimization by month 12; ex vivo optimization by month 24; nonsurvival efficacy testing by month 42; toxicity testing by month 48.

Aim 3: Fluid Resuscitation

Goal: to determine the optimal fluid resuscitation regimen for uncontrolled exsanguinating intraabdominal hemorrhage in a subject who cannot undergo immediate laparotomy/intervention (e.g., a warfighter injured in the field). This Aim will utilize an established swine model of noncompressible hemorrhage to compare fluid resuscitation regimens that would vary by fluid type (crystalloid *vs*. Hetastarch *vs*. Albumin), infusion rate (rapid/unrestricted vs. slow/restricted), and total volume ("high" vs. "low").
Duration: 36 months (1-36).
Cost: 10% of total $5.0M budget = $0.5M
Milestones: fluid type comparison by month 12; infusion rate comparison by month 24; volume comparison by month 36.

III. Significance and/or Uniqueness of the Proposed Effort [character count with spaces = 3,211]

The significance of Aims 1 is the development of a therapy for noncompressible intraabdominal hemorrhage that can be administered in the prehospital setting; at present, no such therapy exists. In order to decrease early mortality from truncal injury, an effective therapy for uncontrolled hemorrhage from truncal injury is needed; this need is specifically stated in BAA 15-1 (section II.A.2; p. 9, paragraph ***a***). Aim 1 will develop a injectable, expansile, resorbable foam, that also will contain multiple human clotting factors, for field treatment of uncontrolled/noncompressible intraabdominal hemorrhage. This will be accomplished in an established preclinical (porcine) model of noncompressible intraabdominal hemorrhage.

The significance of Aim 2 is the development of a bandage therapy for treatment of hemorrhage in a cold, coagulopathic subject; at present, no effective local therapy for this problem exists. Improved treatments for this clinical scenario is a stated priority in BAA 15-1 (ibid.). Aim 2 will focus on the problem of coagulopathic hemorrhage in the hypothermic, hemodiluted subject, using a resorbable nanoengineered bandage that is coated with recombinant fibrin sealant for enhanced wound adhesion. This will be accomplished in an established preclinical (porcine) model of hypothermic hemodilutional hemorrhage.

A unique aspect of both Aims 1 and 2 is that a recombinant fibrin sealant containing fibrinogen, thrombin, and activated Factor XIII will be used in all hemostatic devices. The recombinant fibrin sealant forms a kinetically fast and dense thin film with extraordinary covalent cross-linking derived from a novel activated Factor XIII. Clots normally gain strong adherence to wounds via the transglutaminase activity of Factor XIII. This minimizes the amount of sealant, and therefore cost, needed to gain hemostasis. We uniquely possess an abundant supply of recombinant components in the fibrin sealant necessary to develop an optimized hemostatic device in a preclinical large animal model study. Together with our coagulation science, biochemical/material engineering, and surgical experience, we are uniquely enabled to work on the difficult hemorrhagic problems described in this preproposal.

The significance of Aim 3 is the intent to study and improve prehospital fluid resuscitation strategies. Field observations and some preclinical data have suggested that the patient or warfighter with hypovolemic shock in the prehospital setting will have a better chance of survival with restricted fluid resuscitation. In fact, the current TCCC Guidelines (October 28, 2014 version) recommend that if a subject is in shock and blood products are not available, then resuscitation fluid (hetastarch or crystalloid) should be given only in 500 mL boluses, with reassessment after each bolus, until improved mental status, a palpable radial pulse, or a systolic pressure of 80-90 mm Hg is obtained. Improvement of prehospital resuscitation strategies is a stated priority in BAA 15-1 (ibid.). In Aim 3, various fluid resuscitations strategies will be compared in the preclinical (porcine) model of uncontrolled noncompressible intraabdominal hemorrhage utilized in Aim 1.

IV. Military Relevance and Impact [character count with spaces = 3,222]

The military relevance and impact of Aim 1 will be in the locoregional treatment of noncompressible truncal hemorrhage. Currently, no effective clinical therapy exists for this problem other than rapid evacuation to a forward surgical unit. Noncompressible hemorrhage is a stated priority in BAA 15-1 (see section III). An effective therapy for noncompressible truncal hemorrhage should improve early battlefield survival, because about half of early battlefield deaths in modern warfare have been attributed to uncontrolled hemorrhage. The cause of hemorrhage from many if not most of these fatalities has been truncal injury. Importantly, our approach is unique in its forward-looking engineering and coagulation biotechnology that enables creation of hemostatic solutions which will be cost-effective.

The military relevance and impact of Aim 2 is in the locoregional treatment of hemorrhage in the cold coagulopathic subject (i.e., a subject suffering from the coagulopathy of trauma, or the "lethal triad" of trauma). Improved therapy for this clinical scenario is a stated priority in BAA 15-1 (see section III). An effective therapy for hemorrhage in the cold, coagulopathic subject would be welcome tool for the trauma surgeon faced with this scenario, either in a military forward surgical unit or a civilian trauma center.

The military relevance and impact of Aim 3 is in the prehospital fluid resuscitation of the patient in hypovolemic shock. This scenario is of particular relevance to the injured warfighter, who may be injured on the battlefield in a hostile environment without rapid access to blood products or an operating room. In this circumstance it would be vital to know the optimal fluid resuscitation regimen. Improved prehospital fluid resuscitation strategies is a stated priority in BAA 15-1 (see section III). While clinical and preclinical data have guided TCCC Guidelines to some extent in this area, more preclinical data is needed to inform fluid resuscitation strategy in the critically injured subject with uncontrolled hemorrhage who does not have immediate access to definitive therapy.

It is important to note that all of the proposed research described in this preproposal, along with any resultant hemostatic technology or devices, would have crossover applications to (1) non-combat military trauma and (2) the civilian sector. This would include hemorrhage incurred during military training or other non-combat activities, and also for management of hemorrhage from blunt injury in any setting. Of note, a market with great potential revenue from sales of hemostatic products based on the proposed research would be elective surgery, either within the military or in the civilian sector. Hemostatic devices developed from the results of the proposed research could be used in many elective open and minimally invasive surgical procedures, including thyroidectomy, hepatic resection, pulmonary resection, aortic aneurysm repair, carotid endarterectomy, lower extremity arterial bypass, splenorrhaphy, cholecystectomy, low anterior resection of the rectum, and any of a myriad of surgical procedures in which an effective, fibrin sealant-supplemented hemostatic aid would be useful.

V. Brief Description of Animal and/or Human Use [character count with spaces = 2,869]

No human subjects will be utilized in the proposed work. Swine will be used for the hemorrhage modeling. The assembled research team has over six years' experience with ~300 pigs in both nonsurvival and survival models of hemorrhage in the PI's laboratory, and has a well-established and collaborative relationship with the local IACUC. All of the models to be utilized in this proposal have already been developed, and can be immediately utilized to generate data.

Domestic swine (3 months old, 35-40 kg) will first undergo splenectomy and then standardized hepatic injury while under general anesthesia. Hepatic injury will be applied by (1) lobectomy; (2) stellate laceration of the dome with a special clamp; or (3) an electric drill with a 1.25 in bit, all under an existing protocol. After application of a specific test treatment, nonsurvival subjects will be followed for up to 180 min, and then euthanized while under deep general anesthesia by exsanguination. Nonsurvival subjects in Aim 2 will be made coagulopathic by a exchange of 60% of their blood volume with cold hetastarch solution under an existing protocol. Survival subjects will be recovered, supported as necessary by existing protocols (including

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VI. Plans and Strategy for Translation, Implementation, and/or Commercialization
[character count with spaces = 1,540]

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2015 Update on Hemostasis Project

Mark A. Carlson, MD

University of Nebraska Medical Center
VA Nebraska Western Iowa Health Care System

Omaha, Nebraska, USA

ISR, San Antonio, May 29, 2015

I. Noncompressible Torso Hemorrhage

Experimental Design

- Arterial/jugular lines, splenectomy/warm LR replacement
- Noncompressible liver injury (LM lobectomy)
- Begin treatment at 1 min post-injury
- Resuscitate with 10 mL/min warm LR
- Monitor x 180 min

Treatment groups

1. No treatment
2. Calcium alginate foam alone (no clotting factors)
3. Calcium alginate foam + Factors IIa & XIIIa
4. Calcium alginate foam + FS (fibrinogen/IIa/XIIIa)

Injury Mechanism

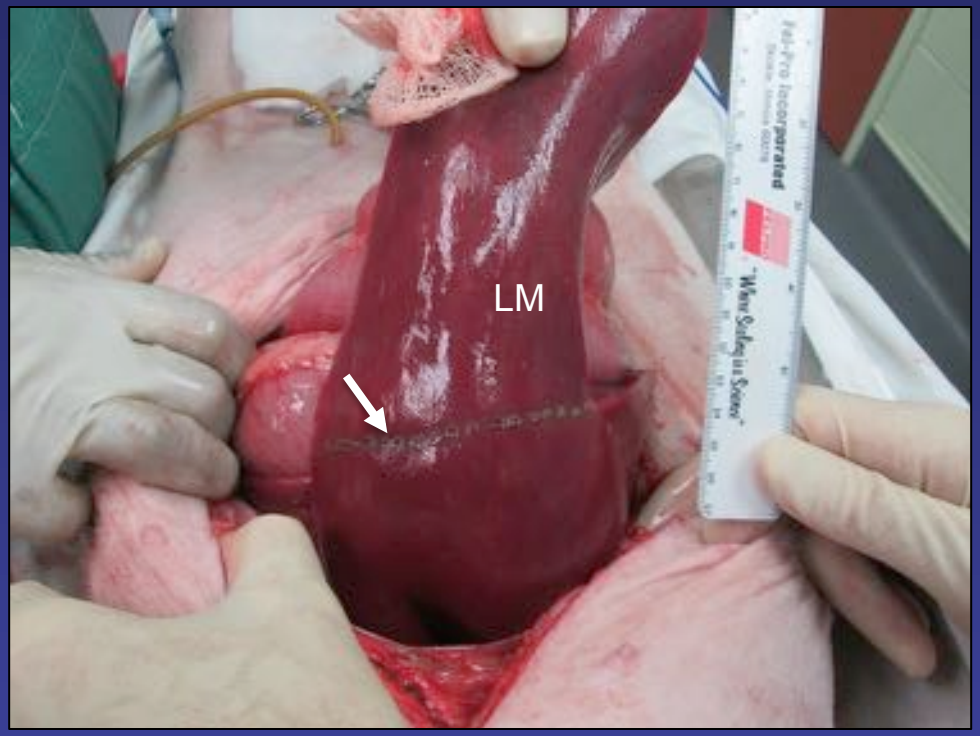


Figure 1, swine 241. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).

Injury Mechanism

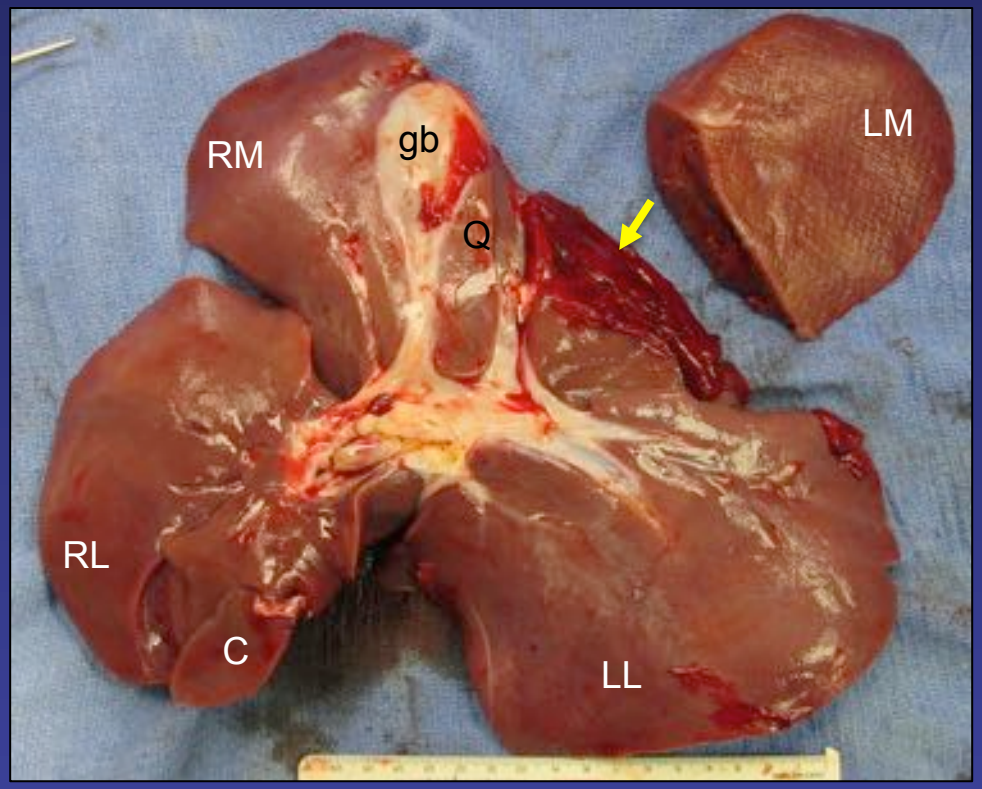


Figure 4, swine 246. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Arrow = large clot overlying surface of injury. Anterior is at top of image.

Injury Mechanism

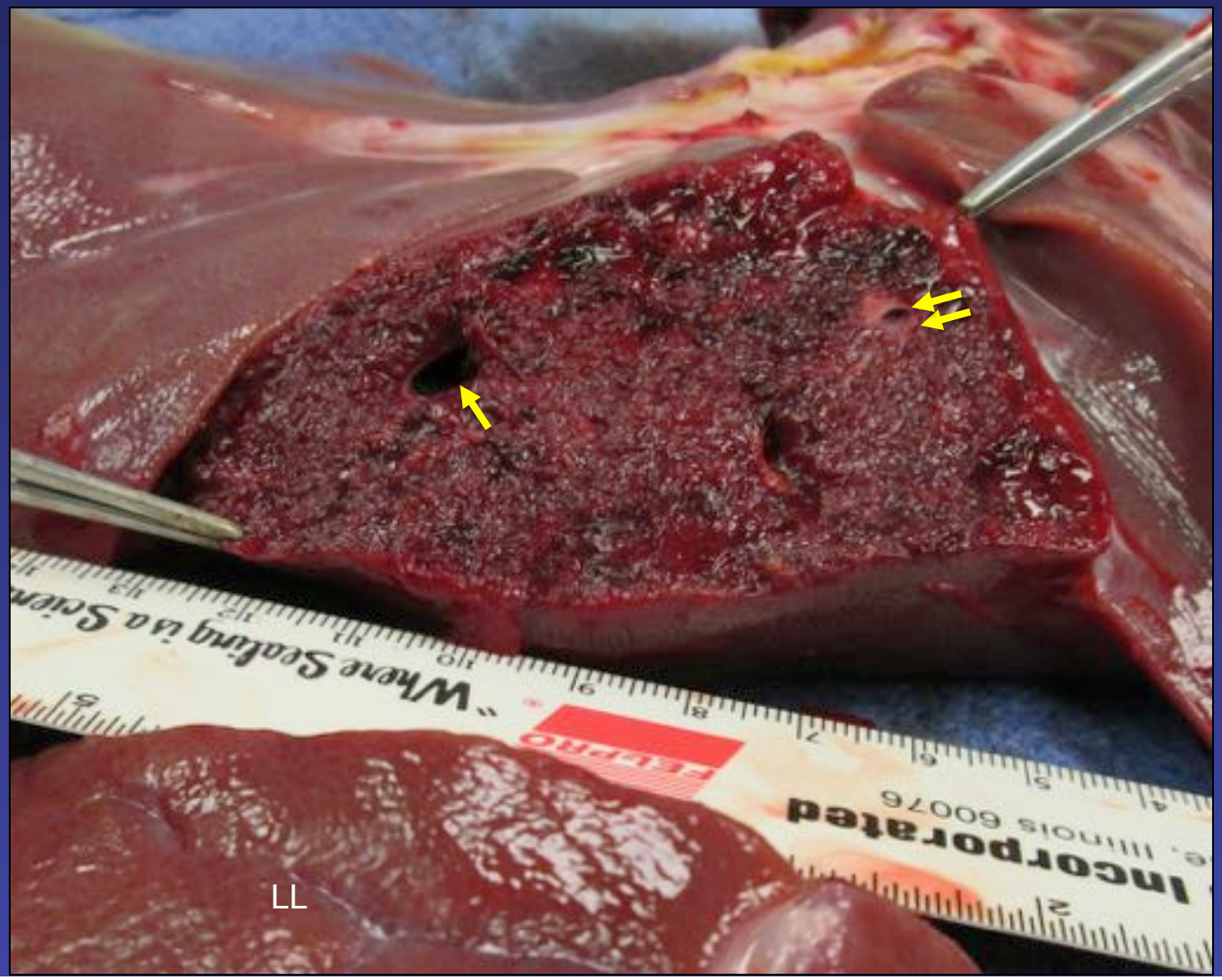


Figure 5, swine 246. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). The clot has been removed from the cut liver surface. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates orifice of transected hepatic vein to LM lobe; double arrow indicates orifice of transected PV branch to LM lobe. These transected veins were all occluded by clot at the time of necropsy.

Foam Injector

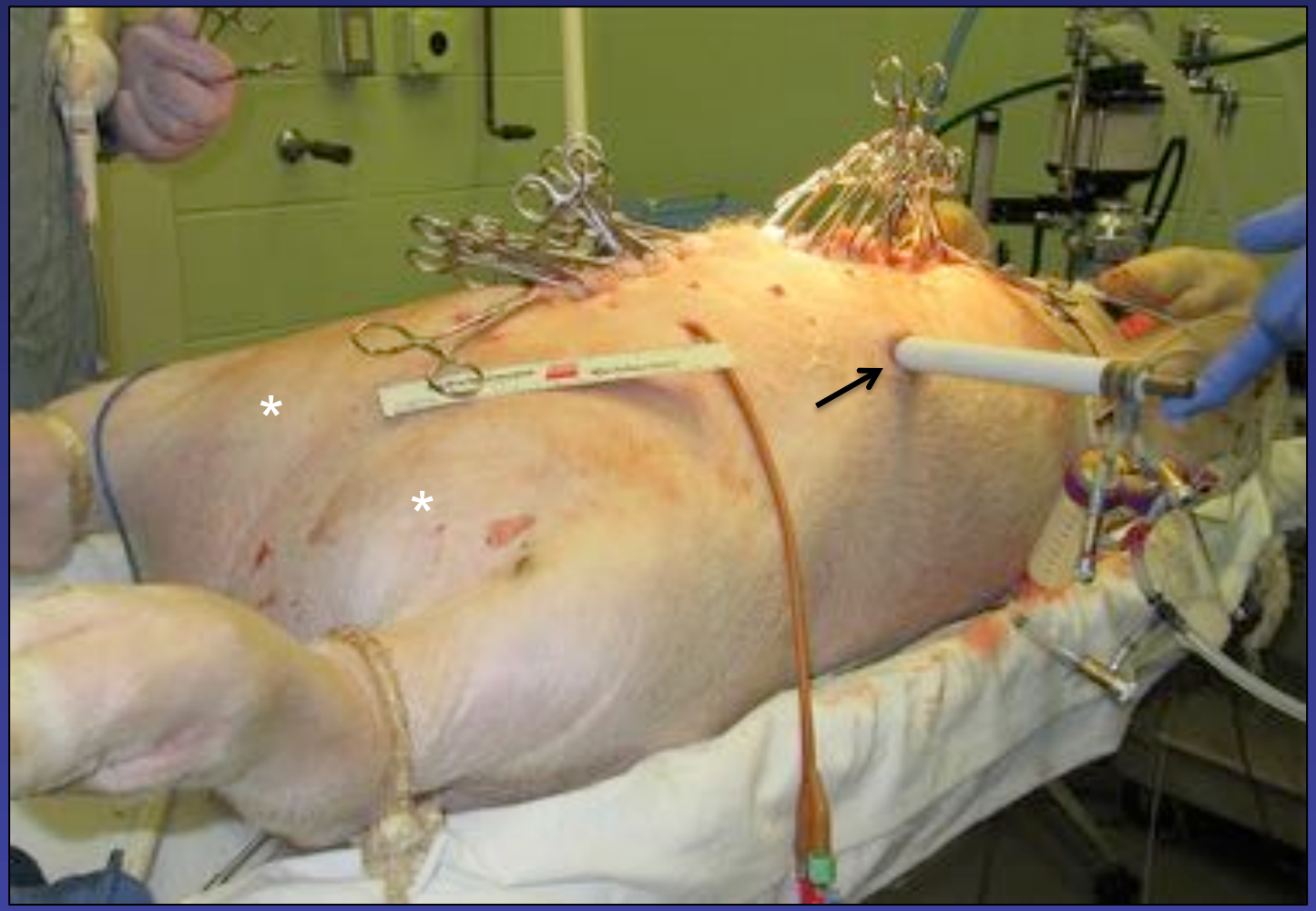


Figure 1, swine 216. View of left side of subject, looking from feet up to head, shortly after completion of the foam injection (time is ~5 min after injury). Midline incision has been closed with towel clips. Note placement of injector (arrow) through a separate stab incision in the left lateral abdominal wall. The bilateral groin in this subject became markedly distended with subcutaneous emphysema (*), caused by the injection. IAP at this point in time ~40 mm Hg.

Endpoints

- Survival @ 180 min
- Blood loss
- Vital signs
- Hematology (Hb & platelets)
- Coagulation (PT/PTT, INR, fibrinogen, TEG)
- ABGs
- Fluid administration
- Subject weight, organ weights
- Intraabdominal pressure (IAP)

Cross-group comparisons:

No Rx

vs.

foam alone

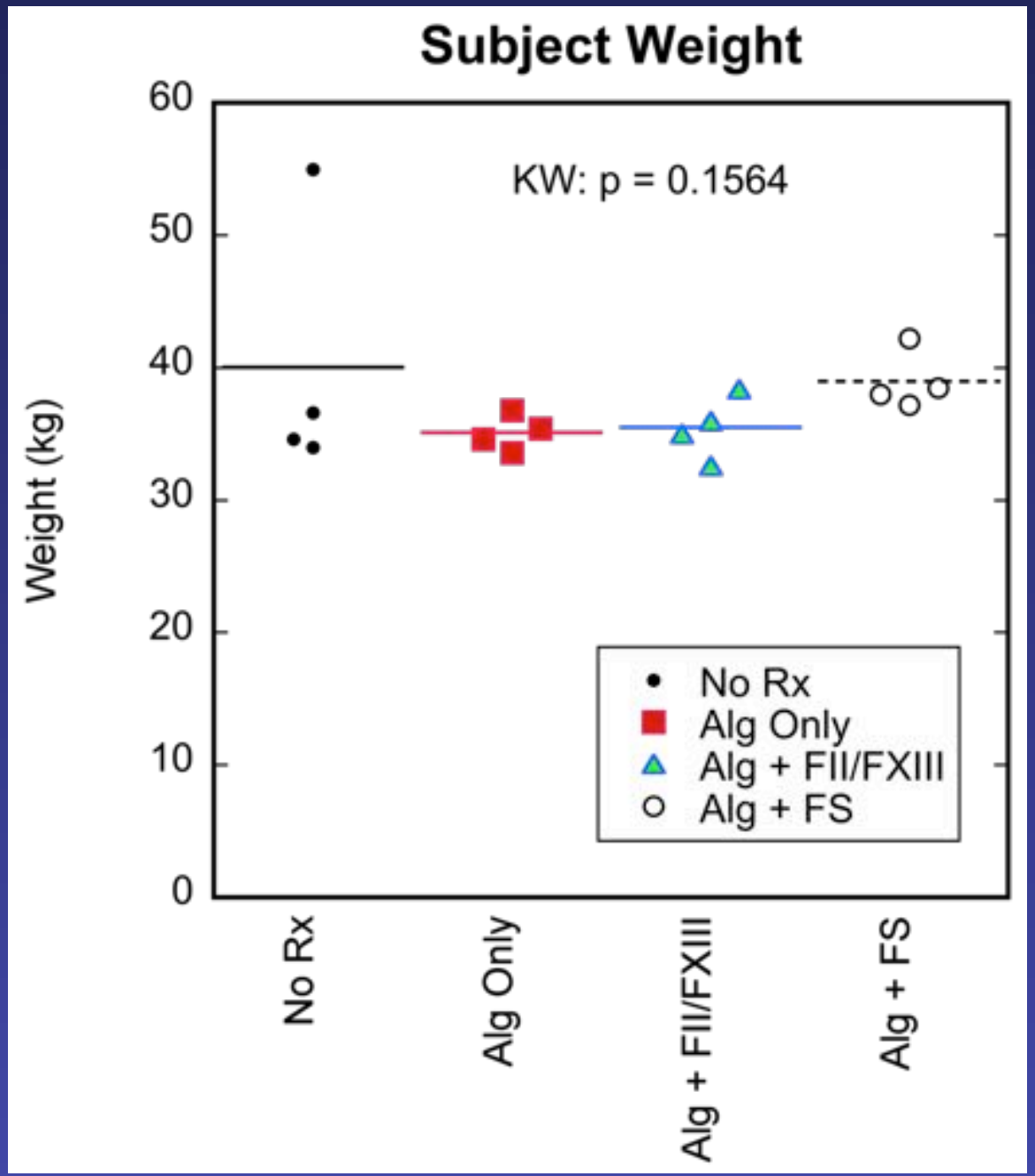
vs.

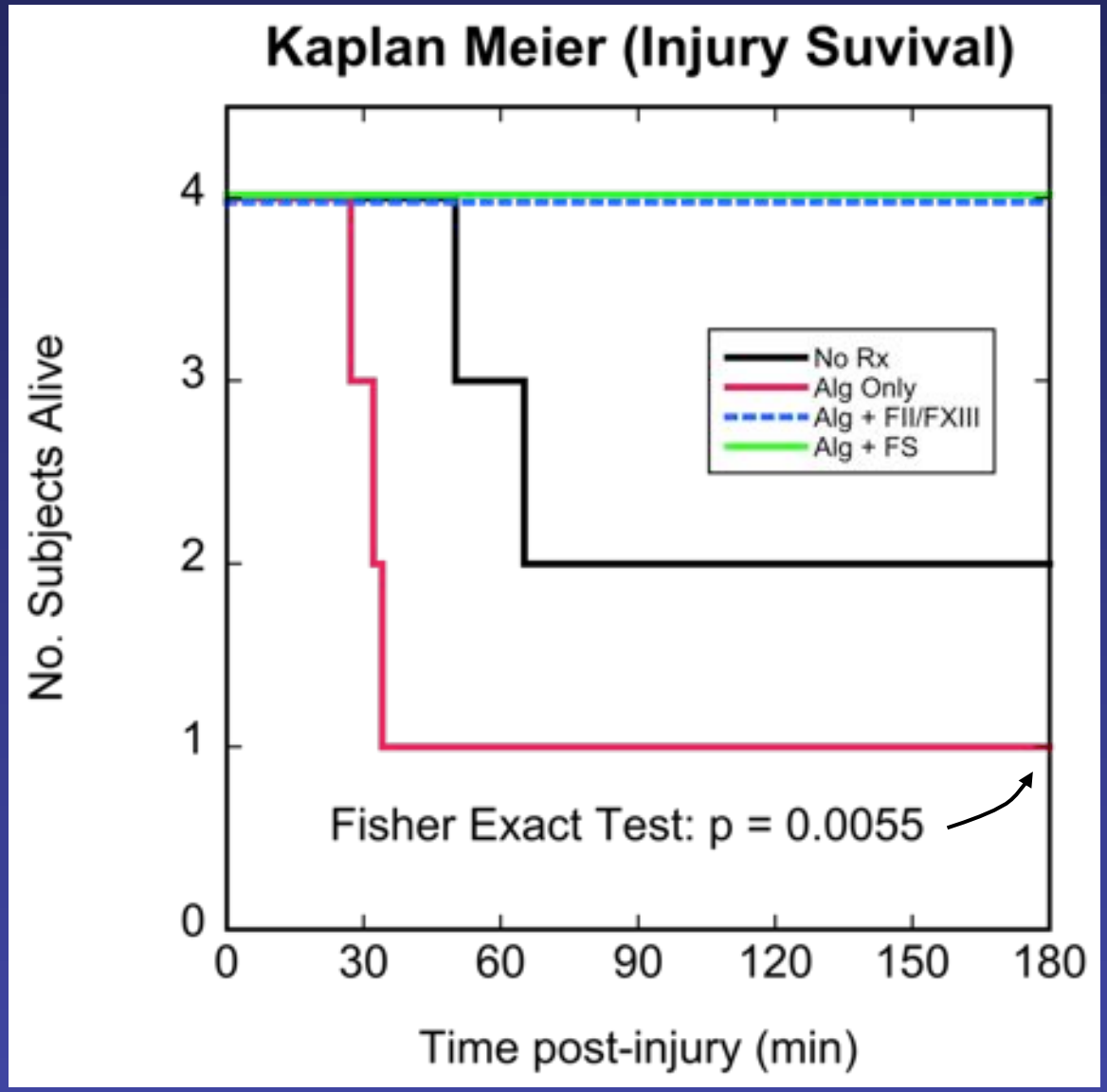
foam + FII/FXIII

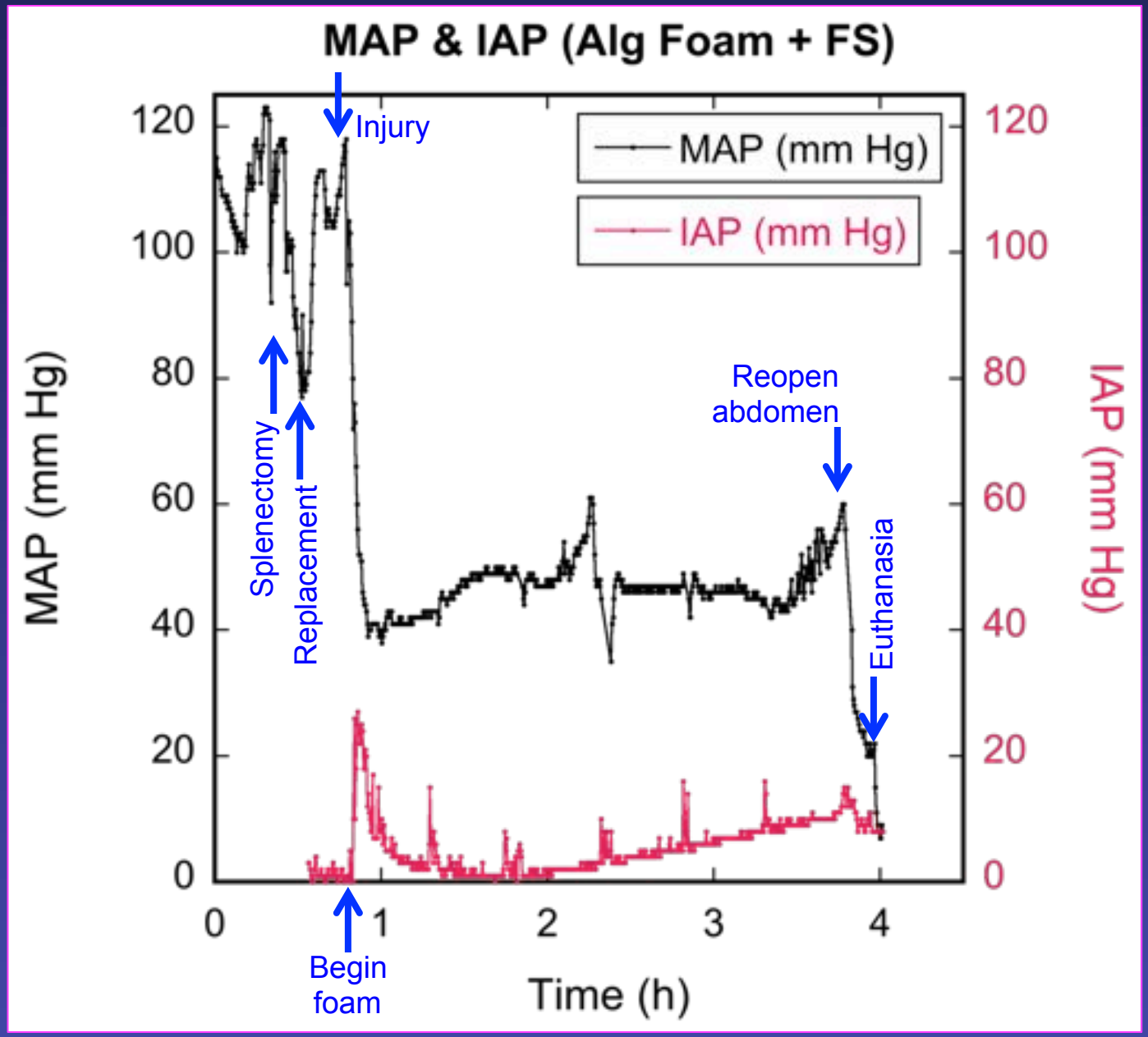
vs.

foam + FS

- N = 4 per group
- Preliminary analysis only
- Final N per group will be ≥10
- Nonparametric testing


Subject Weight
KW: p = 0.1564
Weight (kg)
60
50
40
30
20
10
0
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS


Kaplan Meier (Injury Suvival)
No. Subjects Alive
4
3
2
1
0
0
30
60
90
120
150
180
Time post-injury (min)
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
Fisher Exact Test: p = 0.0055


MAP & IAP (Alg Foam + FS)
MAP (mm Hg)
IAP (mm Hg)
Injury
Splenectomy
Replacement
Reopen abdomen
Euthanasia
Begin foam
Time (h)
MAP (mm Hg)
IAP (mm Hg)
0
20
40
60
80
100
120
0
1
2
3
4

3 h postinjury & foam injection

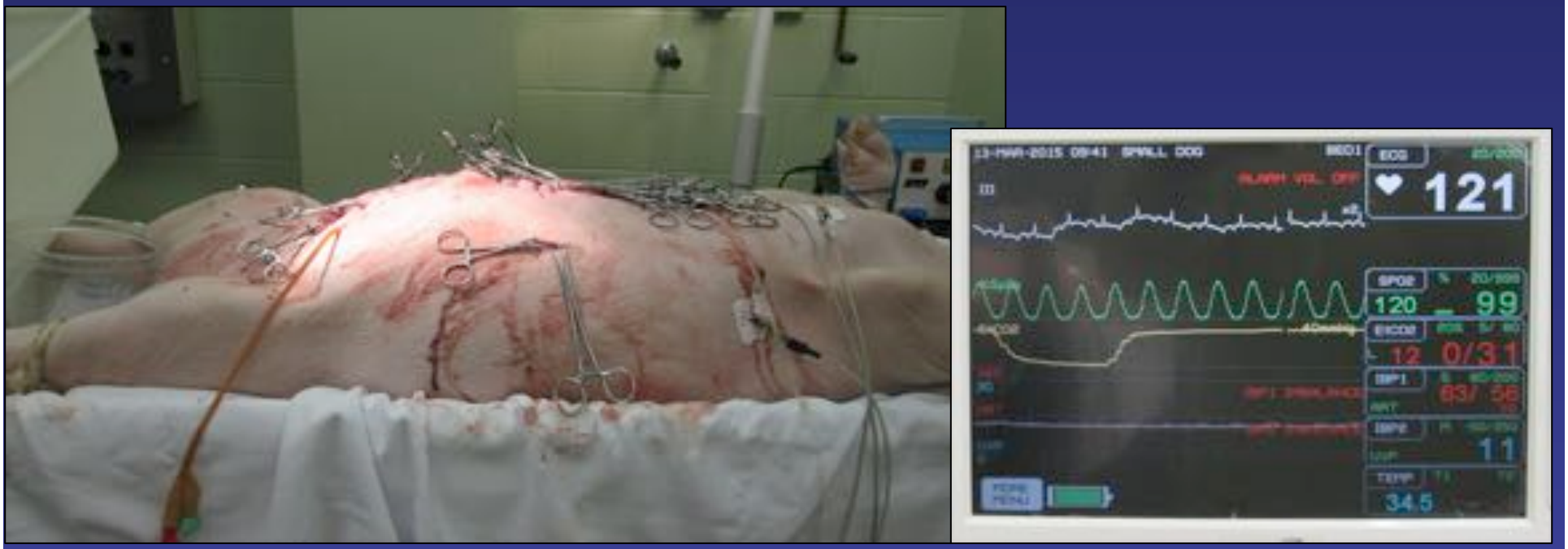


Figure 1, swine 263. Lateral view of swine after completion of 3 h observation period. Subject alive & well, with pulse = 121, MAP = 59 mm Hg, O2 sat = 99%, EtCO2 = 31 mm Hg, temp = 34.5˚C, IAP = 11 mm Hg (see monitor inset).

Abdomen with foam, 3 h post injury

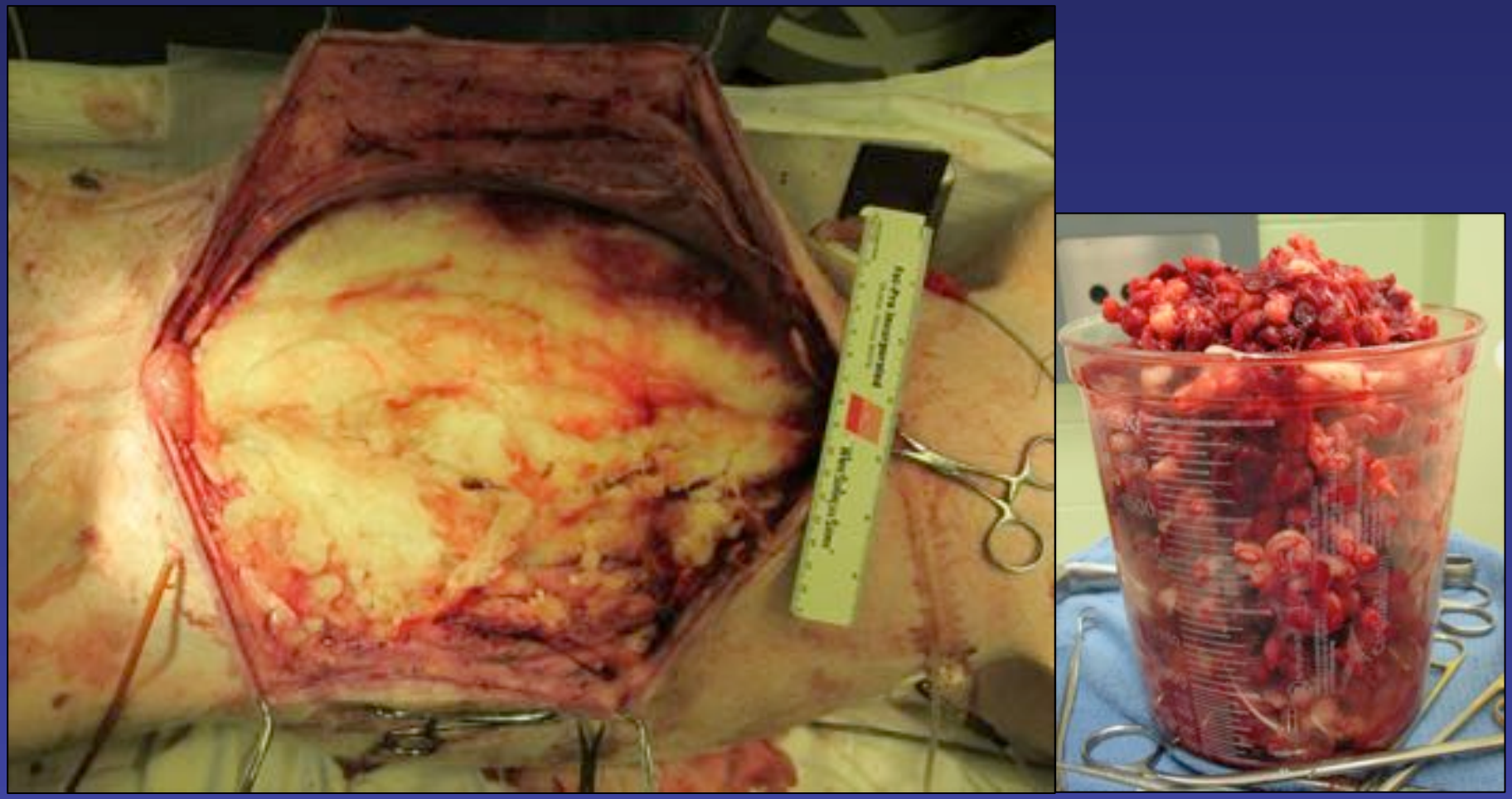


Figure 2, swine 263. Overhead view of re-opened abdomen immediately after Fig. 1 image. Subject alive & well. Cephalad is to right. Foam is overlying the intestines. No clot visible; small amount blood present at periphery. Inset: foam subsequently was removed, and placed into this 2 L canister.

Injury site 3 h post injury, foam removed

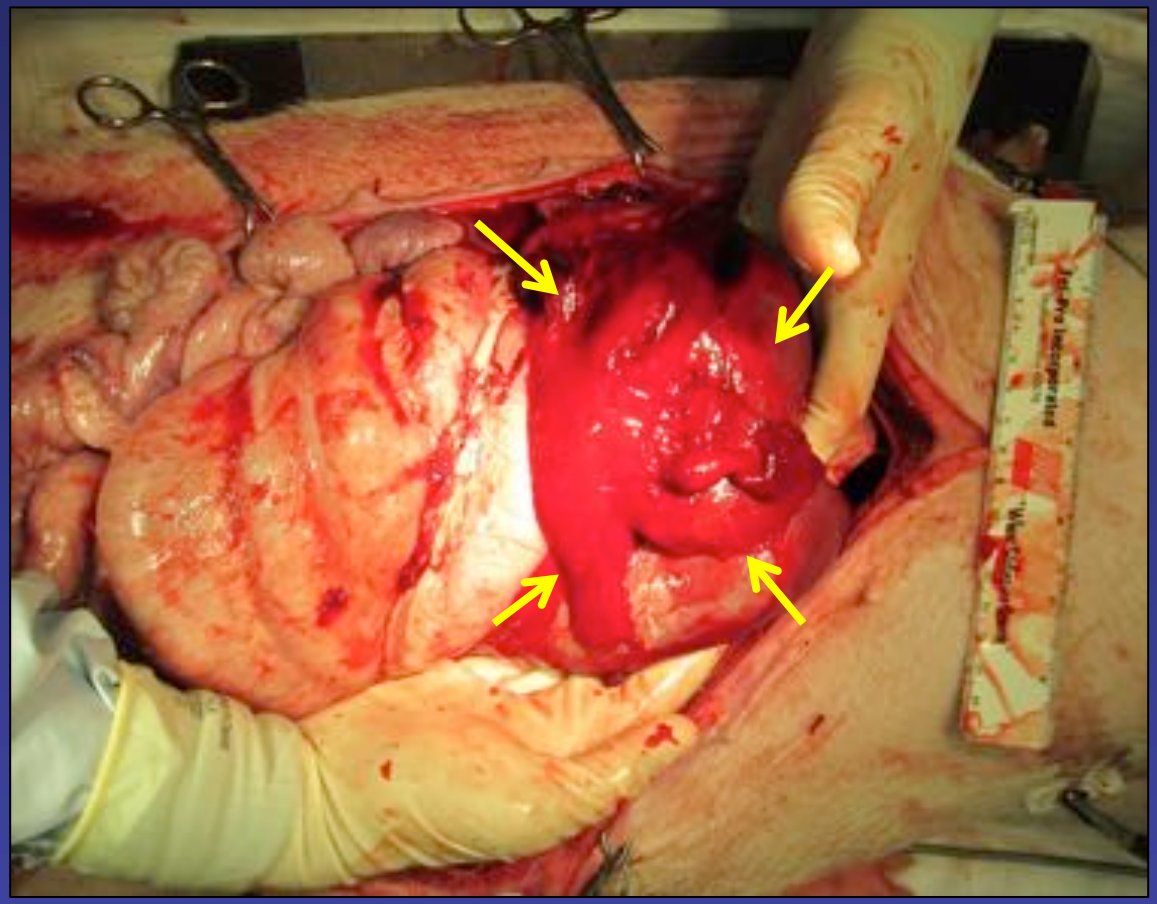


Figure 4, swine 263. View of superior abdomen immediately after Fig. 3 image; liver has been elevated into the wound. Subject alive & well. Cephalad is to right. There was a large clot overlying injury site (arrows) with no active bleeding.

Liver ex vivo

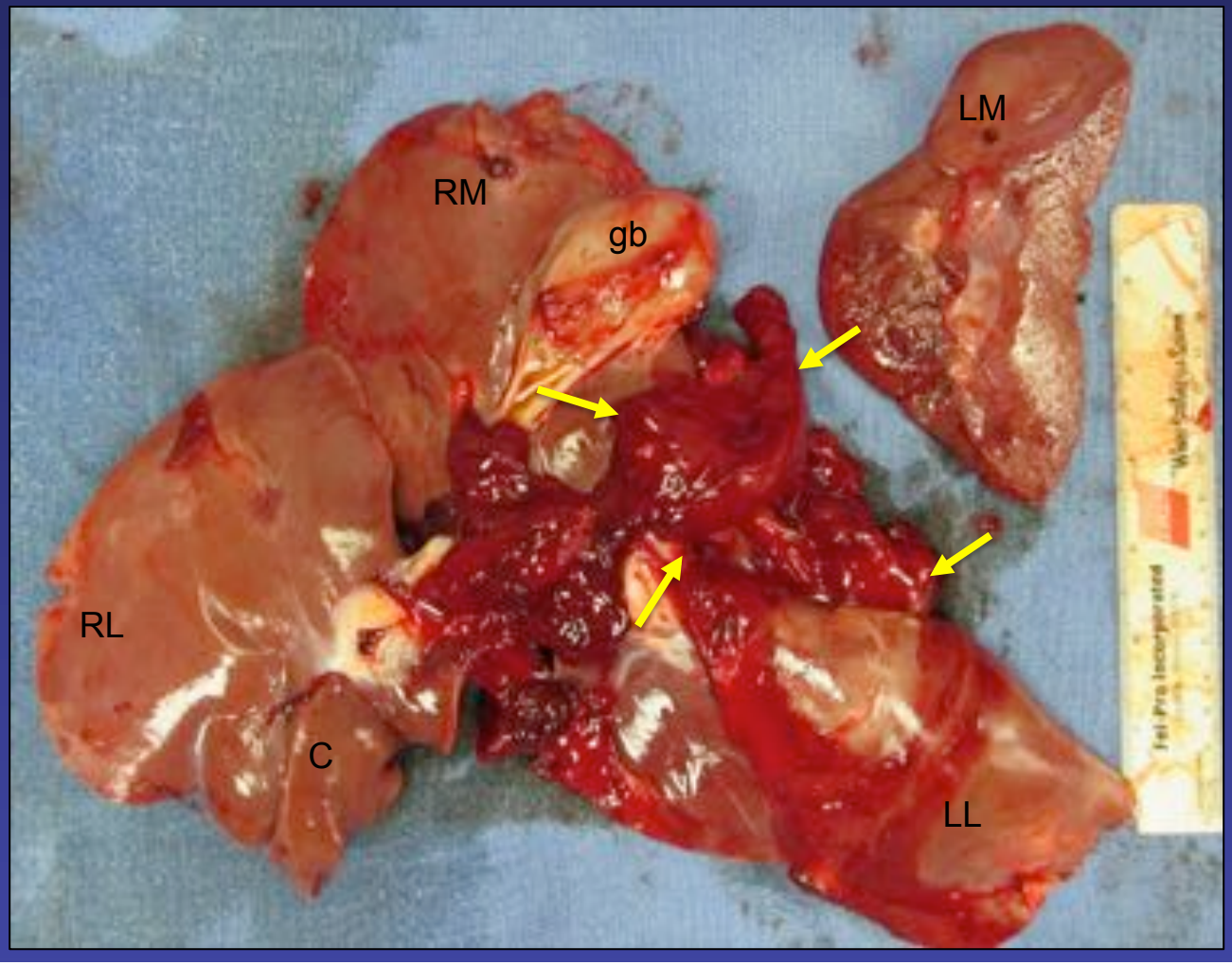


Figure 5, swine 263. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Arrows = large clot overlying surface of injury. Anterior is at top of image. Color of liver appears good (contrast #262).

Clot Adherence: Ca Alg Only

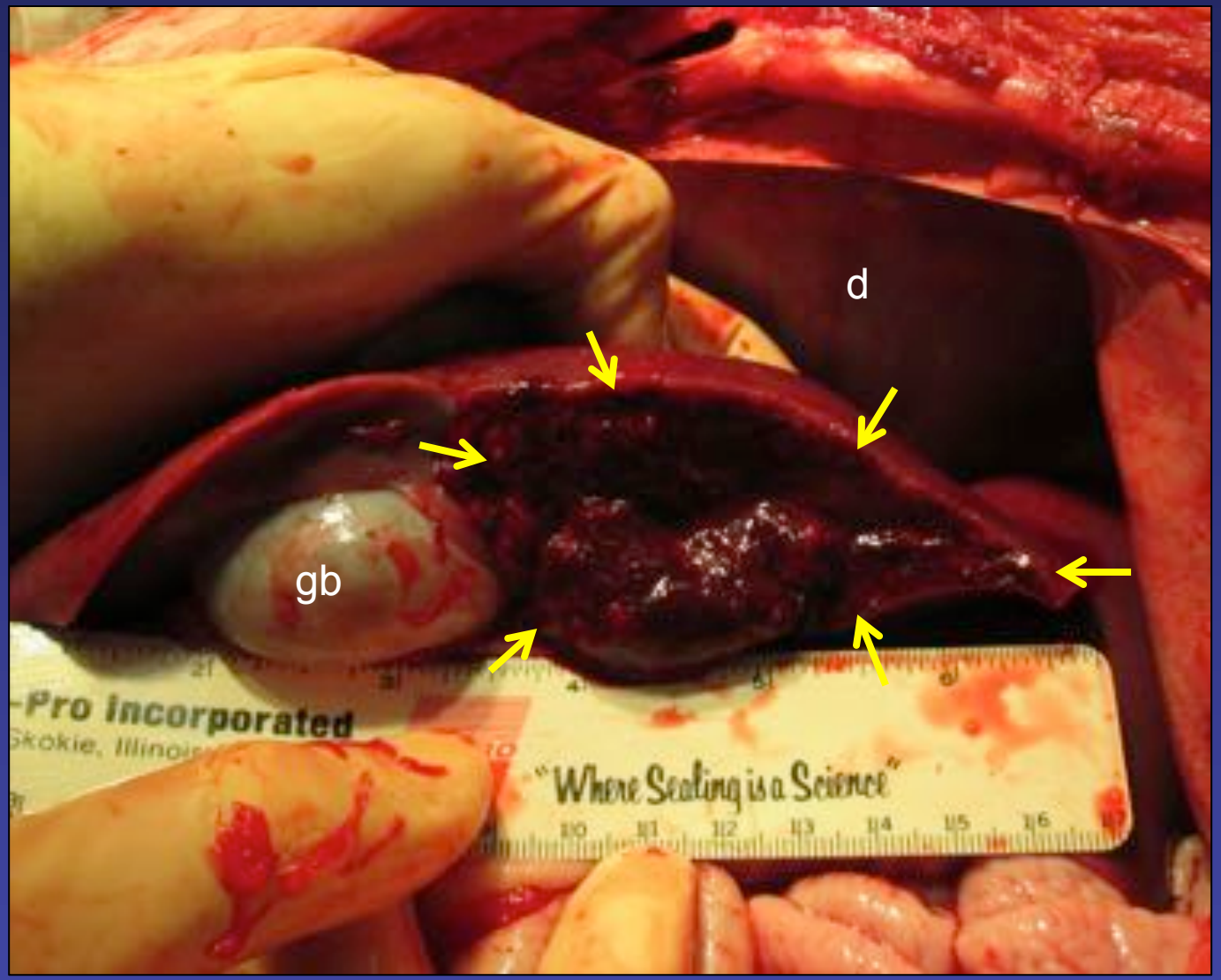
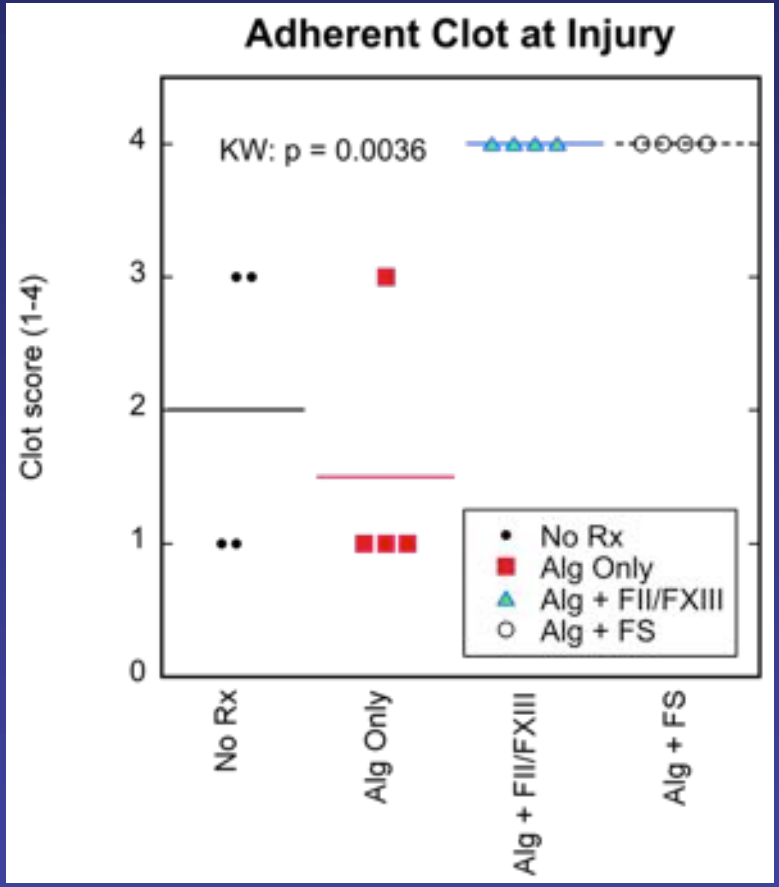


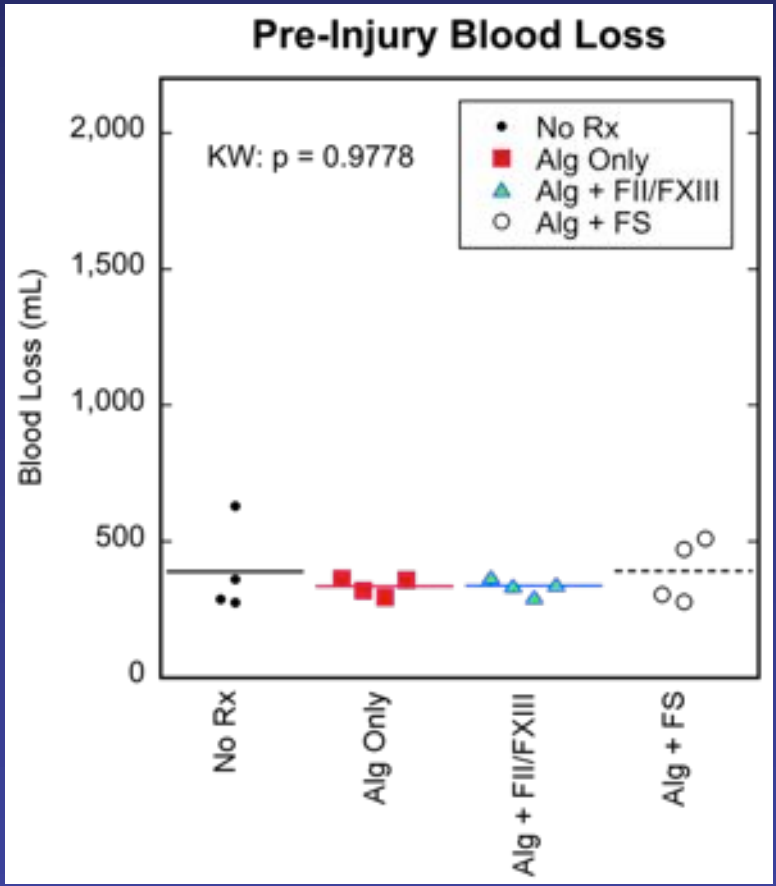
Figure 4, swine 281. Injury site (base of left medial lobe, arrows) after removal of all foam, which was not adherent to the injury. gb = gallbladder; d = diaphragm. Cephalad is at top of image.

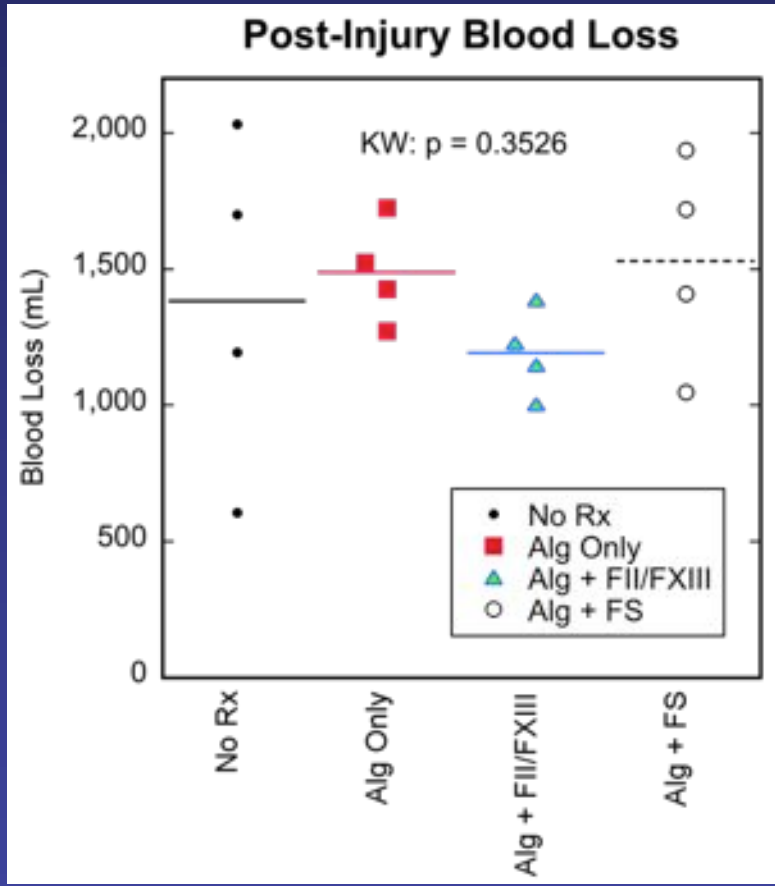
Clot Adherence to Wound Site

Swine No.	Treatment	180 min Survival	Adherent Clot (score 1-4)
237	No Rx	NO	1
238	No Rx	NO	1
241	No Rx	YES	3
246	No Rx	YES	3
280	Alg Only	NO	1
281	Alg Only	NO	1
283	Alg Only	NO	1
284	Alg Only	Yes	3
273	Alg + FII/FXIII	YES	4
274	Alg + FII/FXIII	YES	4
277	Alg + FII/FXIII	YES	4
278	Alg + FII/FXIII	YES	4
262	Alg + FS	YES	4
263	Alg + FS	YES	4
264	Alg + FS	YES	4
265	Alg + FS	YES	4

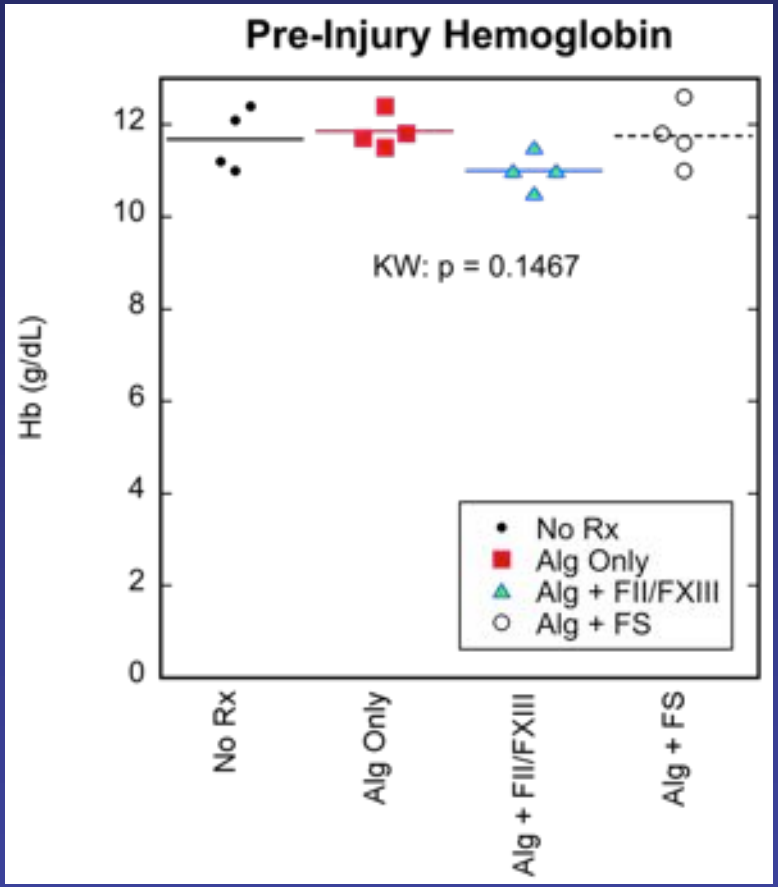


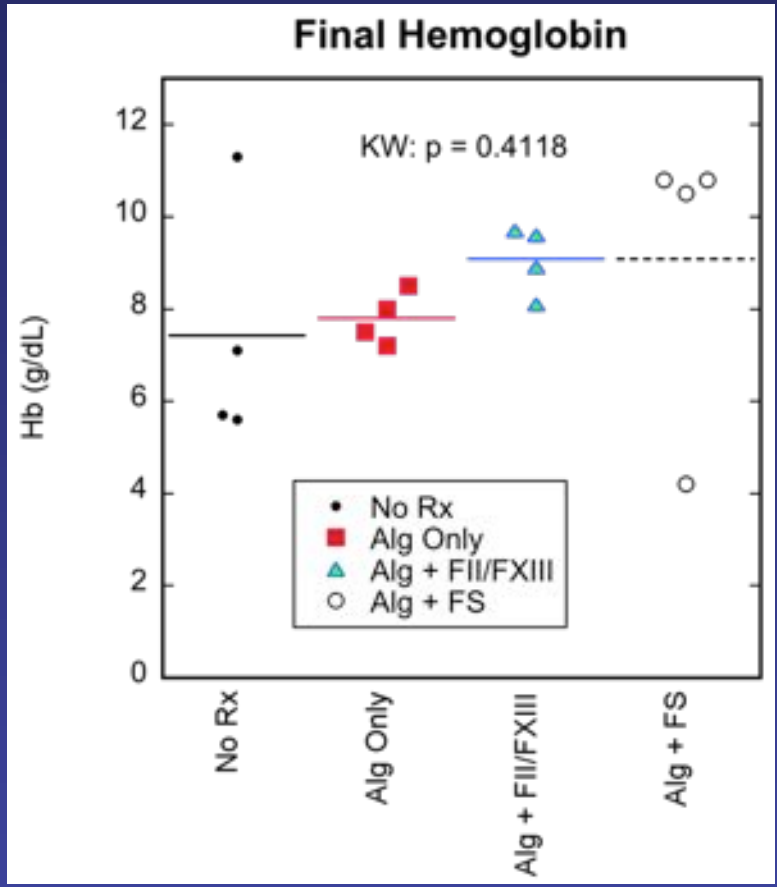
Blood Loss


Pre-Injury Blood Loss
KW: p = 0.9778
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
Blood Loss (mL)
2,000
1,500
1,000
500
0
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

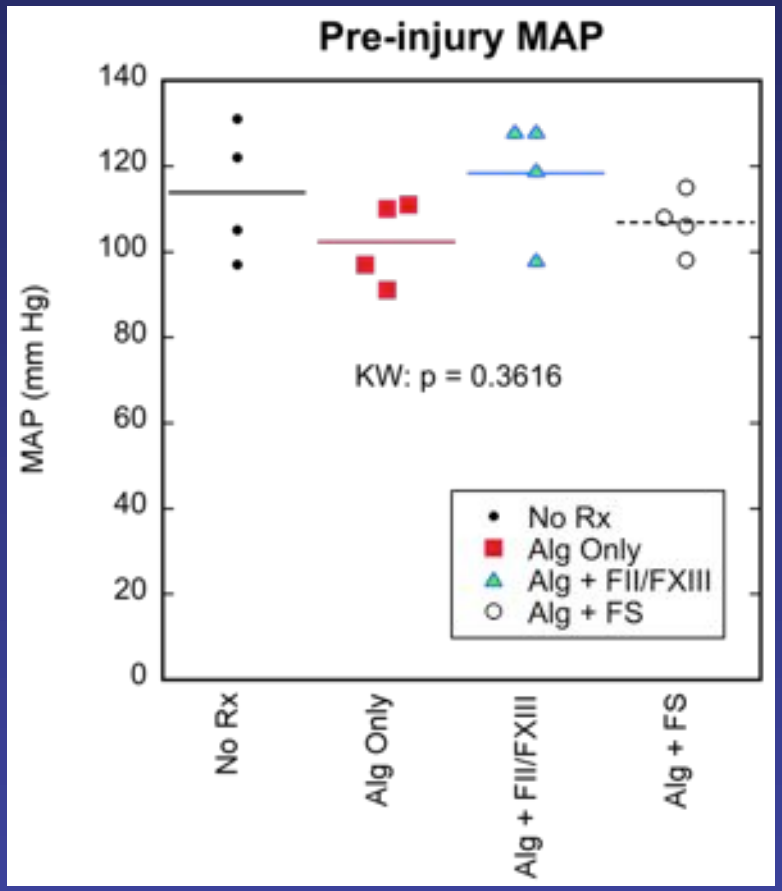

Post-Injury Blood Loss
KW: p = 0.3526
Blood Loss (mL)
2,000
1,500
1,000
500
0
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

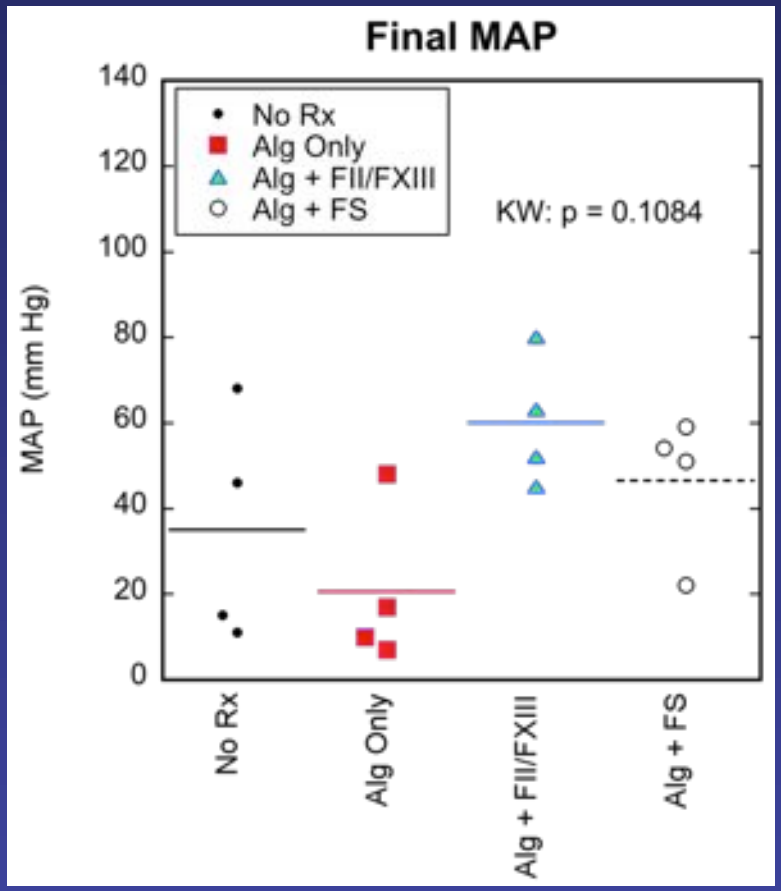
Hemoglobin


Pre-Injury Hemoglobin
Hb (g/dL)
0
2
4
6
8
10
12
KW: p = 0.1467
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

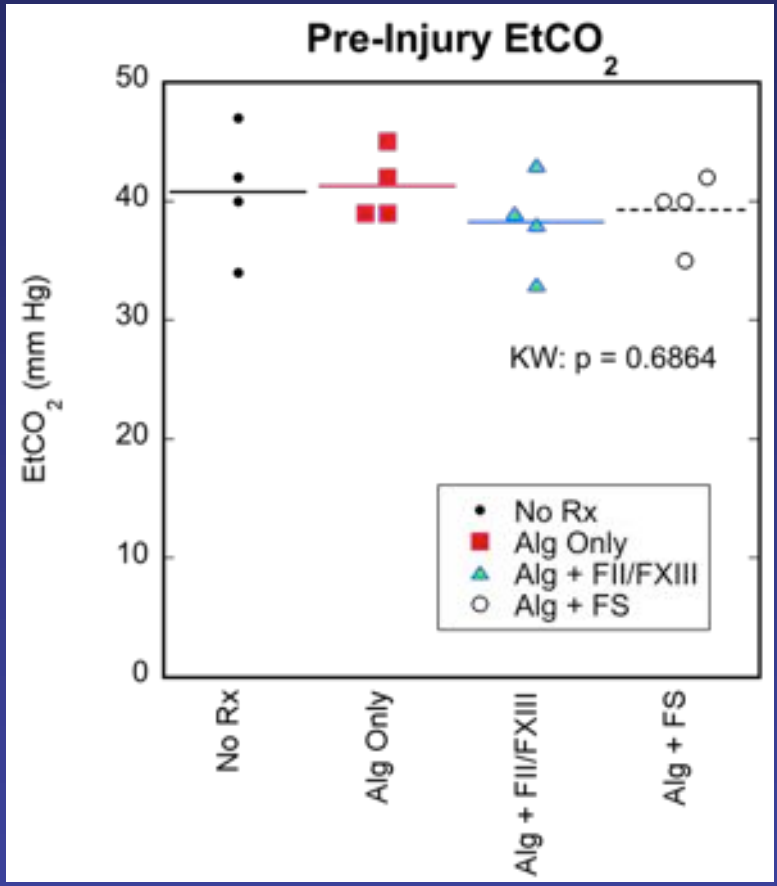

Final Hemoglobin
Hb (g/dL)
0
2
4
6
8
10
12
KW: p = 0.4118
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

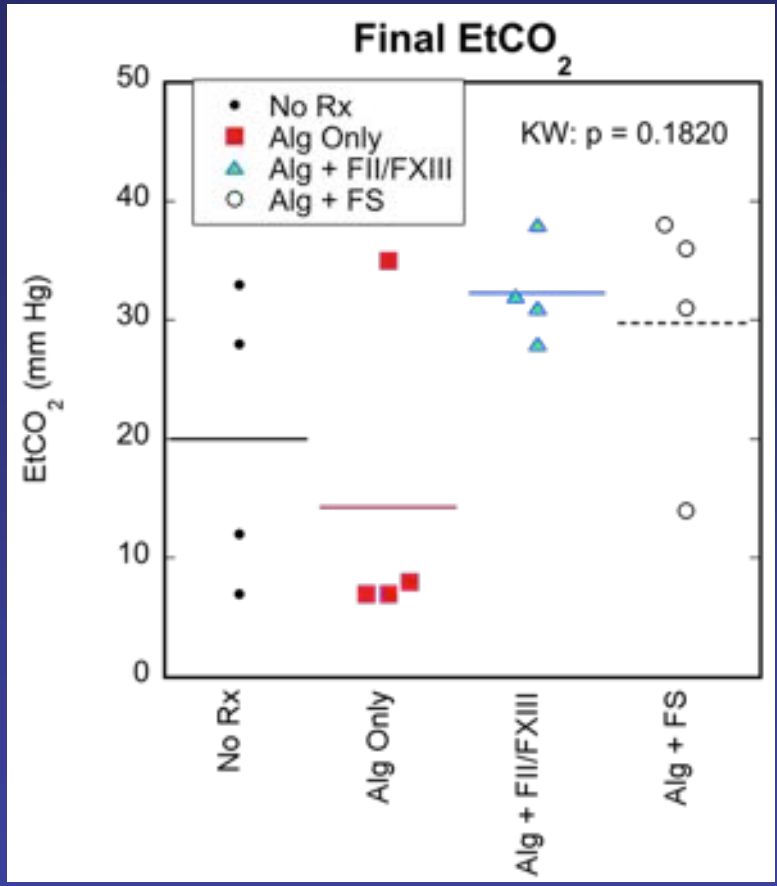
Mean Arterial Pressure



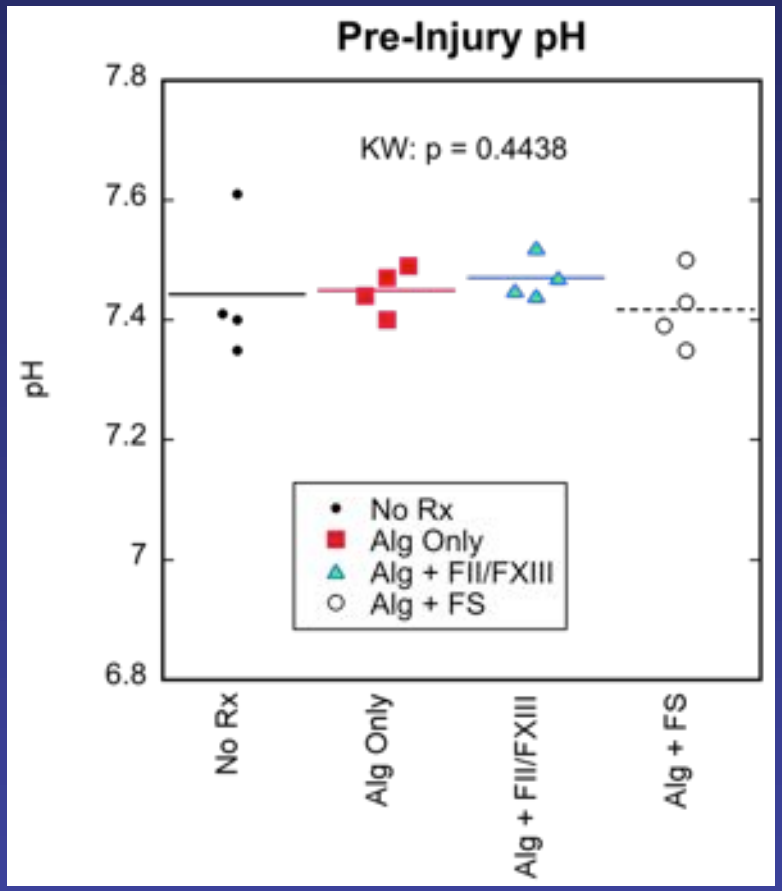


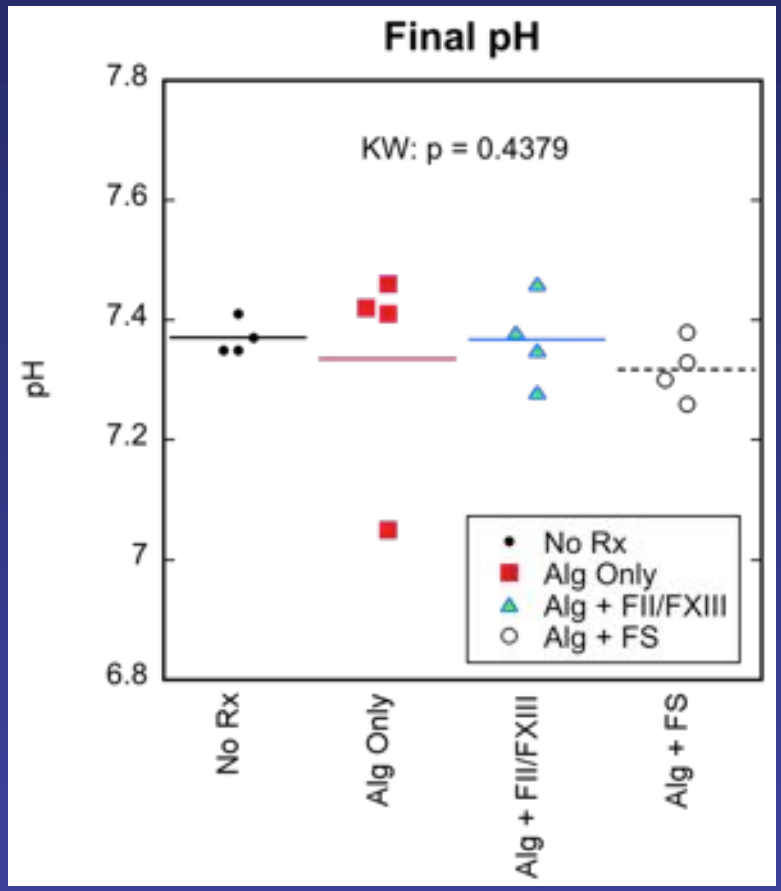
Endtidal CO_2



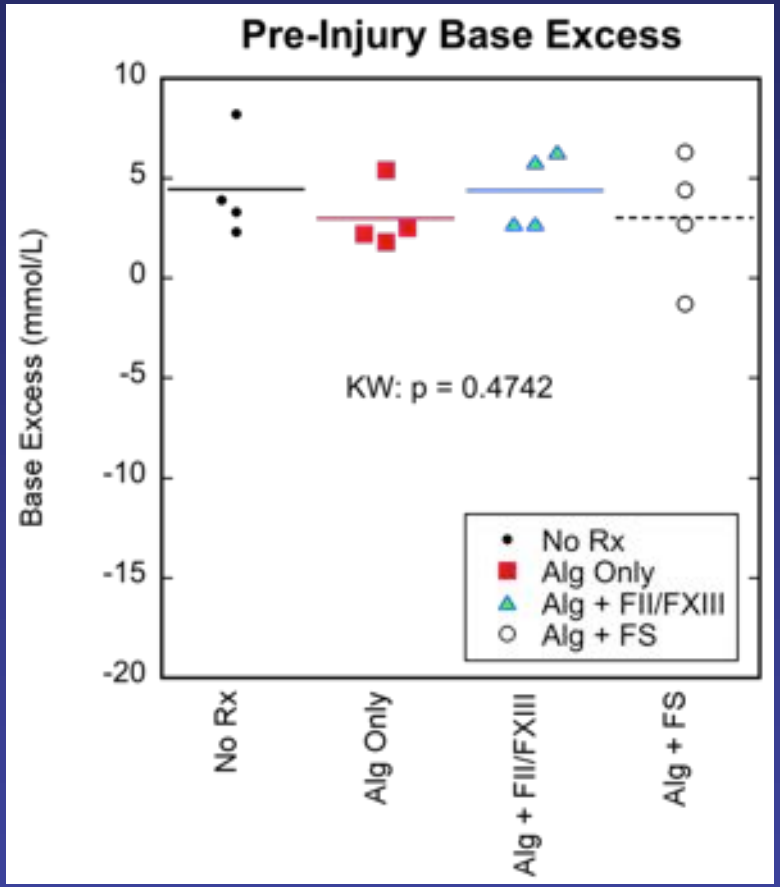


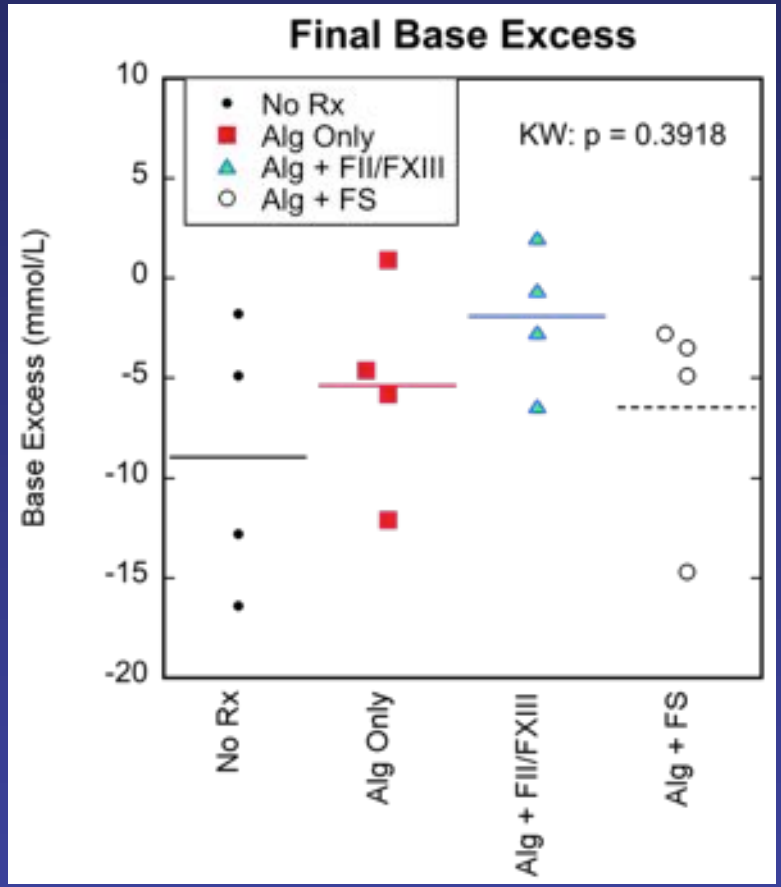
Arterial pH


Pre-Injury pH
KW: p = 0.4438
pH
7.8
7.6
7.4
7.2
7
6.8
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

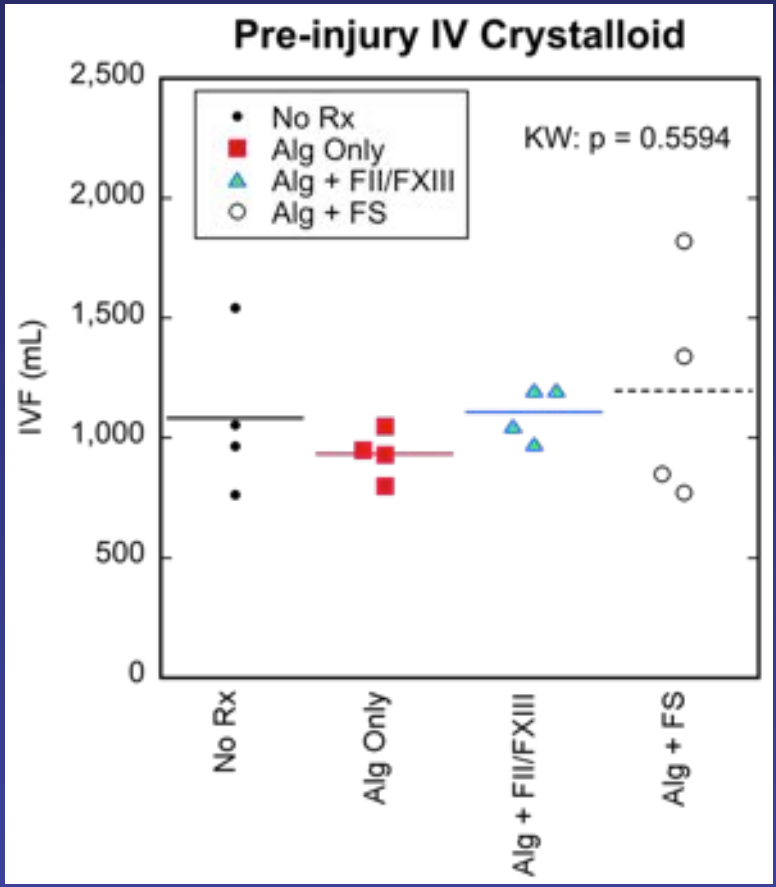

Final pH
KW: p = 0.4379
pH
7.8
7.6
7.4
7.2
7
6.8
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS

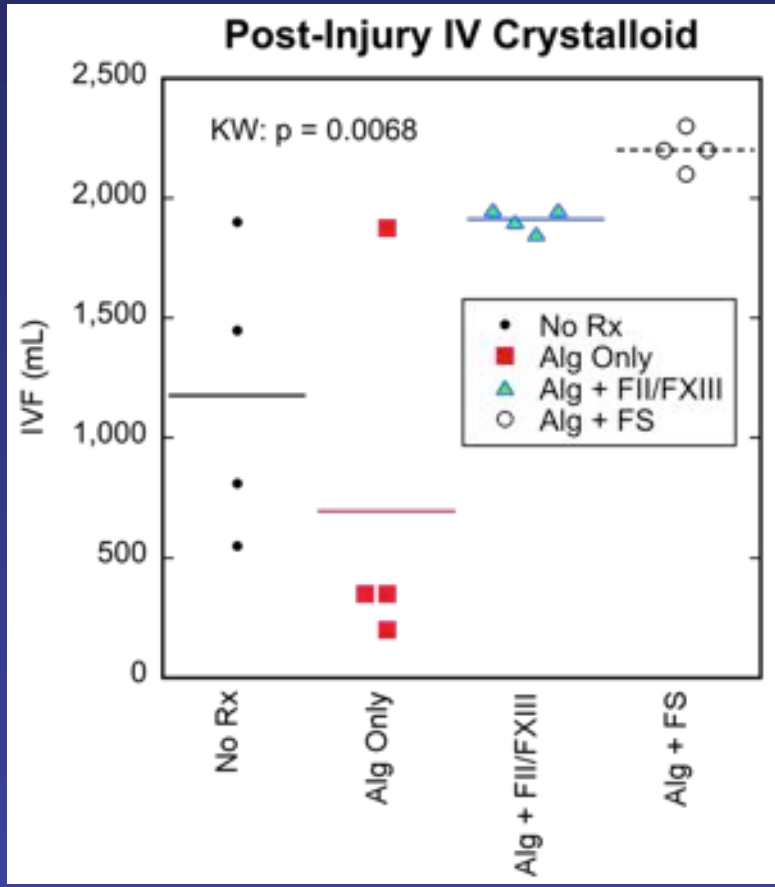
Arterial Base Excess



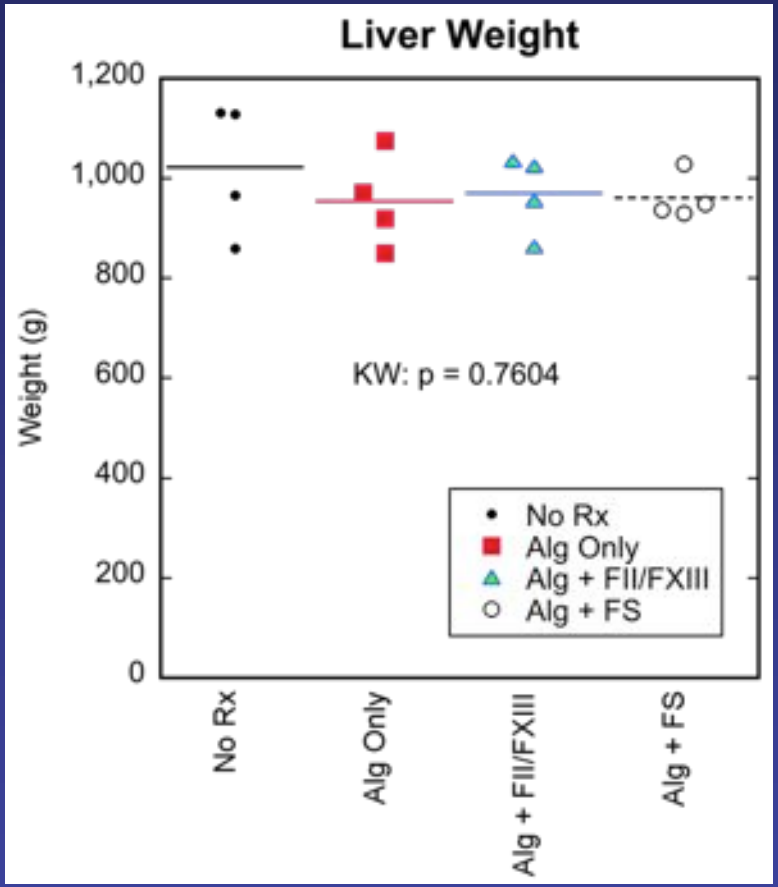


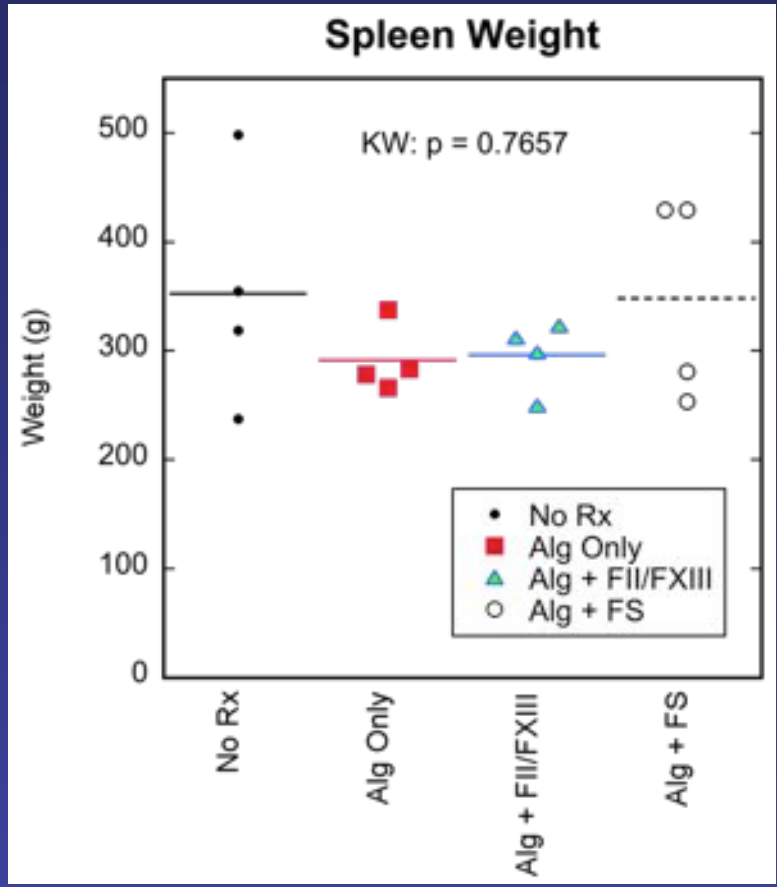
Crystalloid Administration





Organ Weights



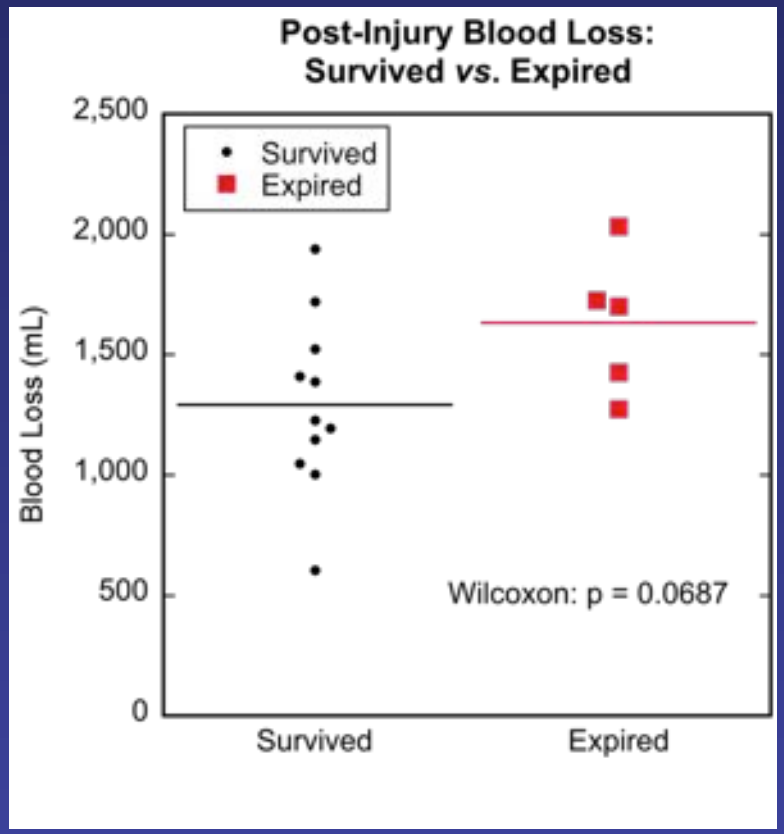


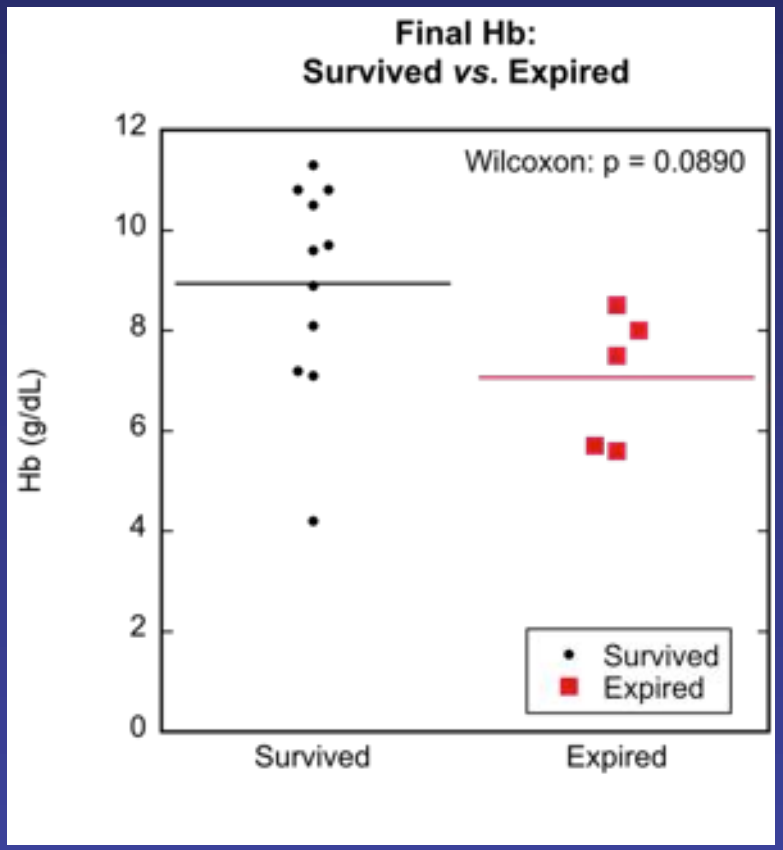
Pair-wise comparisons:

Survivors
vs.
Non-survivors

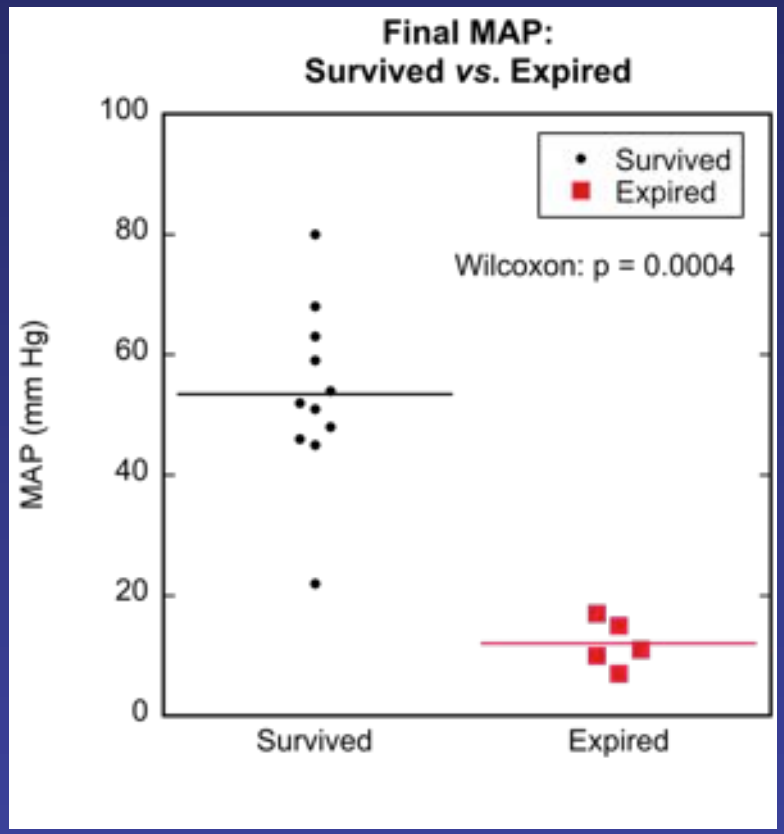
- N = 16 total
- Preliminary analysis only
- Final total N will be ≥40
- Nonparametric testing

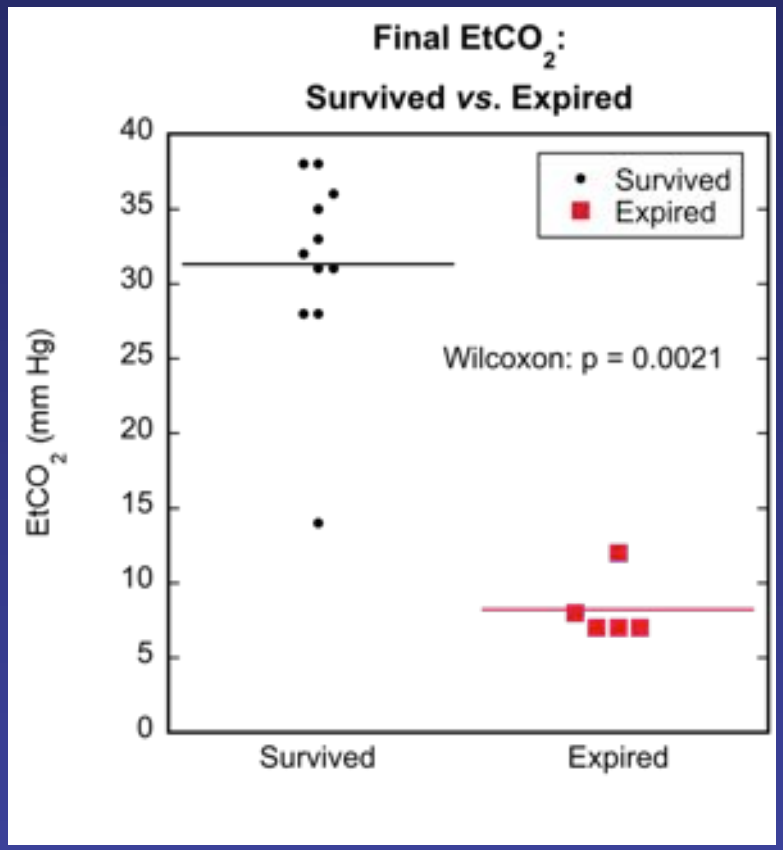
Blood Loss & Hemoglobin



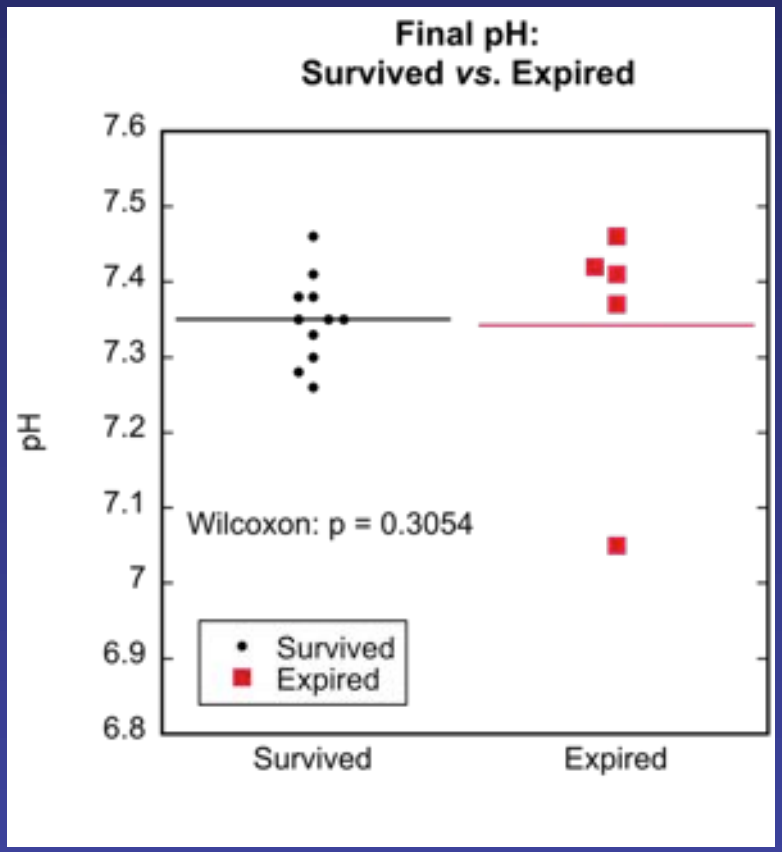


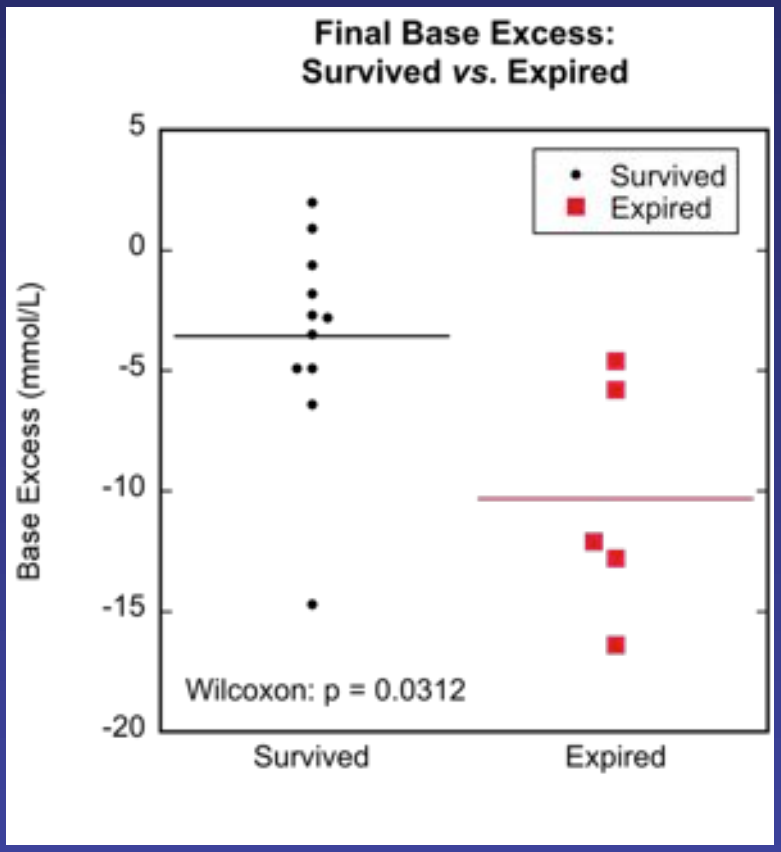
MAP & $EtCO_2$


Final MAP:
Survived vs. Expired
MAP (mm Hg)
100
80
60
40
20
0
Survived
Expired
Wilcoxon: p = 0.0004
Survived
Expired
Survived
Expired

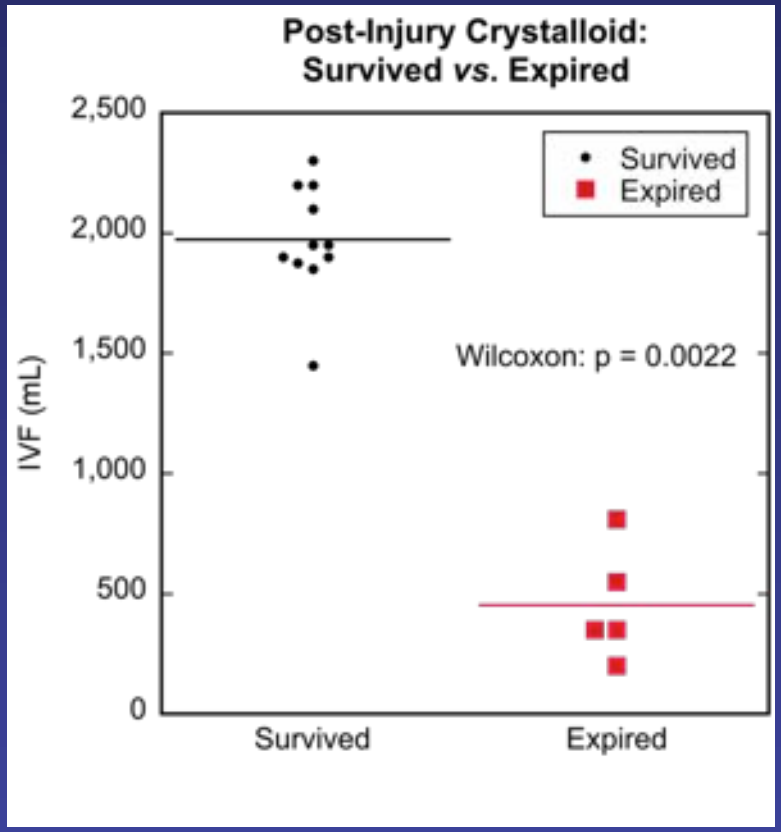

Final EtCO2:
Survived vs. Expired
EtCO2 (mm Hg)
40
35
30
25
20
15
10
5
0
Survived
Expired
Wilcoxon: p = 0.0021
Survived
Expired
Survived
Expired

pH & BE


Final pH:
Survived vs. Expired
pH
7.6
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
Wilcoxon: p = 0.3054
Survived
Expired
Survived
Expired


Final Base Excess:
Survived vs. Expired
Base Excess (mmol/L)
5
0
-5
-10
-15
-20
Survived
Expired
Wilcoxon: p = 0.0312
Survived
Expired

Crystalloid



I. Noncompressible Torso Hemorrhage

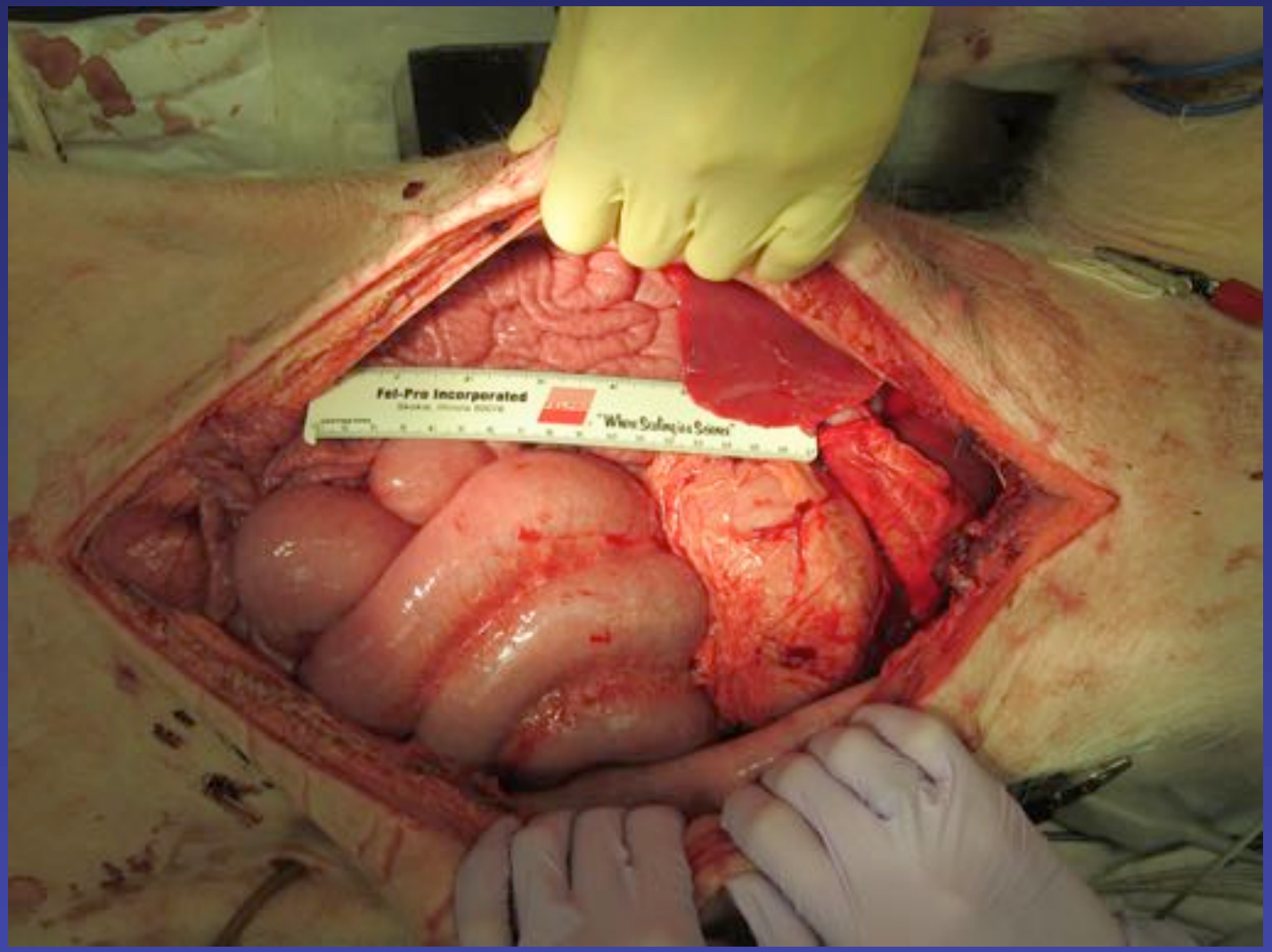
Preliminary Thoughts & Plans

II. Hypothermic Hemodilutional Hemorrhage

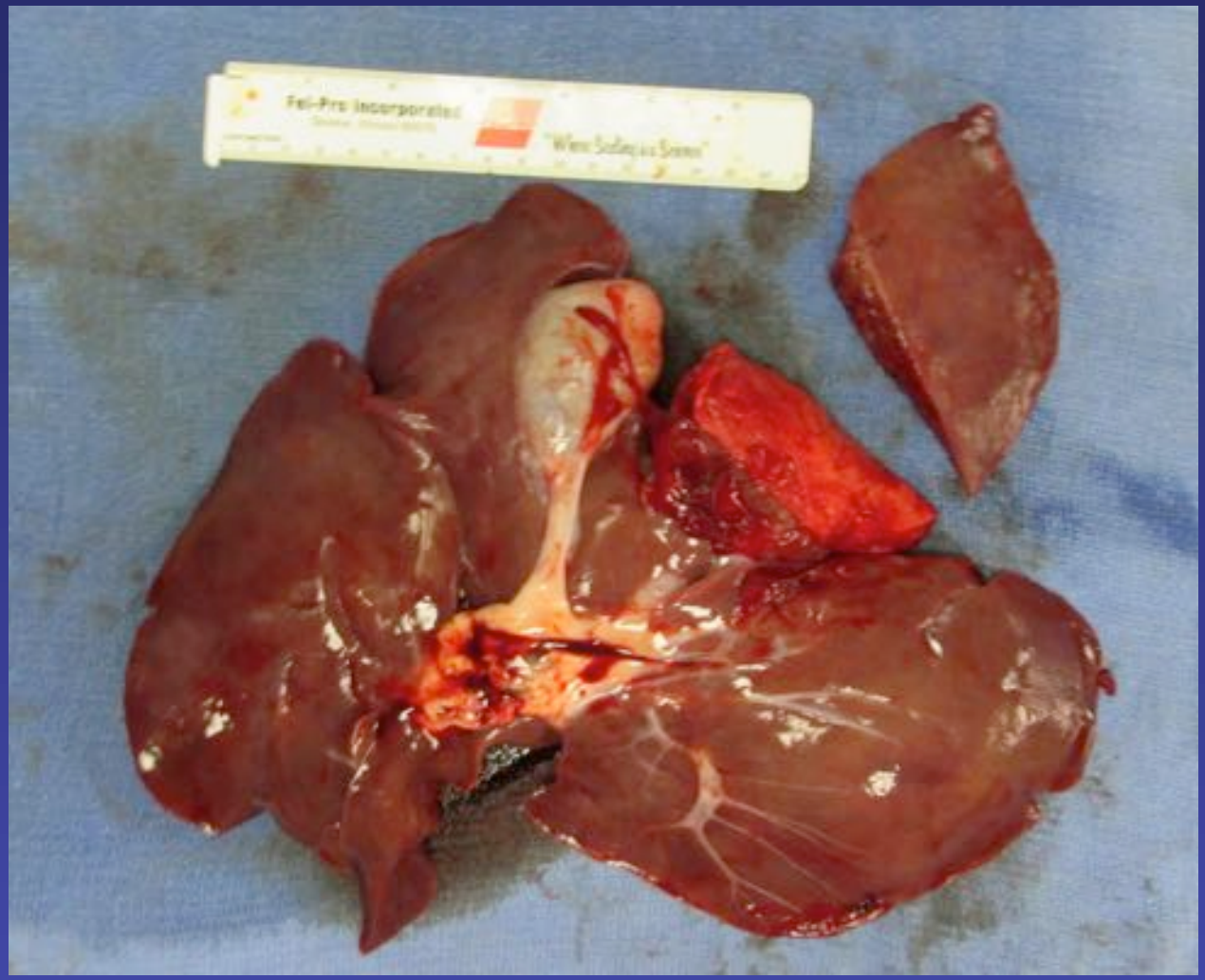
III. Resuscitation Protocols

IV. NE Dept Econ Develop (NEDED) Project

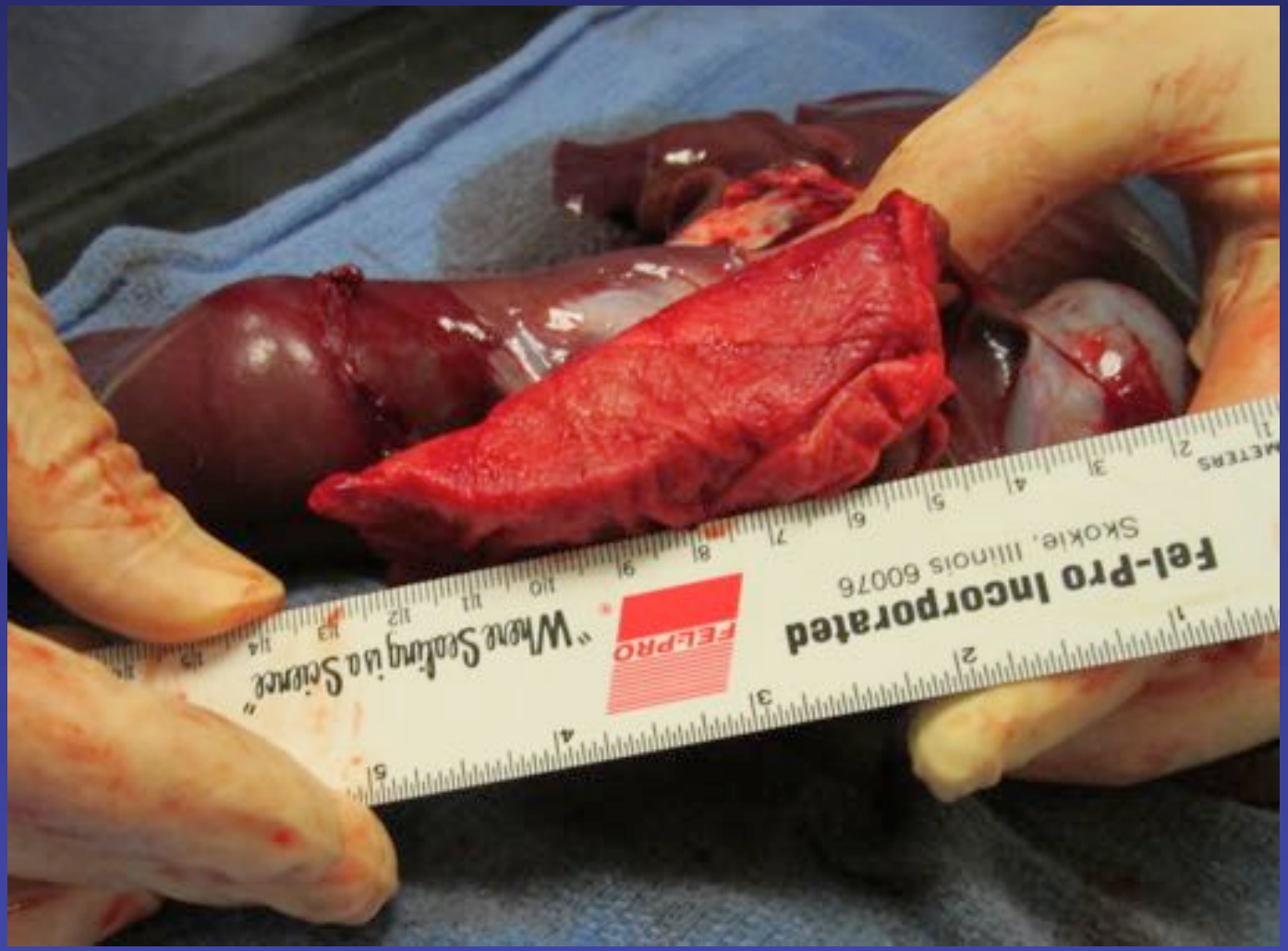
PCL bandage + FS (NEDED)



PCL bandage + FS (NEDED)



PCL bandage + FS (NEDED)



Research Update – May 29, 2015

for

Technologies for Hemostasis and Stabilization of Acute Traumatic Wound

Prime Award #: W81XWH-11-1-0836

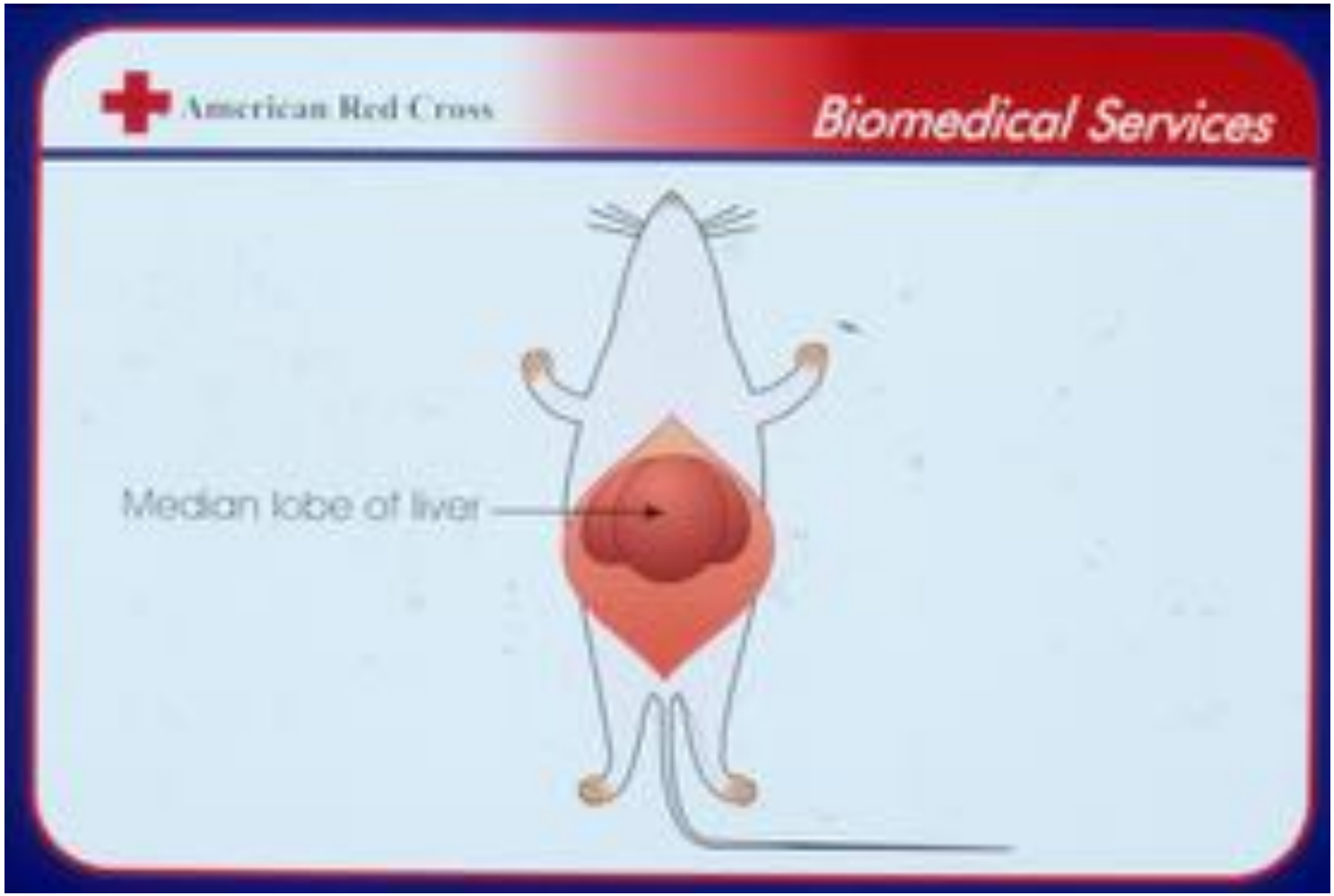
Awarding Agency: U.S. Army Medical Research Acquisition Activity

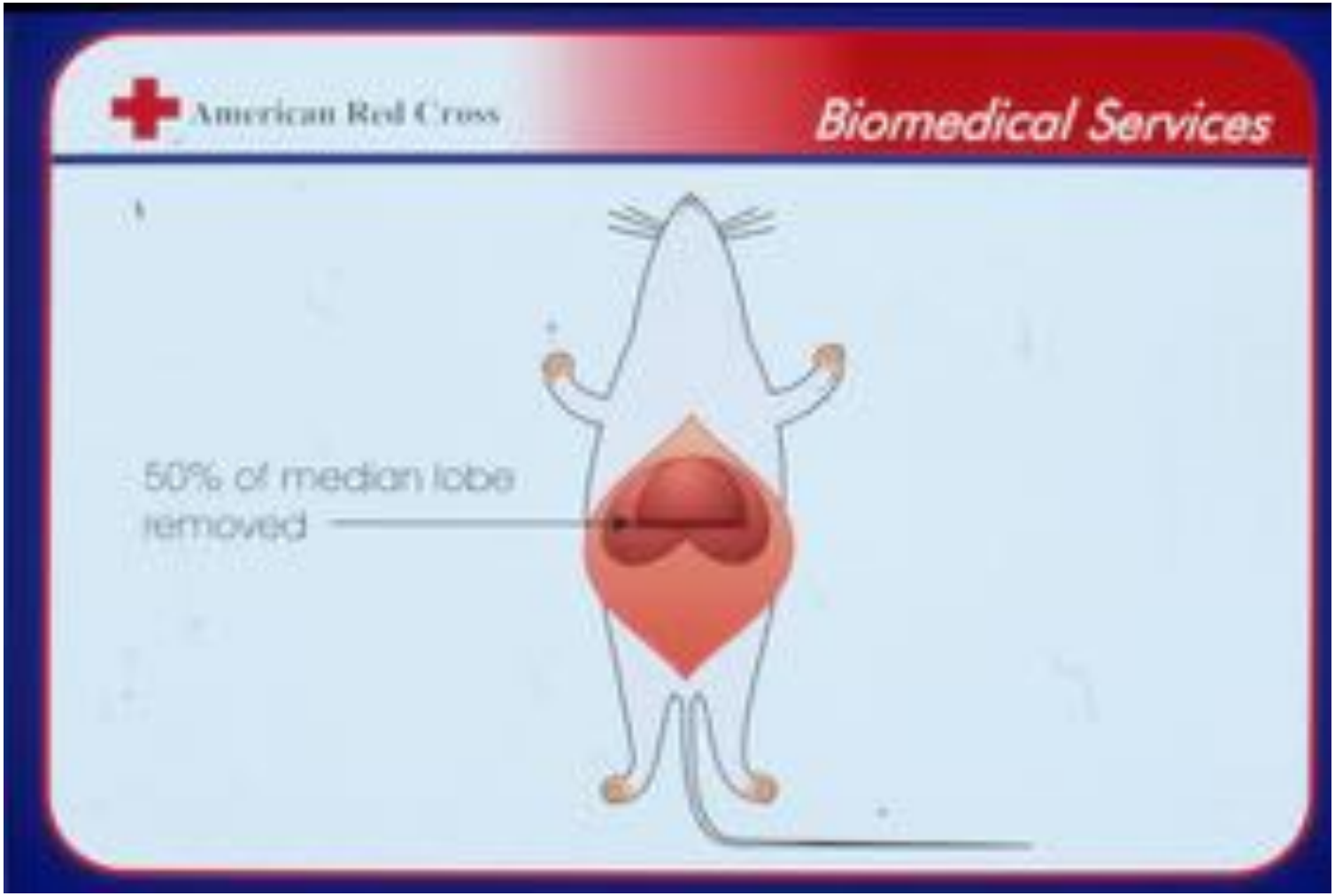
Research Team Leaders

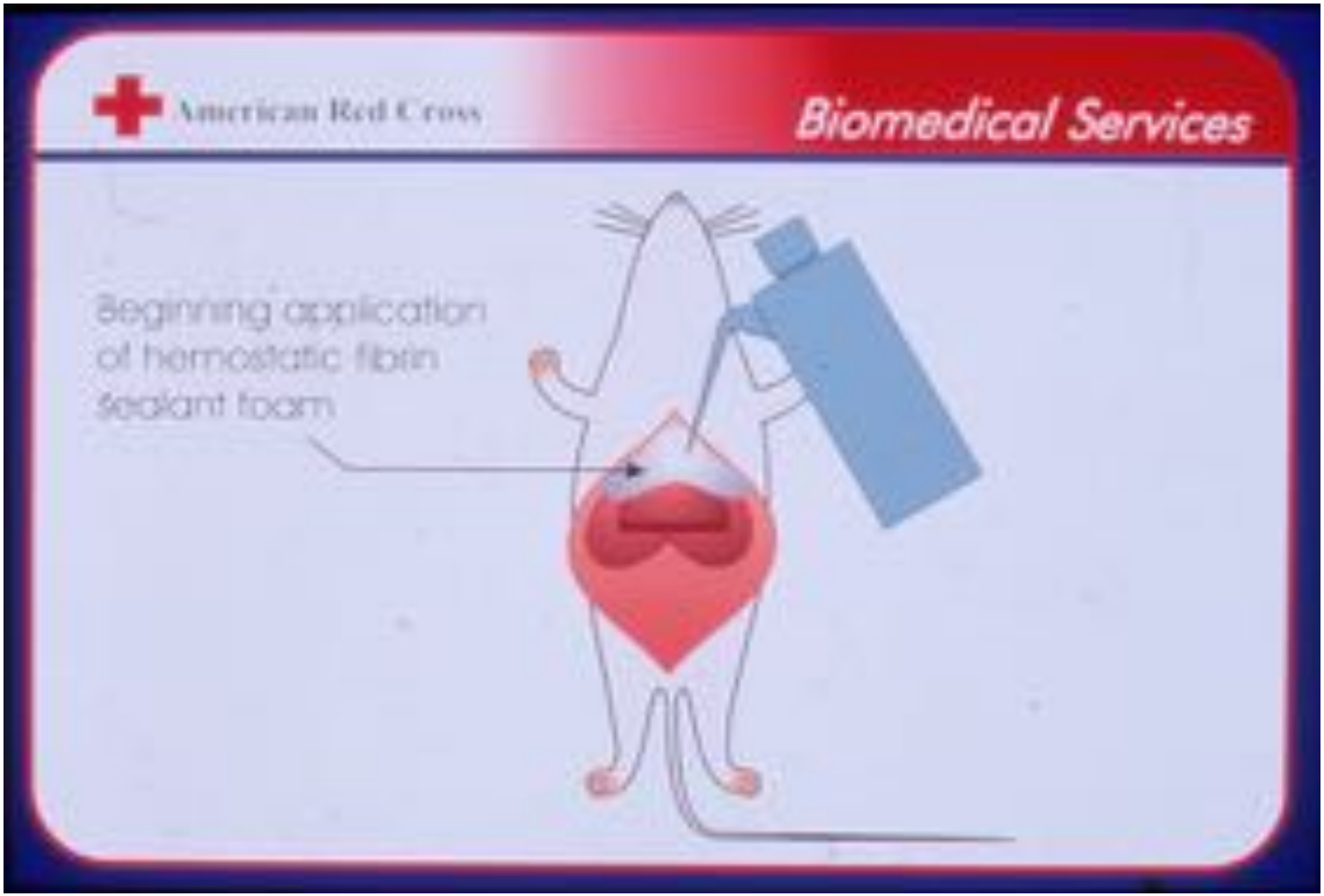
- Wilson Burgess(passed away February 04, 2015)
- Bill Velander (UNL): Biologics and Device Design
- Gustavo Larsen (LNK): Alginate Foam, Resorbable mesh
- Mark Carlson (UNMC): Surgical Trauma Model

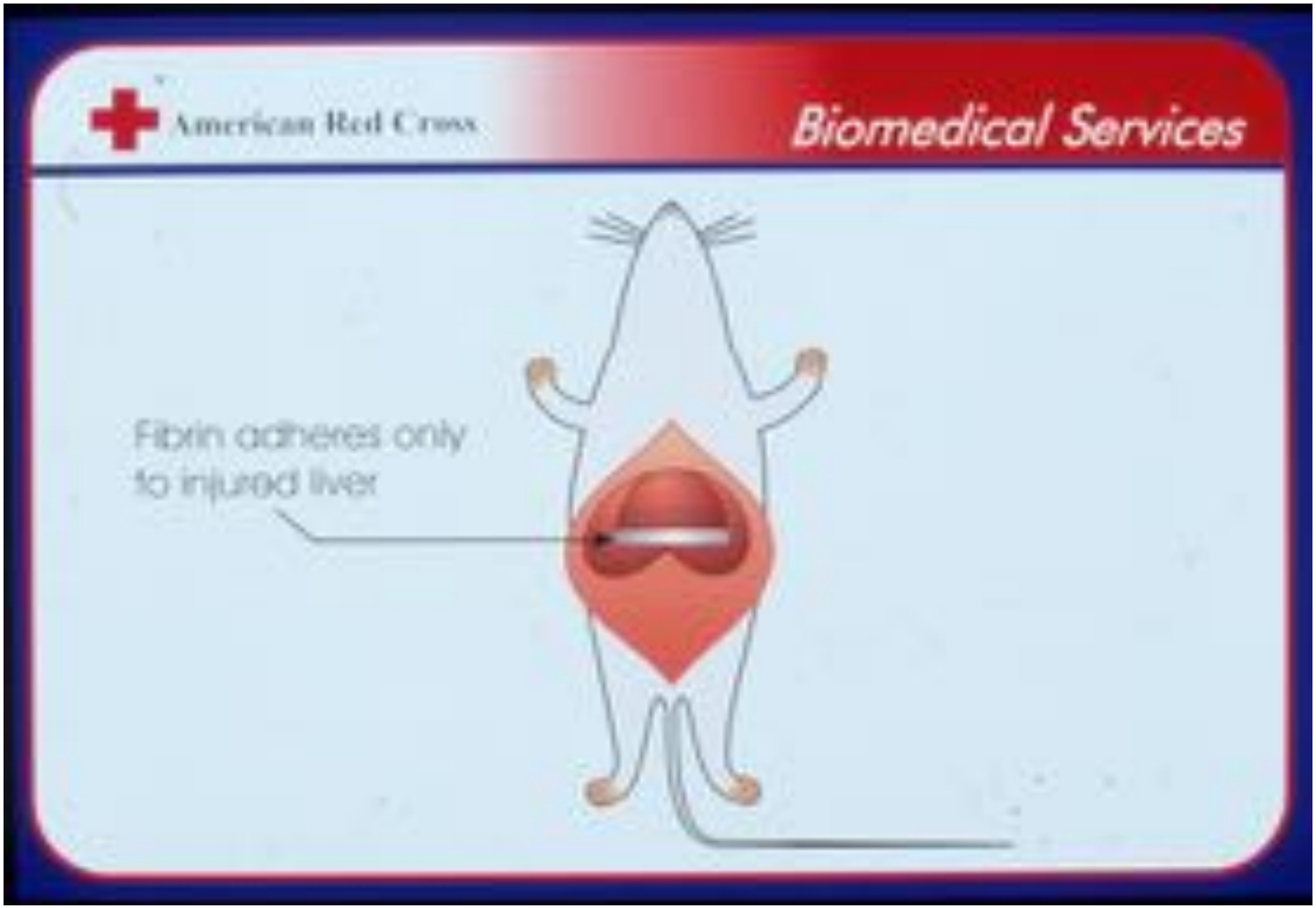
What we know from previous ARC work on fibrin foams…


200ml


American Red Cross
Biomedical Services
Median lobe of liver


American Red Cross
Biomedical Services
50% of median lobe
removed


American Red Cross
Biomedical Services
Beginning application of hemostatic fibrin sealant foam


American Red Cross
Biomedical Services
Fibrin adheres only
to injured liver

Product Feasibility of Hemostatic “100% FS Foam”:

100% FS foam for noncompressible wounds in severe parenchymal hemorrhage:

- partial liver resection in rat and rabbit models.
- effective in the rabbit noncompressible model at 300 mg fibrinogen/50 mL foam
- 4-6 L of expanding FS foam to fill an adult abdomen: $7,200 to $10,800 of fibrinogen, thereby rendering it economically infeasible.

- **Proof of principle that combination of tamponade and fibrin sealant is hemostatic**
- Balancing tamponade effects, exogenous and endogenous coagulation not studied.

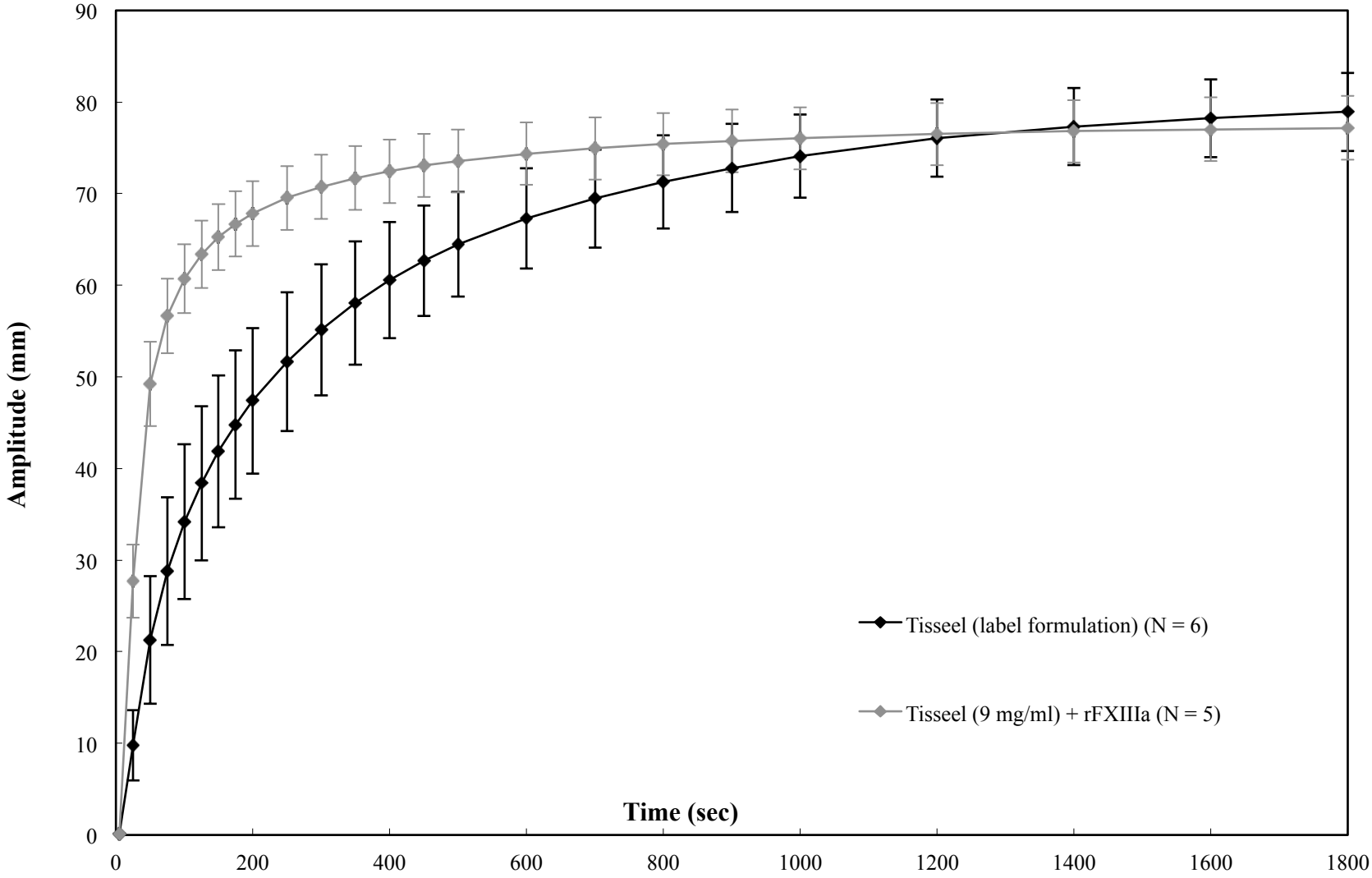
Holcomb JB, McClain JM, Pusateri AE, Beall D, Macaitis JM, Harris RA, et al. Fibrin sealant foam sprayed directly on liver injuries decreases blood loss in resuscitated rats. *J Trauma* 2000 Aug;49(2):246-50.

Kheirabadi BS, Sieber J, Bukhari T, Rudnicka K, Murcin LA, Tuthill D. High-pressure fibrin sealant foam: an effective hemostatic agent for treating severe parenchymal hemorrhage. *J Surg Res* 2008 Jan;144(1):145-50.

What we know from our work using our abundant supplies of pdF1, rF1, rFIIa, rFXIIIa:

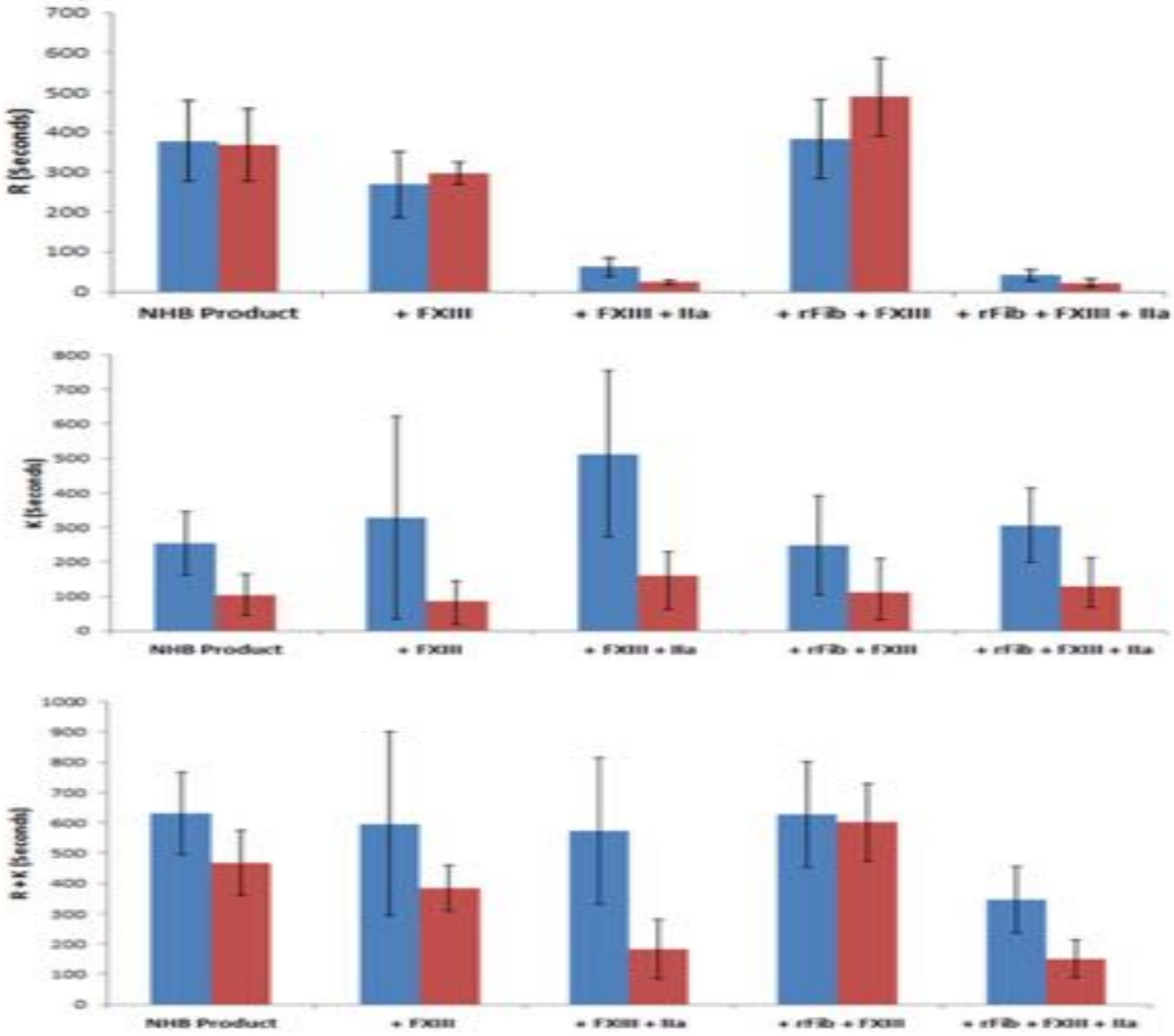
- **clotting kinetic and strength enhancements for fibrin formation**
- **clotting kinetic enhancements in whole human blood**
- **clotting kinetic enhancements in whole pig blood**
- **enhanced fibrin sprays onto wounds**

Viscosity effects: rFXIIIa relieves diffusion slowed crosslinking reactions during fibrin formation



rFXIIIa can decrease the amount of pdF1 needed while improving kinetics

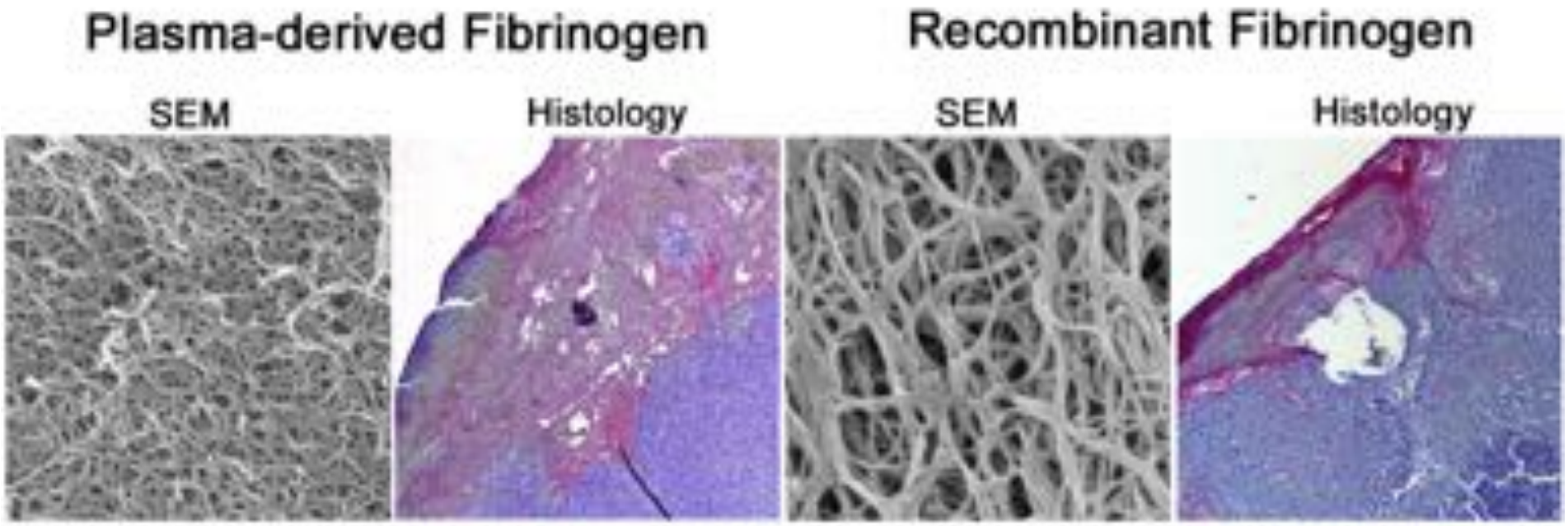
Clotting Kinetic Effects of r-F1,r-FXIII, rFIIa on NHB



Normal Human Blood
Platelet Rich Plasma

- R: Time until clot Initiation
- K: Time from clot initiation to maximum clot Formation

Characteristic Adhesiveness of rFI



- rF1 solates the effect of fibrin polymer structure
- Our gold standard fibrinogen for making FS

Key Engineering features to optimize device design

1. Hemostasis data on wound interfacial indicates that hemostasis is achievable in a 100 um layer of high density fibrin clot
2. Tamponade effect at minimal compartmental pressure is a key effect
3. Delivery of procoagulants (F1 and FIIa:FXIIIA) are key biologics
4. Role of exogenous versus endogenous procoagulation is engineerable.

FS FOAM DEVICE R&D PATHWAY

- Key engineering focus: cost minimization by biologic minimization
- Engineer device to deliver biologics and provide tamponade effect.
- Engineer biologics component mixing and delivery
- Use pdFS with key strength and kinetic enhancement by rFIIa-rFXIIIa (prefer rFS in long term)
- Study in closed cavity wound pig model
- Iterative redesign of FS-Foam nozzle with large animal surgery
- Engineer appropriate foam compressibility

Hemostatic Foam Discussion Points

- Research Enterprise Pathways and Timelines
- Foam and tamponade effects from endogenous clotting potential
- Foam and enhanced tamponade effects from exogenous procoagulants (speed, strength of clotting kinetics)
- Likely Ca^{2+} toxicity from vascular uptake at wound site
  1. current solution by dilution
  2. future solution by sequestration

Table 1. Per Dose Manufacturing Costs Estimated from Preclinical Data Using the Swine Liver Injury Model

Injury	Protein	Concentration in FS (mg/mL or U/mL	Amt FS needed (mL)	Total per Wound (mg or U)	Cost ($/mg or $/U)[1]	Cost per protein per Treatment	Total Estimated Cost per Treatment
Wedge Excision 2 (treat with 2 mL FS alone	FI or rFI	9 mg/mL	2	18 mg	$0.30	$5.40	$9.96
	rFXIIIA2-a	2,460 U/mL	2	4,920 U	$0.00007	$0.32	
	rFIIa	106 U/mL	2	212 U	$0.02	$4.24	
Grade V Laceration[2] (treat with 6 mL FS alone)	FI or rFI	9 mg/mL	6	54 mg	$0.30	$16.20	$29.89
	rFXIIIA2-a	2,460 U/mL	6	14,760 U	$0.00007	$0.97	
	rFIIa	106 U/mL	6	636 U	$0.02	$12.72	
Lobe Resection[2] (treat with 6mL + FS Resorbable, Pliable, Nanofibrous PLA Dressing)	FI or rFI	9 mg/mL	6	54 mg	$0.30	$16.20	$29.89
	rFXIIIA2-a	2,460 U/mL	6	14,760 U	$0.00007	$0.97	
	rFIIa	106 U/mL	6	636 U	$0.02	$12.72	
Dual Foam Technology[3] (8mL FS: used in 4L of Dual Foam)	FI or rFI	9 mg/mL	8	72 mg	$0.30	$21.60	$40.11
	rFXIIIA2-a	2,460 U/mL	8	19,680 U	$0.00007	$1.30	
	rFIIa	106 U/mL	8	848 U	$0.02	$16.96	

[1] Purchased (market) cost of rFI or FI, rFXIIIA2-a, and rFIIa. For rFIIa we use the actual market cost from Zymogenetics (0.02/U which translates to $4/mg). The rFXIIIA2-a will have an estimated market cost of $4/mg which translates to $0.00007/U. The rFI has an estimated purchased market cost of $300/g which is comparable to current purchase market cost of plasma-derived FI.

[2] Estimates from our preliminary results obtained in non-coagulopathic swine hepatic injury models.

[3] Estimates from closed cavity swine hepatic injury model: estimated $0.15 alginate per 4 L dose.

G0,G1,G2 Hemostatic Products and Regulatory Pathway:

Two Combined Biologic and Medical Devices

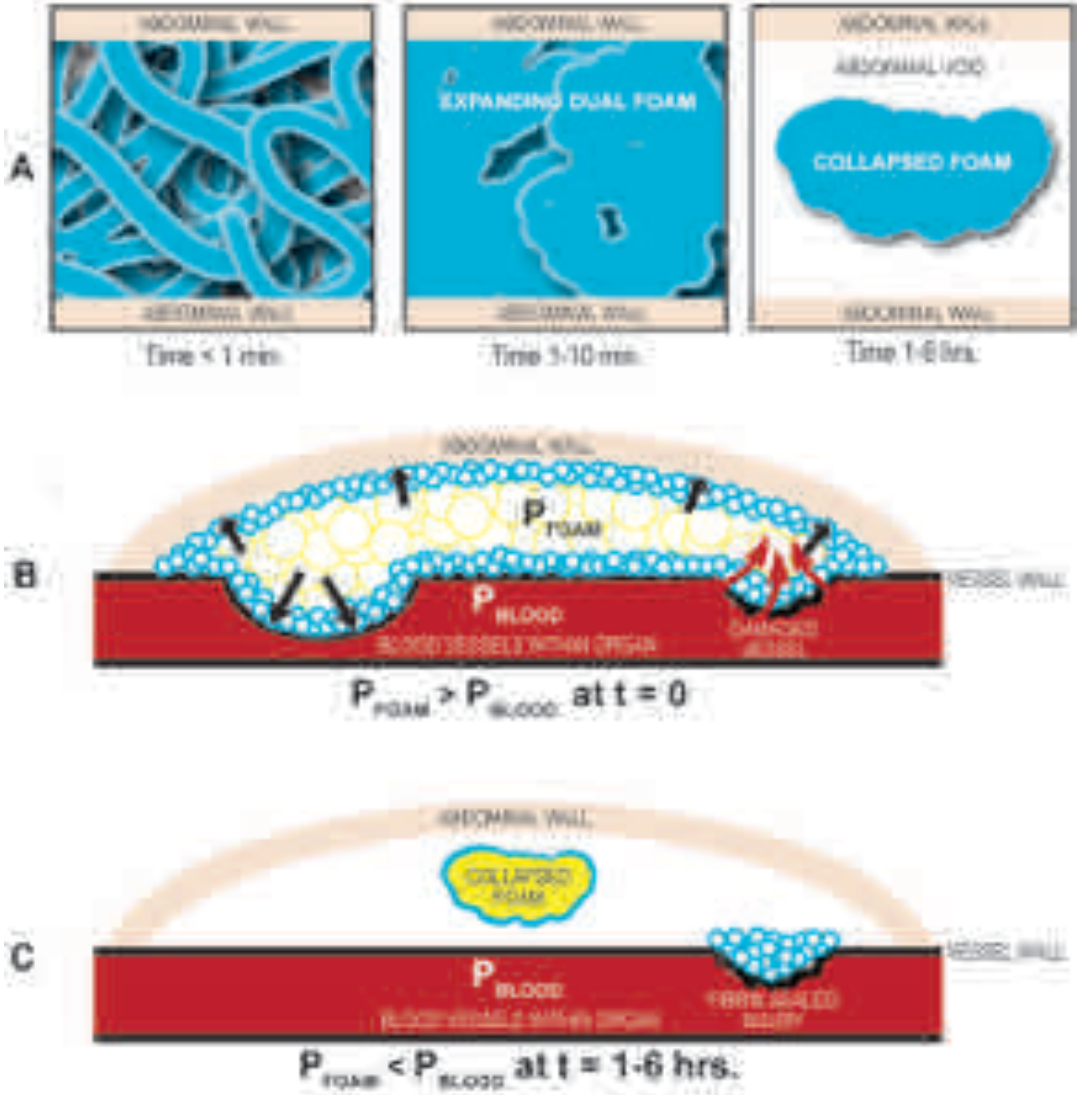
Foam Product Family

- G0 Foam alone (including Ca^{2+} sequestration dilution)
- G1 Foam plus rFIIa-pdF1
- G2 Foam plus rFIIa-rFXIIIa
- G3 Foam plus rFIIa-rFXIIIa-rFI

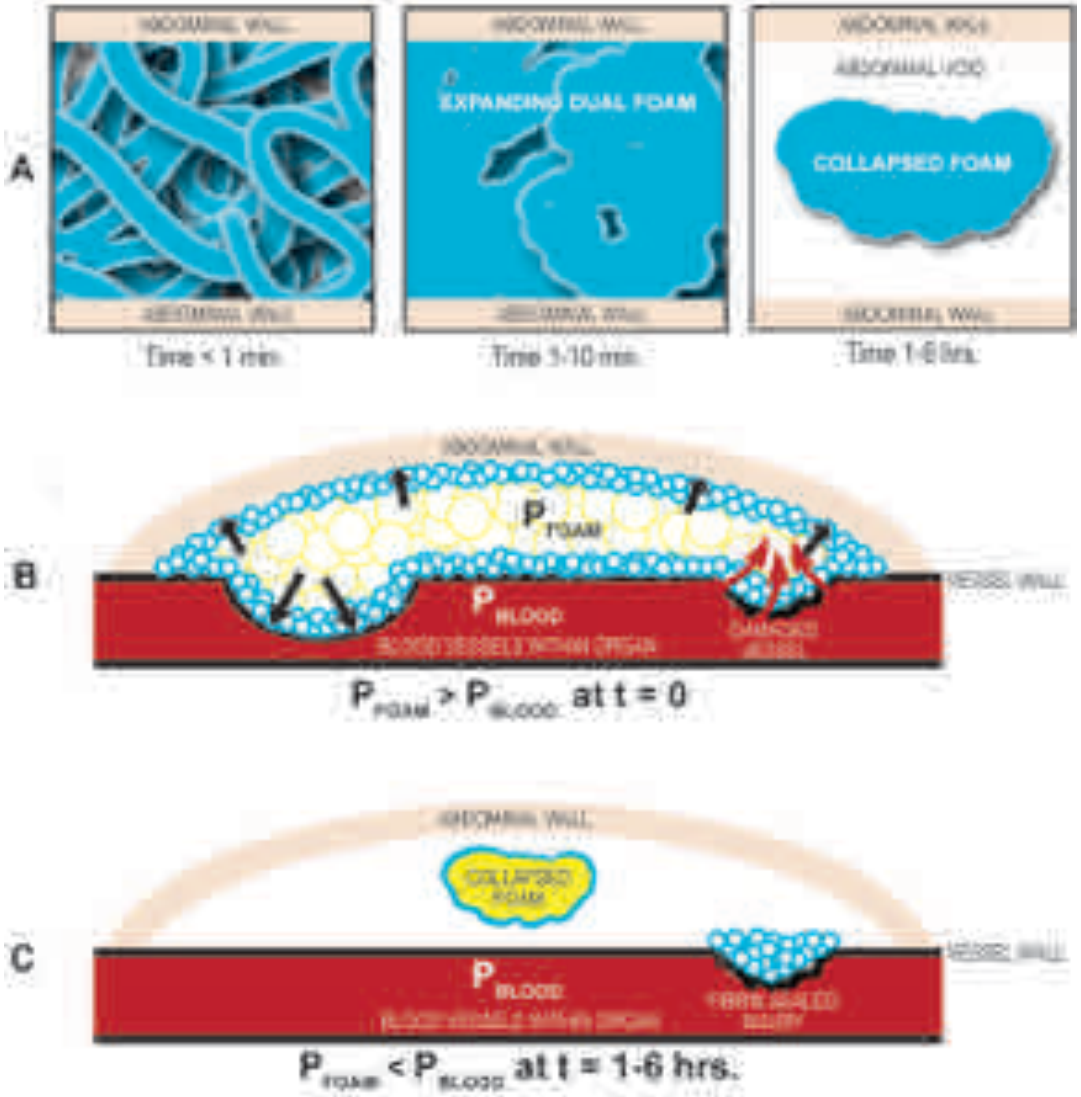
Bandage Product Family

- G0 bandage alone
- G1 bandage plus rFIIa-pdF1
- G2 bandage plus rFIIa-rFXIIIa
- G3 bandage plus rFIIa-rFXIIIa-rFI

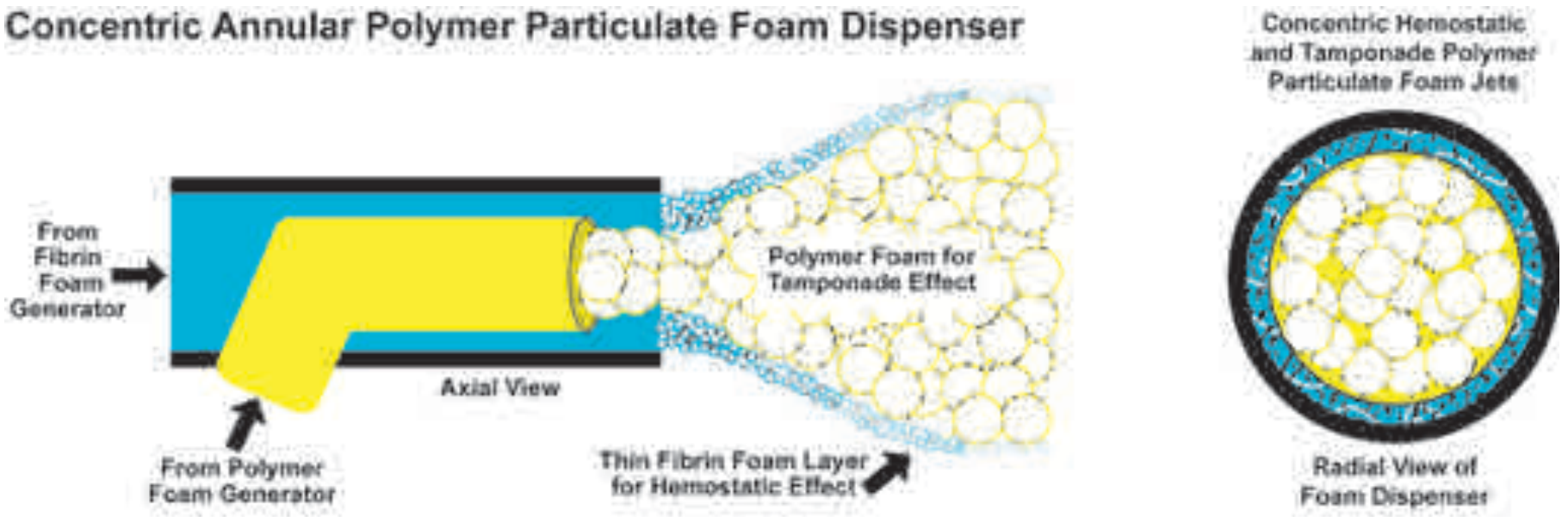
Engineered hydrodynamics/hydrostatics of co-administered resorbable polymer particulate hemostatic and tamponade foams



Current preclinical overview

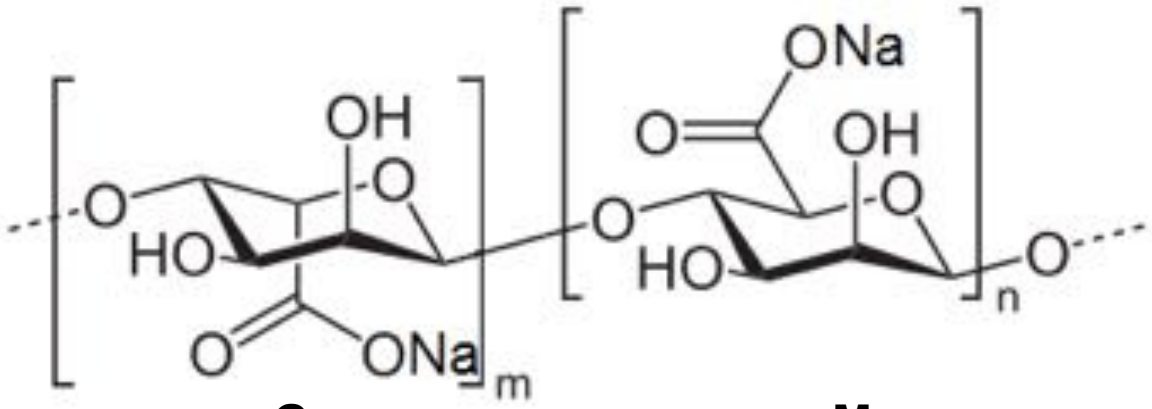


Prototype Foaming Device



Sodium to Calcium Alginate (XL)

- Alginates are binary copolymers, consisting of (1→4) linked β-D-mannuronic acid (**M**) and α-L-guluronic acid (**G**) residues of widely varying composition and sequence.



- Molecular weight range: 10,000 - 600,000 g/mol.
- Soluble sodium alginate forms soft hydrogels in the presence of calcium anions by non-covalent
- Cross-linking done here at 200 mM Ca^{2+}

Ca2+ Alginate Gel degradation

- The major mechanism for degradation *in vivo* (in humans) is disintegration due to gradual exchange of gelling Ca^{2+} with Na^{+}.

- Gentle chelation with citrate buffer can dissolve the gel formed by calcium

Alginate suppliers and cost

- FMC BioPolymer remains the world's largest producer of alginates. Several Chinese producers compete along with Cargill and DuPont.
- Average prices for Sodium Alginates : 18.00 $USD/Kg*.
- Cost per application (at 2wt% in 400mL; expansion ratio = 10-fold): 15 ¢ (8g).

* Quarterly Review of Food Hydrocolloids; end of 2012.

Alginates in regulatory approved products

Product	Company/ responsible	Clinical Trial (last updated)	Description
PROGENIX™ DMB Putty (US: 2006 & 2008)	Medtronic, Inc	-	Putty comprising demineralized bone matrix, sodium alginate and bovine collagen. Bone void filler.
Temporary Ureteral Drainage Stent (US: 2002)	Boston Scientific	-	Degradable stent facilitating passage of urine from kidney to bladder.
Emdogain (All major markets, US: 1996)	Straumann	-	Injectable PEG-alginate and enamel matrix proteins. Stimulates tissue regeneration after loss of periodontal tissue.
FOREseal	Les Laboratoires Brothier S.A.	-	Prevention of air leakage after lung resection of cancer using knitted calcium alginate sleeves.
Algicell	Derma Science Inc	FDA app.2006	Silver, calcium alginate wound dressing.

Alginate products in clinical trials

Product	Company/ responsible	Clinical Trial (last updated)	Description
Monolayer Cellular Device	Université Catholique de Louvain	Phase I (ongoing, Sept. 2009)	Human islets encapsulated in an alginate monolayer cellular device enabling subcutaneous implantation in type 1 diabetes patients
GLP-I CellBeads	CellMed AG(BTG plc.)	Phase I/II (ongoing, April 2011)	Alginate based microcapsules. Implantation into brain tissue cavity after hematoma removal.
IK-5001	Ikaria, Inc.	Phase II (not yet started, Feb. 2011)	Injectable mixture of sodium alginate and calcium gluconate. Prevention and reversal of left ventricular remodeling after myocardial infarction
DIABECELL® 5	Living Cell Technologies, Ltd.	Phase II(ongoing, June 2011)	Immunoprotection of insulin producing cells enabling xenotransplantation into peritoneal cavity of type I diabetes patients.
Algisyl-LVR™	LoneStar Heart, Inc.	Phase II/III (March 2011)	Injectable alginate hydrogel for patients with dilated cardiomyopathy.
CARTIPATCH	TBF Genie Tissulaire	Phase III (ongoing, Oct. 2010)	Implantation of chondrocytes from autologous cartilage.

Source: Andersen et al., *Carbohydr. Chem.,* 2012, **37**, 227-258.

PROJECT R&D BIOENGINEERING EVOLUTION AND GUIDELINES (DO'S AND DON'TS)

1. Use cost per dose to set bioengineering design limits
2. Make all biologics in gram amounts to be able to do large animal studies
3. Shift barrier role to low cost resorbable synthetic polymers
4. Use biologics to achieve covalent and noncovalent adhesion
5. Use bandage meshes as developmental tool to test adhesion

1. Don't throw (lots of) spaghetti at the wall to see if it sticks (one step at a time, don't believe in the lottery)
2. Use transport phenomena to guide positioning and formation of the hemostatic barrier

Table 1. Estimated Costs for FS Biologics per Wound Using the Swine Liver Injury Model

Injury	Protein	Concentration in FS (mg/mL or U/mL)	Amt FS needed (mL)	Total per Wound (mg or U)	Cost ($/mg or $/U)[1]	Cost per protein per Treatment	Total Estimated Cost per Treatment
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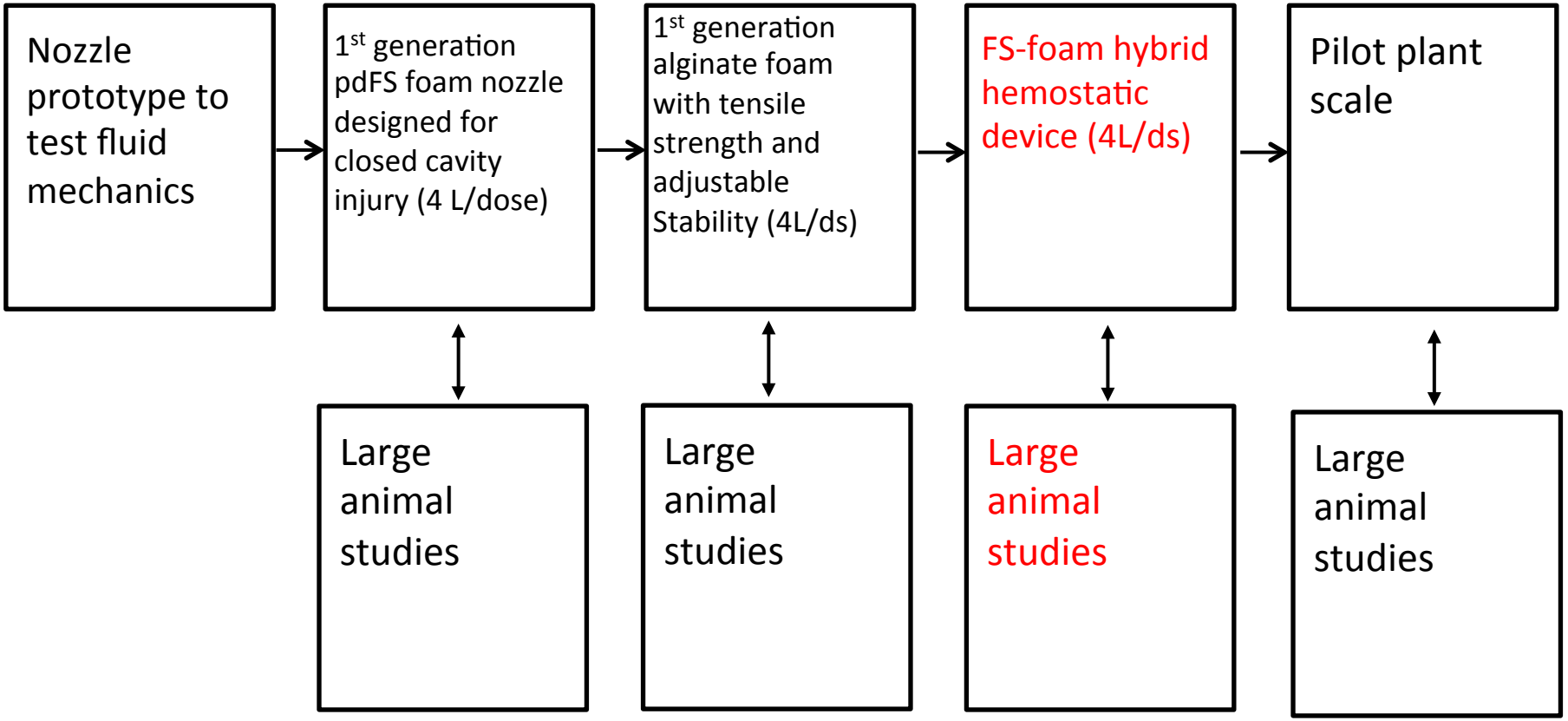
	rFIIa	106 U/mL	6	636 U	$0.02	$12.72	
Dual Foam Technology[3] (8 mL FS: used in 4L of Dual Foam)	FI or rFI	9 mg/mL	8	72 mg	$0.30	$21.60	$39.86
	rFXIIIA2-a	2,460 U/mL	8	19,680 U	$0.00007	$1.30	
	rFIIa	106 U/mL	8	848 U	$0.02	$16.96	

[1] Purchased (market) cost of rFI or FI, rFXIIIA2-a, and rFIIa. For rFIIa we use the actual market cost from Zymogenetics ($0.02/U which translates to $4/mg). The rFXIIIA2-a will have an estimated market cost of $4/mg which translates to $0.00007/U. The rFI has an estimated purchased market cost of $300/g which is comparable to current purchase market costs of plasma-derived FI.

[2] Estimates from our preliminary results obtained in non-coagulopathic swine hepatic injury models.

[3] Estimates for outcome of the proposed work with application to closed cavity swine hepatic injury model.

Evolution and future of FS Foam Hemostatic Device for Noncompressible Closed Cavity Wounds



Consortium Pathway to Commercialization

- US commercial partnership not feasible until early clinical trial
- Federal partnership is needed until clinical trial started
  - DOD funding for next 5 years
  - University small private company partnership has worked well

- US DOD embrace gives confidence to other Federal investment:
  1. French Ministry of Health through LFB
  2. Brazilian Ministry of Health through IVB, Fiocruz, Hemobras

Closed Cavity Hemostasis: Tamponade, Strength, Adhesiveness Effects

- Achieve necessary strength while managing viscosity
  1. Use lowest [F1] possible
  2. Use rFIIa/rFXIIIa to speed fibrin crosslinking
  3. AYMAN's slides on pig blood
  4. Nick's slides on TEG

- Improve adhesion using non and covalent attachment
  1. Use rFXIIIa to crosslink clot to exposed ECM proteins
  2. Use rFXIIIa to clot abdominal blood into a tamponade supplement

Tamponade slides

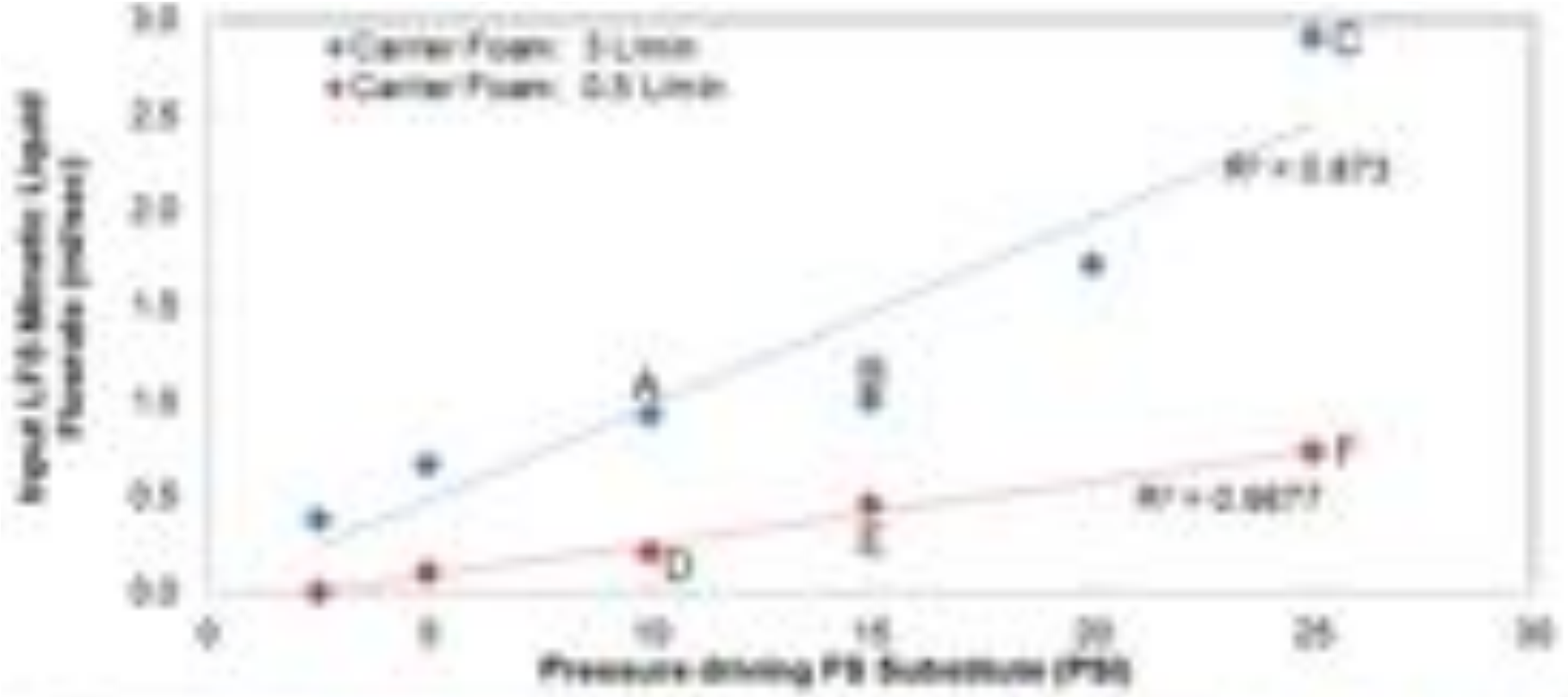
- Hyper Coagulated Blood to form a contour following moderatley compressible layer
- Resorbable foam particulate that gets entrained into the hyper coagulated blood
- <20 mmHg of intrabdominal pressure

- AYMAN's slides !!!

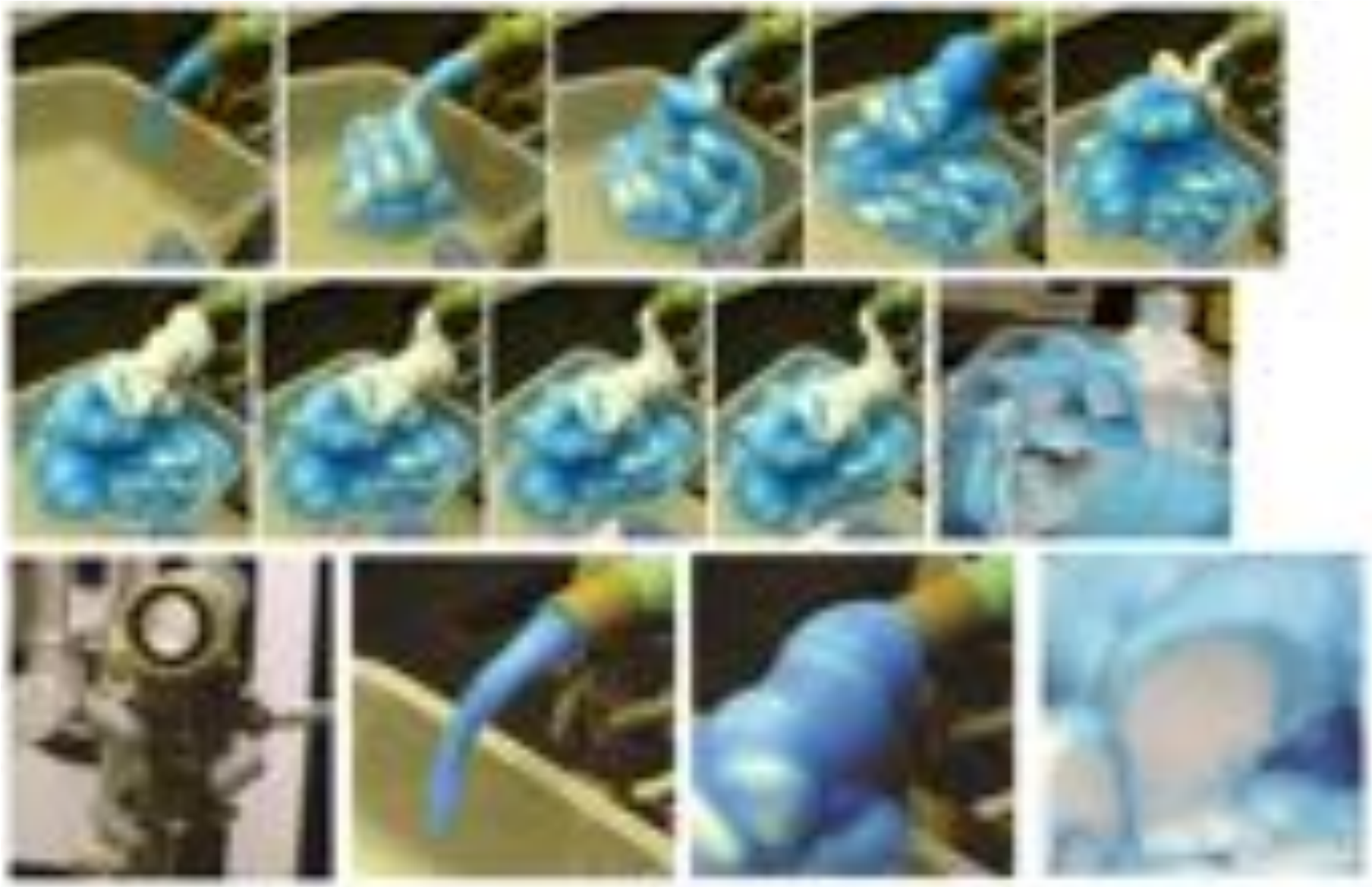
Prototype Device: Carrier Foam + 2 FS substitute inputs

Well mixed FS mimetic (red + blue = purple)

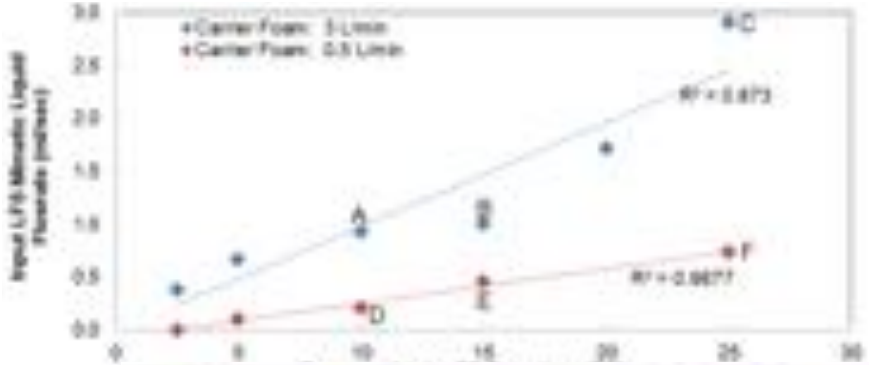
Prototype Device: Carrier Foam + 2 FS substitute inputs

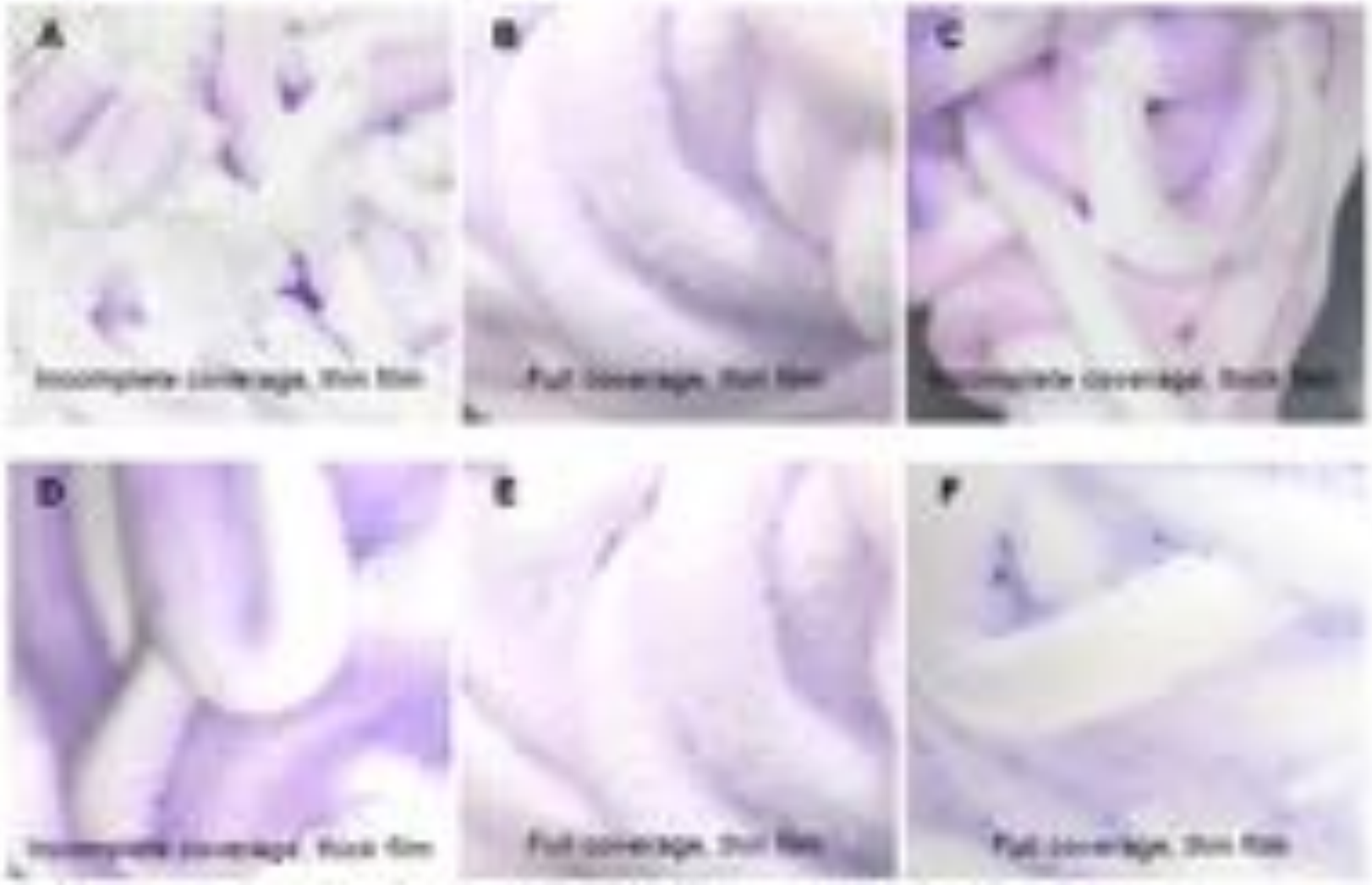


Prototype Device: Carrier Foam + 1 FS input (blue)

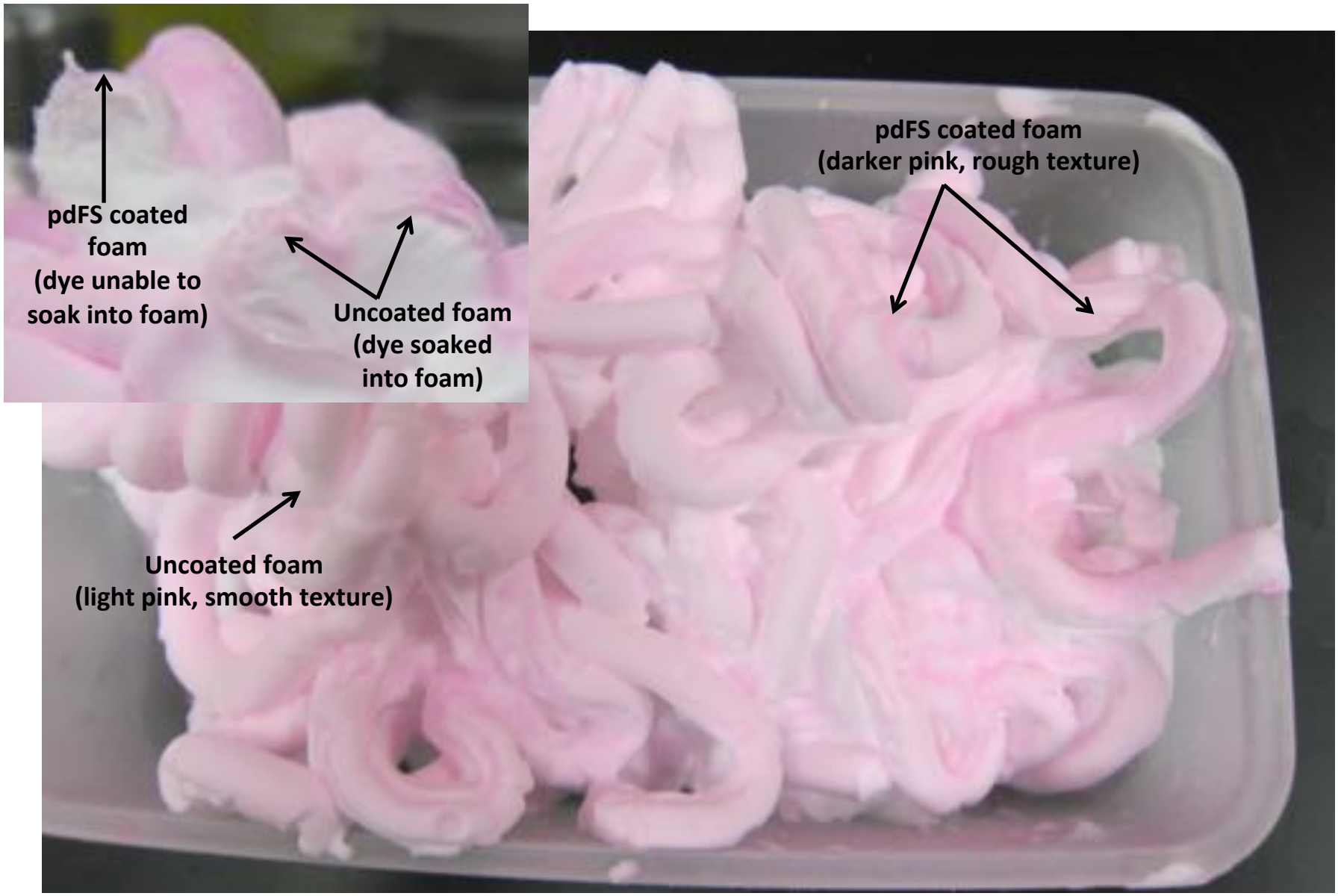


Dye test of fluid mechanics and mixing

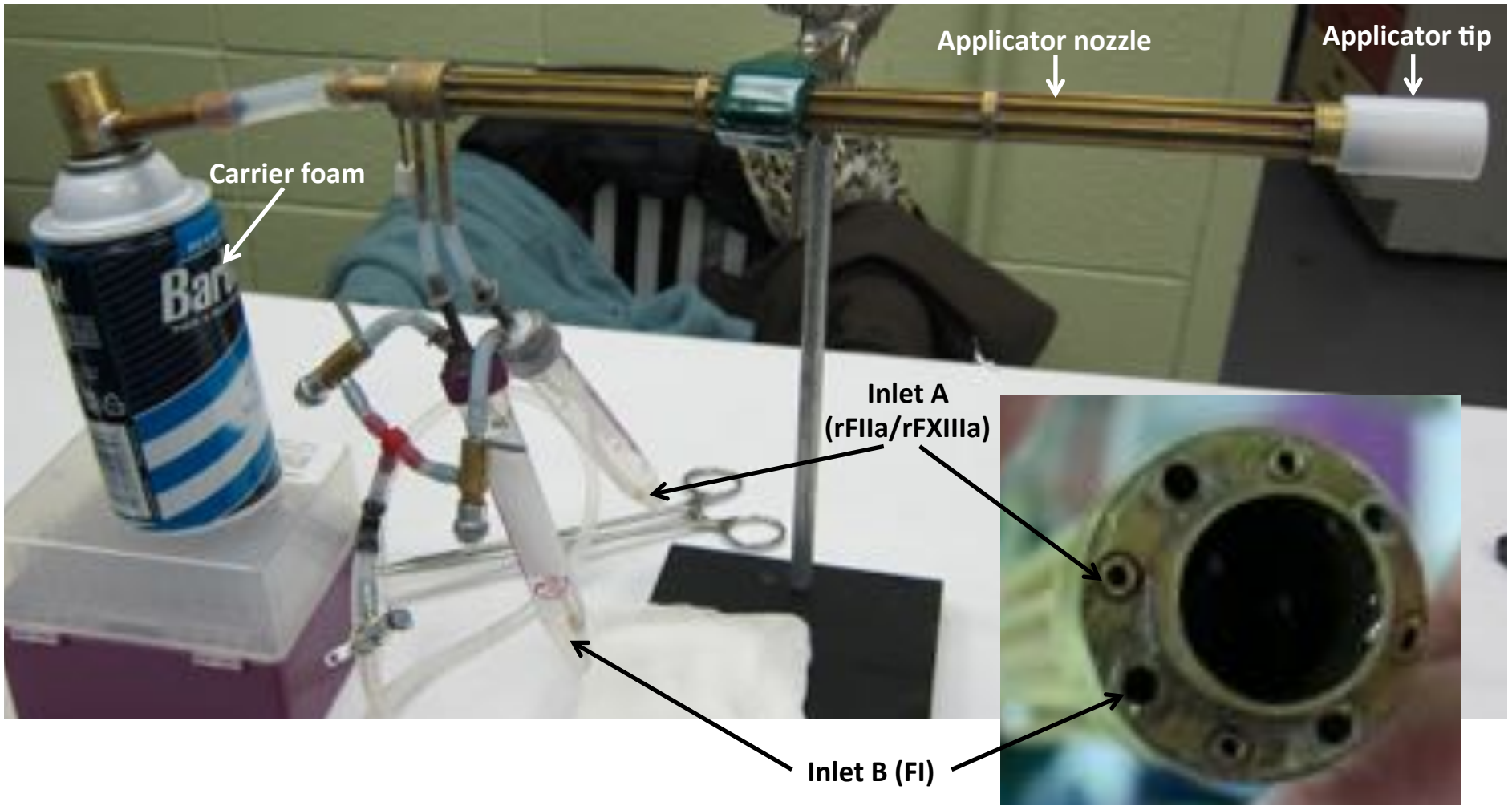




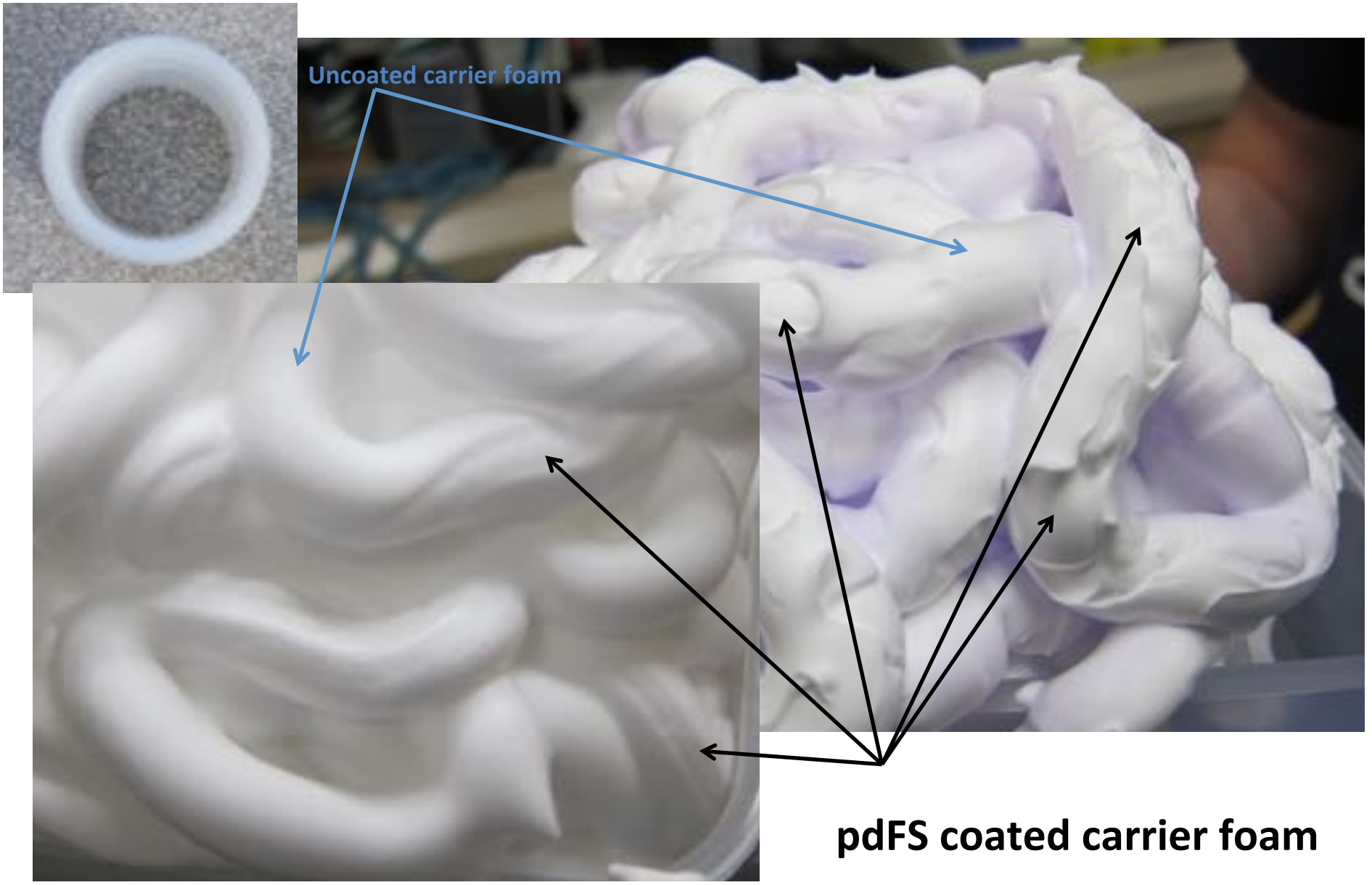
Prototype Nozzle: Carrier Foam + pdFS + dye



2nd Generation Foaming Device



2nd Generation Foaming Device: Unbeveled Tip



Alginate foam slide and movie

Survival stats for three treatment groups

- Intra-abdominal pressure trace

Aymans' slides

- Diagram of intracavity observations (3 phases)
- For treatments group slides
- Presence of foam without amalgam clot (phase I)
- Presence of foam with clot amalgam (phase II)
- Presence of firm adhesive clot at wound surface (phase III)
- TEG of what happens in pig and human blood when you add procoagulants
- TEG of pig blood before and after wounds

UNMC

BREAKTHROUGHS FOR LIFE.®

University of Nebraska Medical Center

Biomedical Porcine Models At The Omaha VAMC

Mark A. Carlson, MD

University of Nebraska Medical Center
VA Nebraska Western Iowa Healthcare System

Omaha, Nebraska, USA

UNMC BREAKTHROUGHS FOR LIFE.

Nebraska Medicine SERIOUS MEDICINE. EXTRAORDINARY CARE.

VA Nebraska-Western Iowa Health Care System

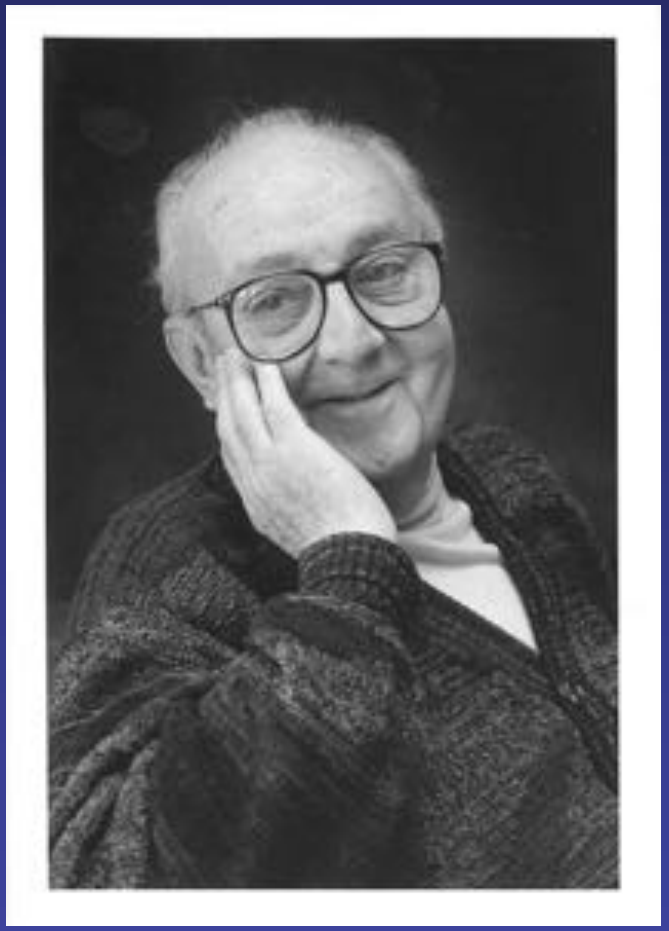
Surgery Research Forum, June 24, 2015

Disclosures:

none

Models…

"Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful."

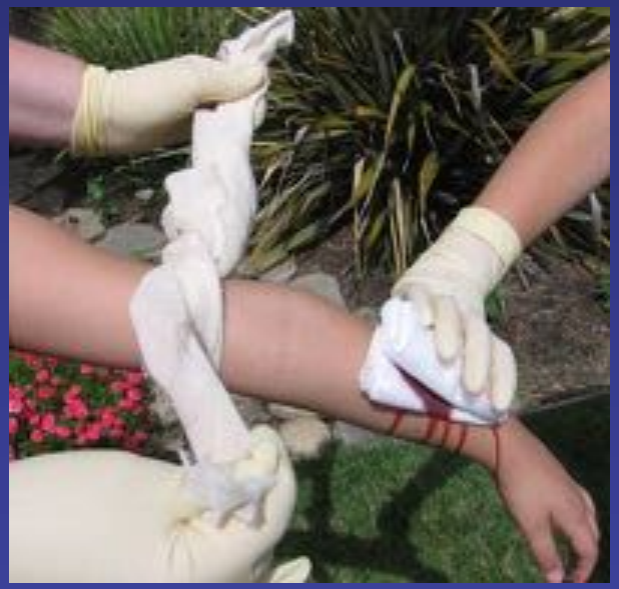


George E. P. Box
1919-2013

Hemostasis

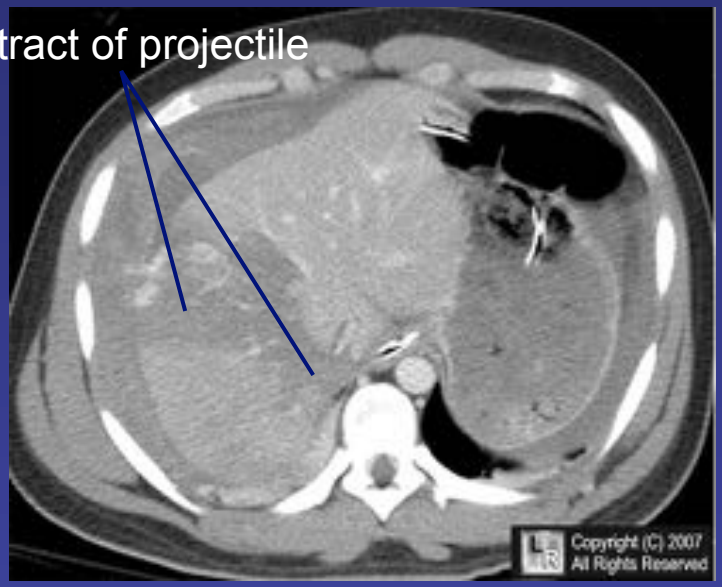
Hemorrhage Types

Compressible



Extremity

Noncompressible



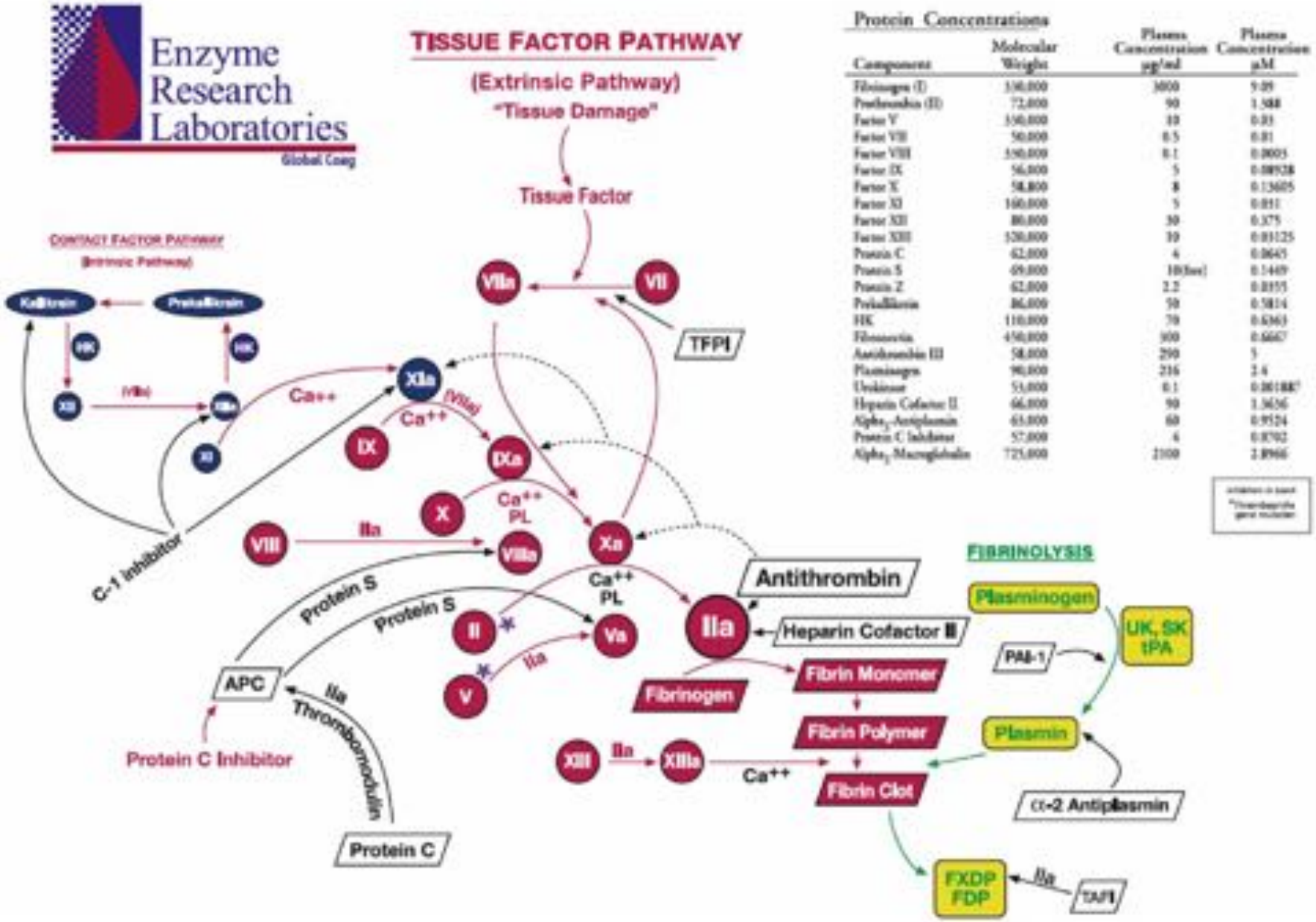
Liver

Noncompressible Hemorrhage

- 80-90% combat-related deaths happen <1 hour after injury
- >50% combat-related deaths 2˚ to uncontrolled bleeding
- Most deaths 2˚to hemorrhage involved noncompressible hemorrhage
- No Rx for noncompressible hemorrhage (except immediate surgery)

Source: US Army CCCRP (Combat Casualty Care Research Program)

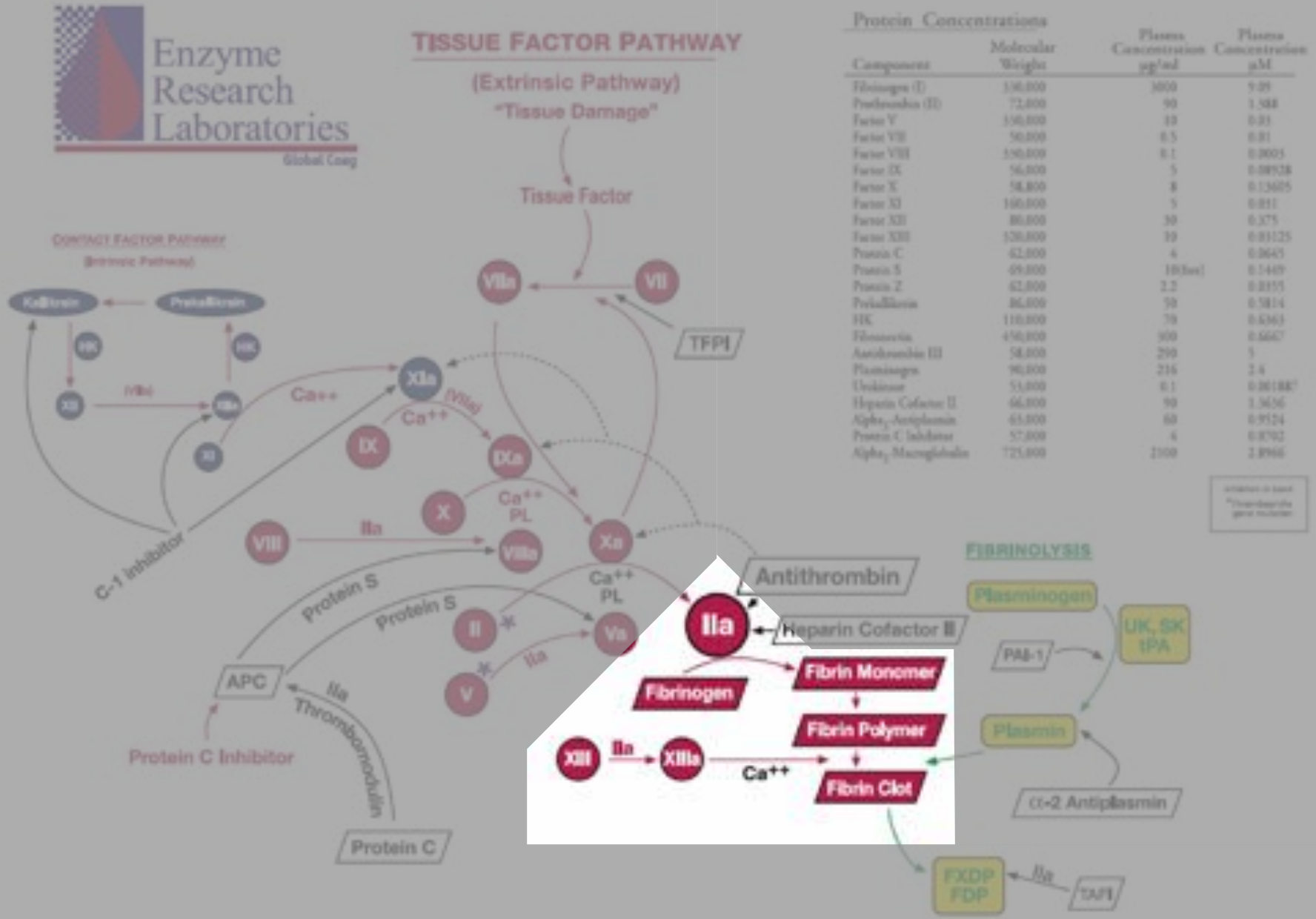
Coagulation Cascade



Protein Concentrations

Component	Molecular Weight	Plasma Concentration µg/ml	Plasma Concentration µM
Fibrinogen (I)	340,000	3000	9.09
Prothrombin (II)	72,000	90	1.388
Factor V	330,000	10	0.03
Factor VII	50,000	0.5	0.01
Factor VIII	330,000	0.1	0.0003
Factor IX	56,000	5	0.08928
Factor X	58,800	8	0.13605
Factor XI	160,000	5	0.031
Factor XII	80,000	30	0.375
Factor XIII	320,000	10	0.03125
Protein C	62,000	4	0.0645
Protein S	69,000	10[free]	0.1449
Protein Z	62,000	2.2	0.0355
Prekallikrein	86,000	50	0.5814
HK	110,000	70	0.6363
Fibronectin	450,000	300	0.6667
Antithrombin III	58,000	290	5
Plasminogen	90,000	216	2.4
Urokinase	53,000	0.1	0.00188*
Heparin Cofactor II	66,000	90	1.3636
Alpha$_2$-Antiplasmin	65,000	60	0.9524
Protein C Inhibitor	57,000	4	0.0702
Alpha$_2$-Macroglobulin	725,000	2100	2.8966

Coagulation Cascade



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Factor X	58,800	8	0.13605
Factor XI	160,000	5	0.031
Factor XII	80,000	30	0.375
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Protein Z	62,000	2.2	0.0355
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Urokinase	53,000	0.1	0.00188*
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Alpha$_2$-Antiplasmin	65,000	60	0.9524
Protein C Inhibitor	57,000	4	0.0702
Alpha$_2$-Macroglobulin	725,000	2100	2.8966

TEG: effect of Factor XIII

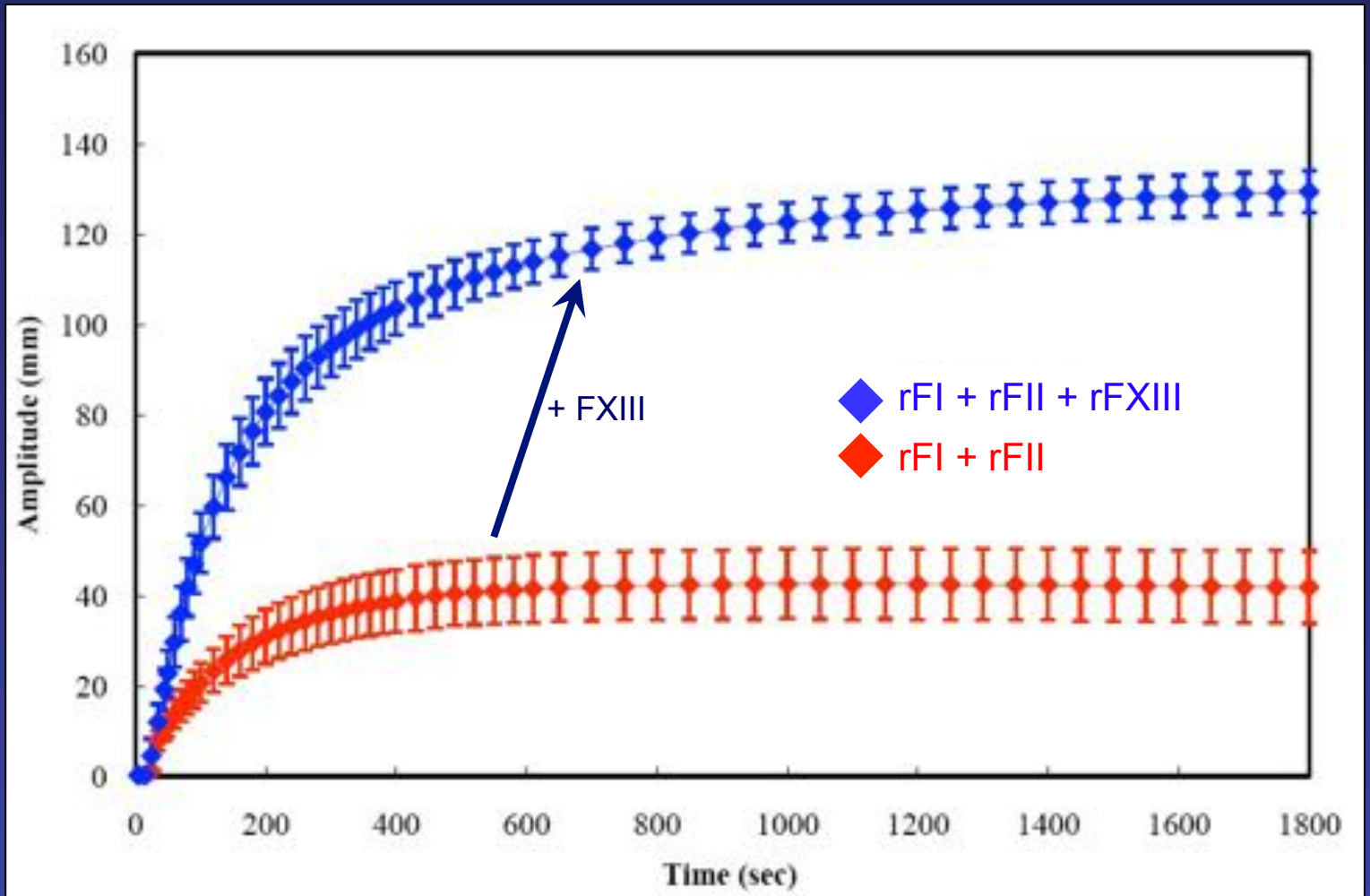


Figure 3.11. Analysis of the effect of rFXIIIa on clot strength by thromboelastography. TEG monitored the change in clot strength over time of recombinant fibrinogen (8.56 mg/ml) activated by recombinant thrombin (52.8 U/ml) with (blue) and without (red) rFXIII (0.35 mg/ml). Data are expressed as mean +/- standard deviation.

TEG: r-FS vs. pd-FS (Tisseel)

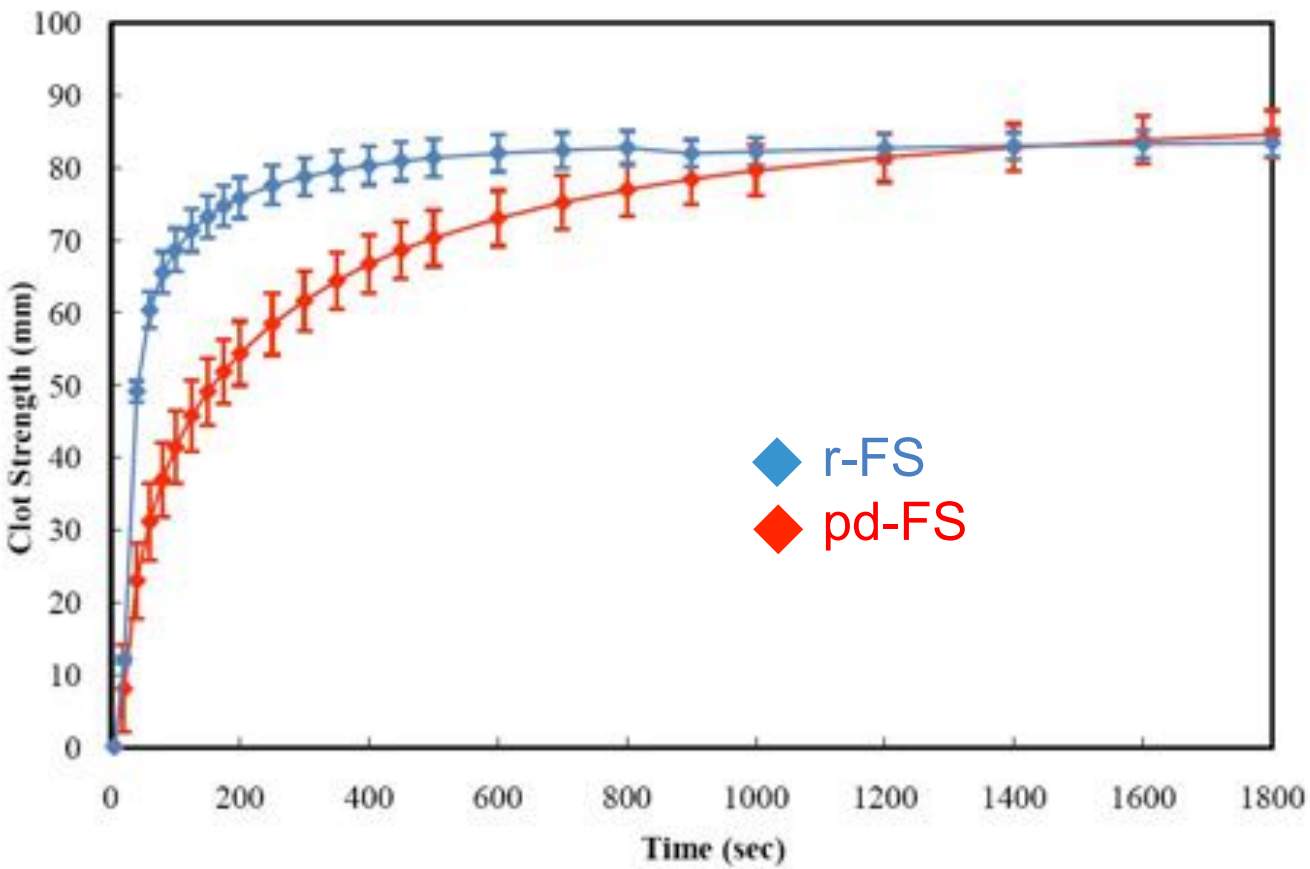
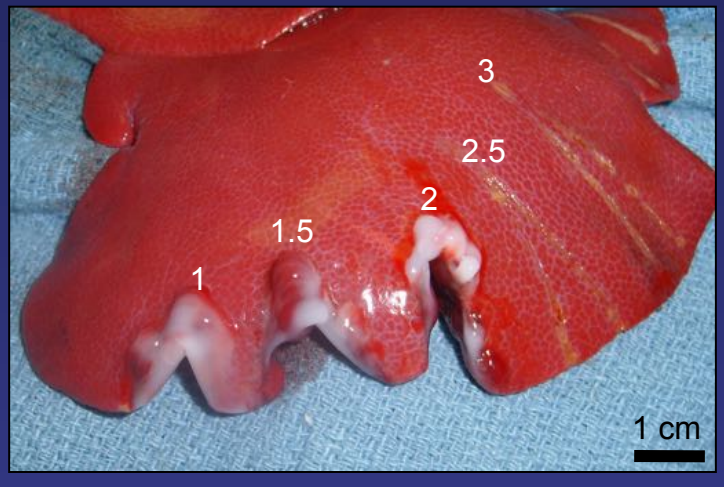
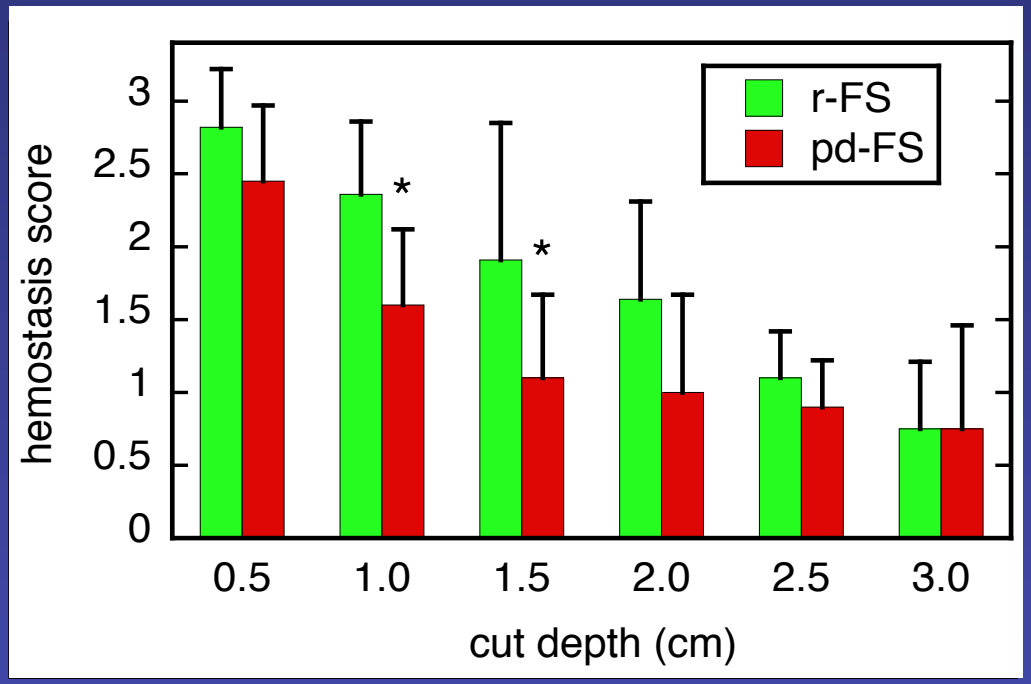


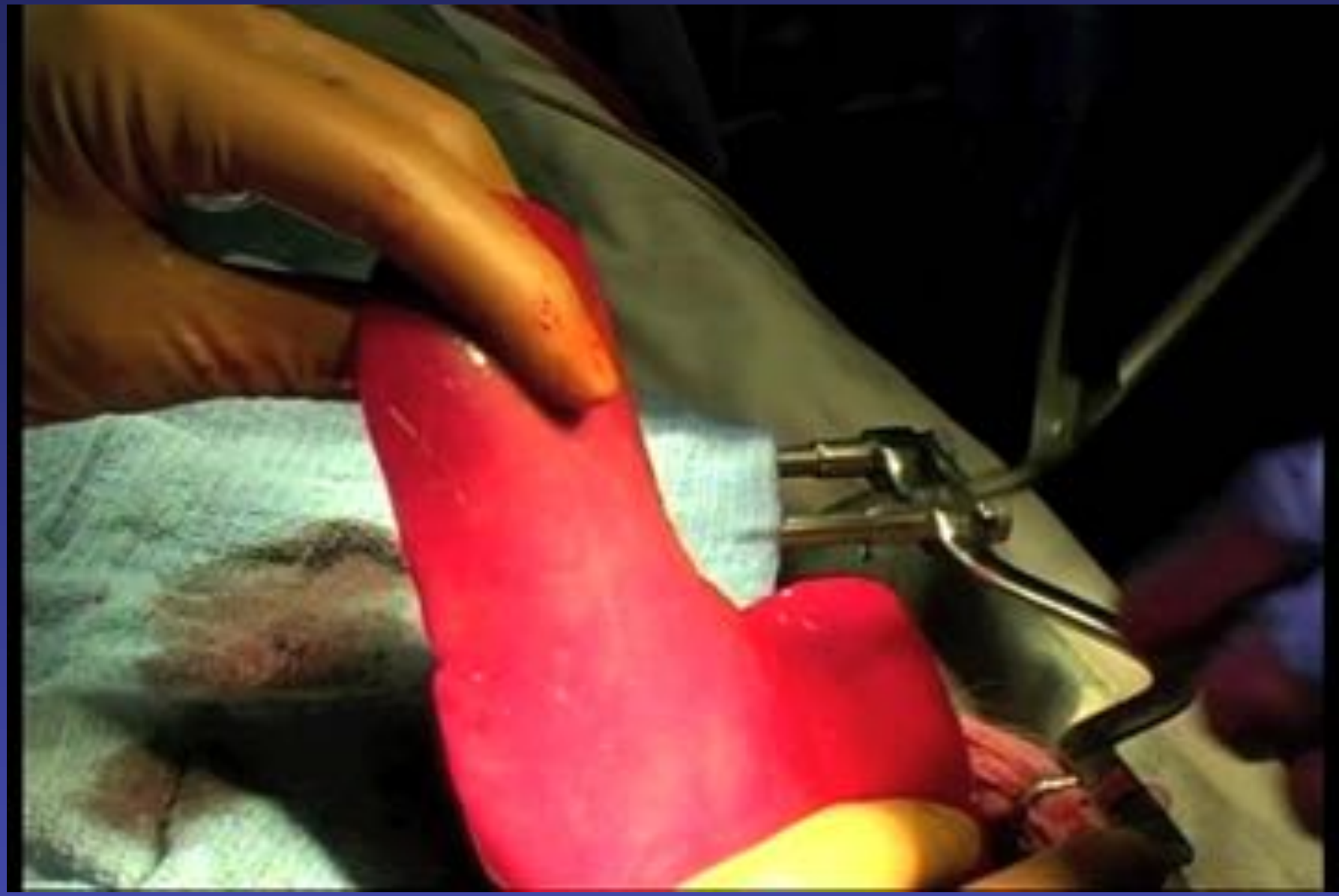
Figure 4.10. Thromboelastographic properties of the optimized tri-component rLFS and a pdLFS (Tisseel®). Clots consisting of 15 mg/ml rFI, 0.59 mg/ml (4,095 U/ml) rFXIIIa and 0.33 mg/ml (176.7 U/ml) rFIIa (FXIIIa/FI = 0.16, FIIa/FI = 0.18) (blue) have faster clot kinetics and equivalent maximal clot strengths as a pdLFS (Tisseel®) as prepared by its manufacturer's instructions (red). Tisseel contains 33.5 to 53 mg/ml pdFI and 200 to 312.5 U/ml pdFIIa. Data were expressed as mean +/- standard deviation.

Fibrin sealants: plasma-derived vs. recombinant



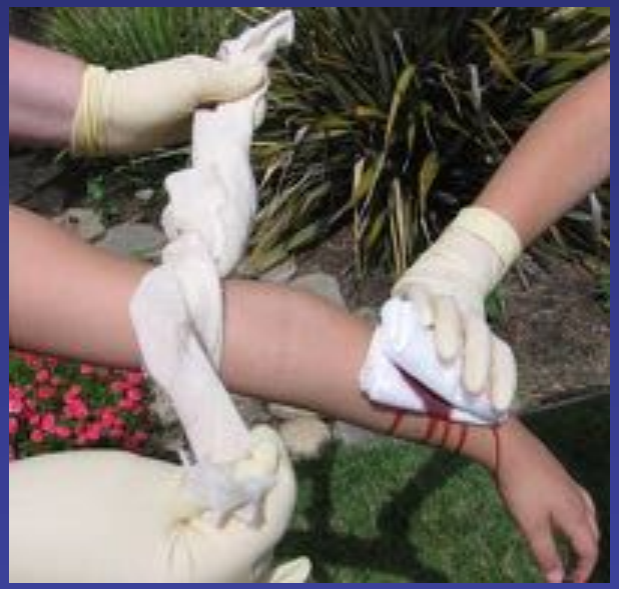


Relative efficacy of plasma-derived vs. recombinant FS



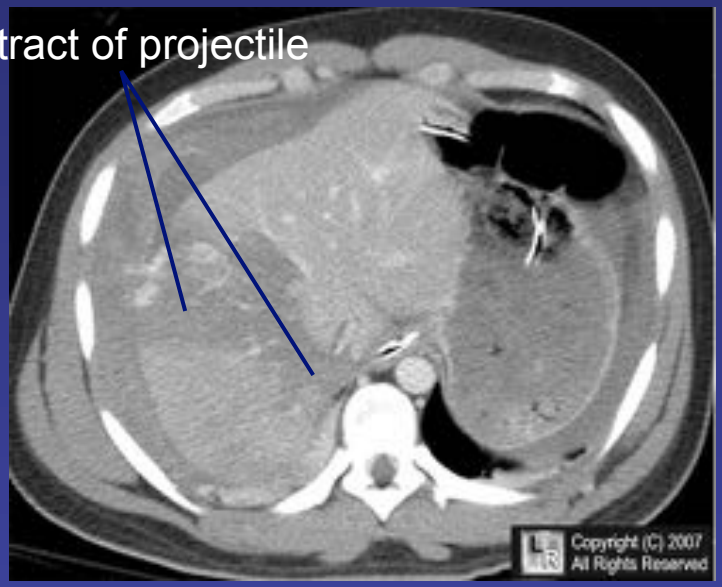
Hemorrhage Types

Compressible

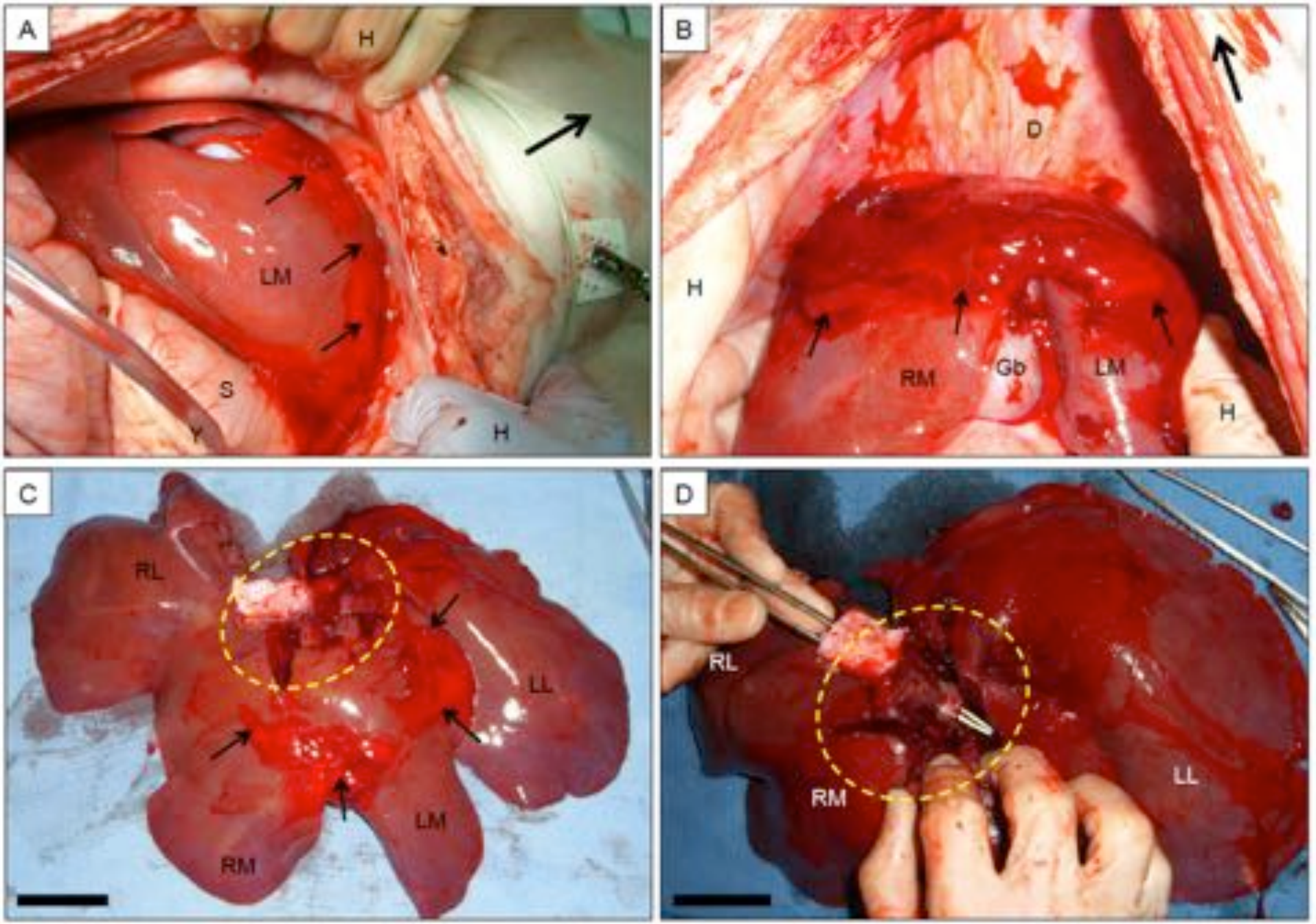


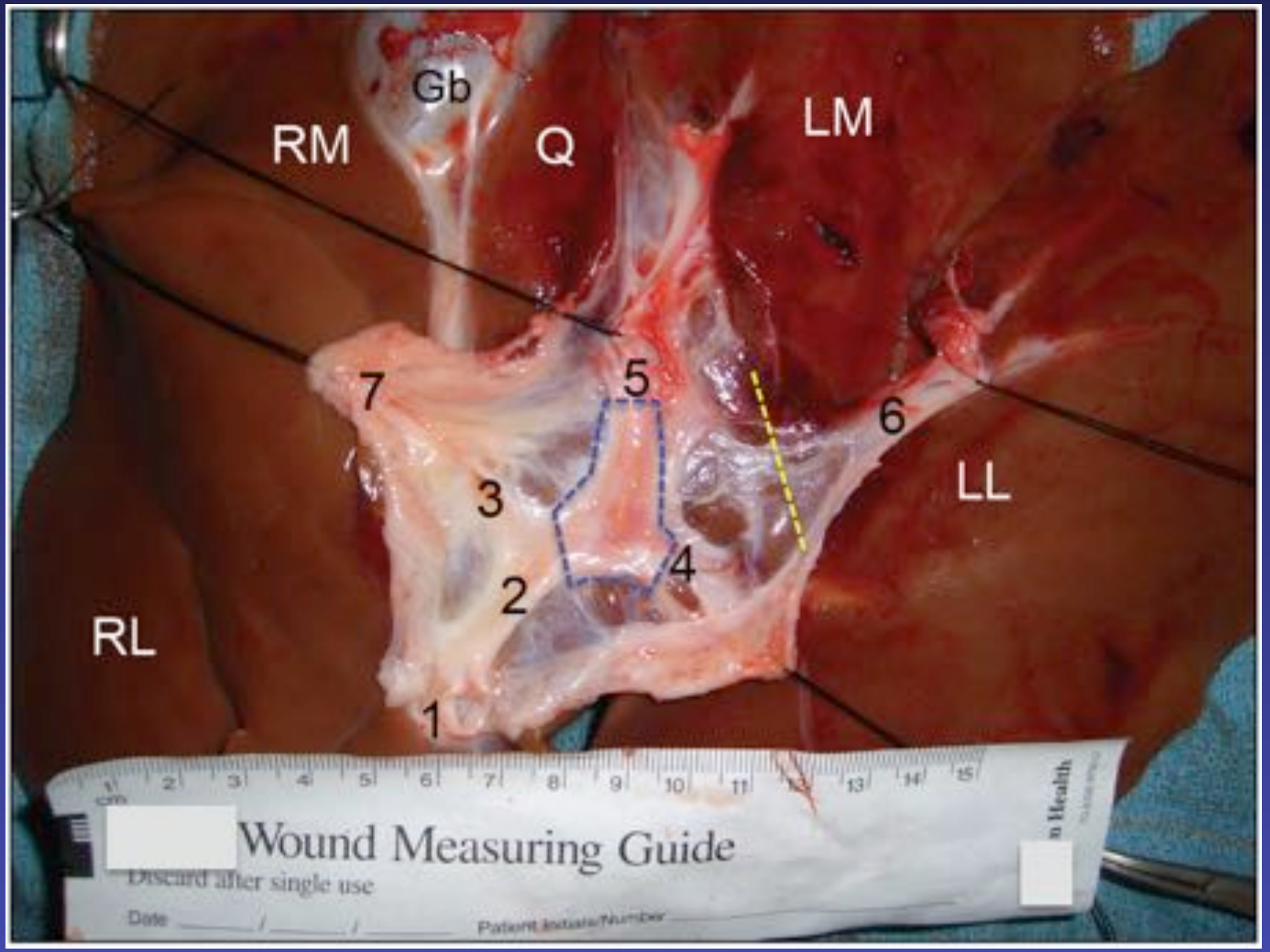
Extremity

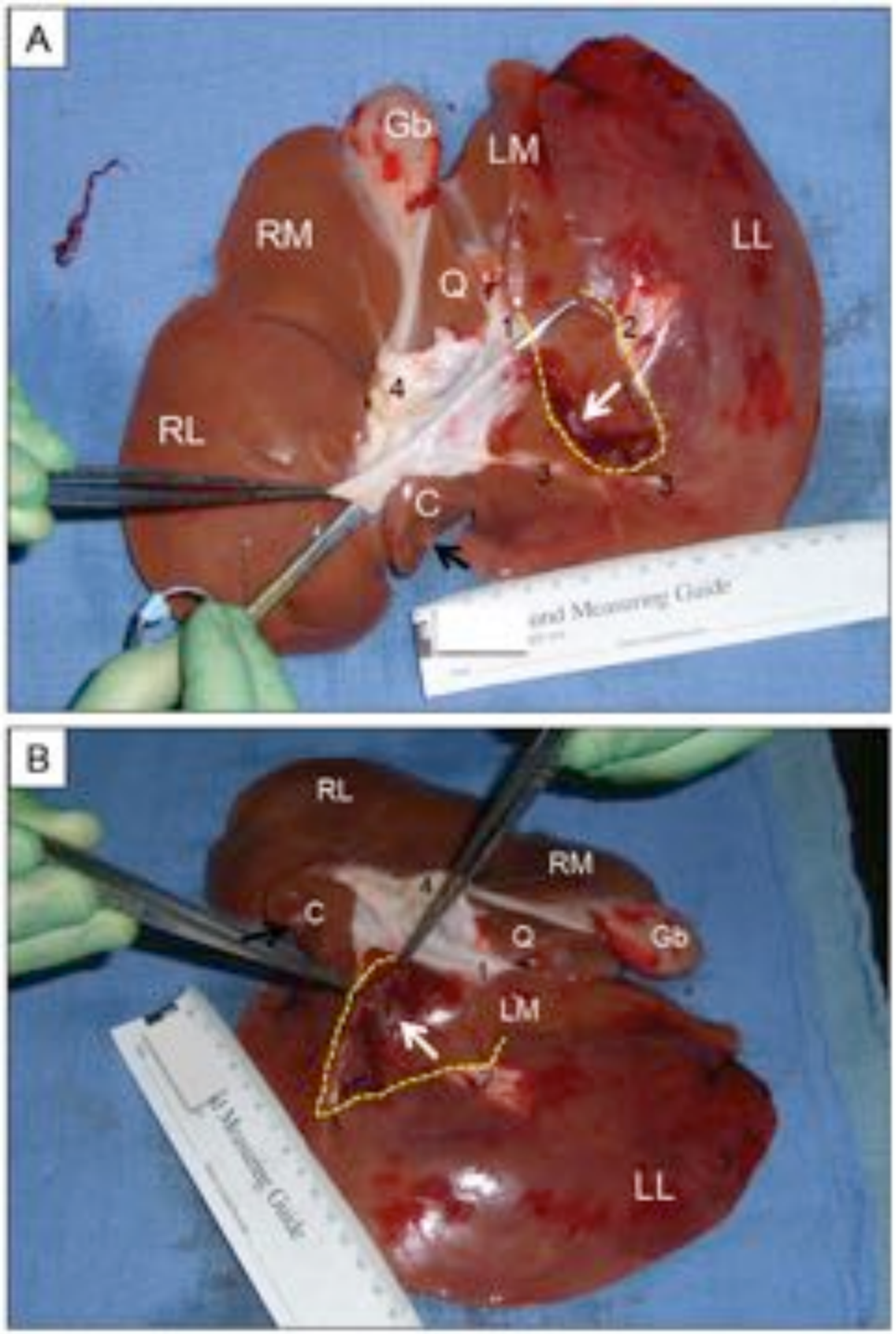
Noncompressible

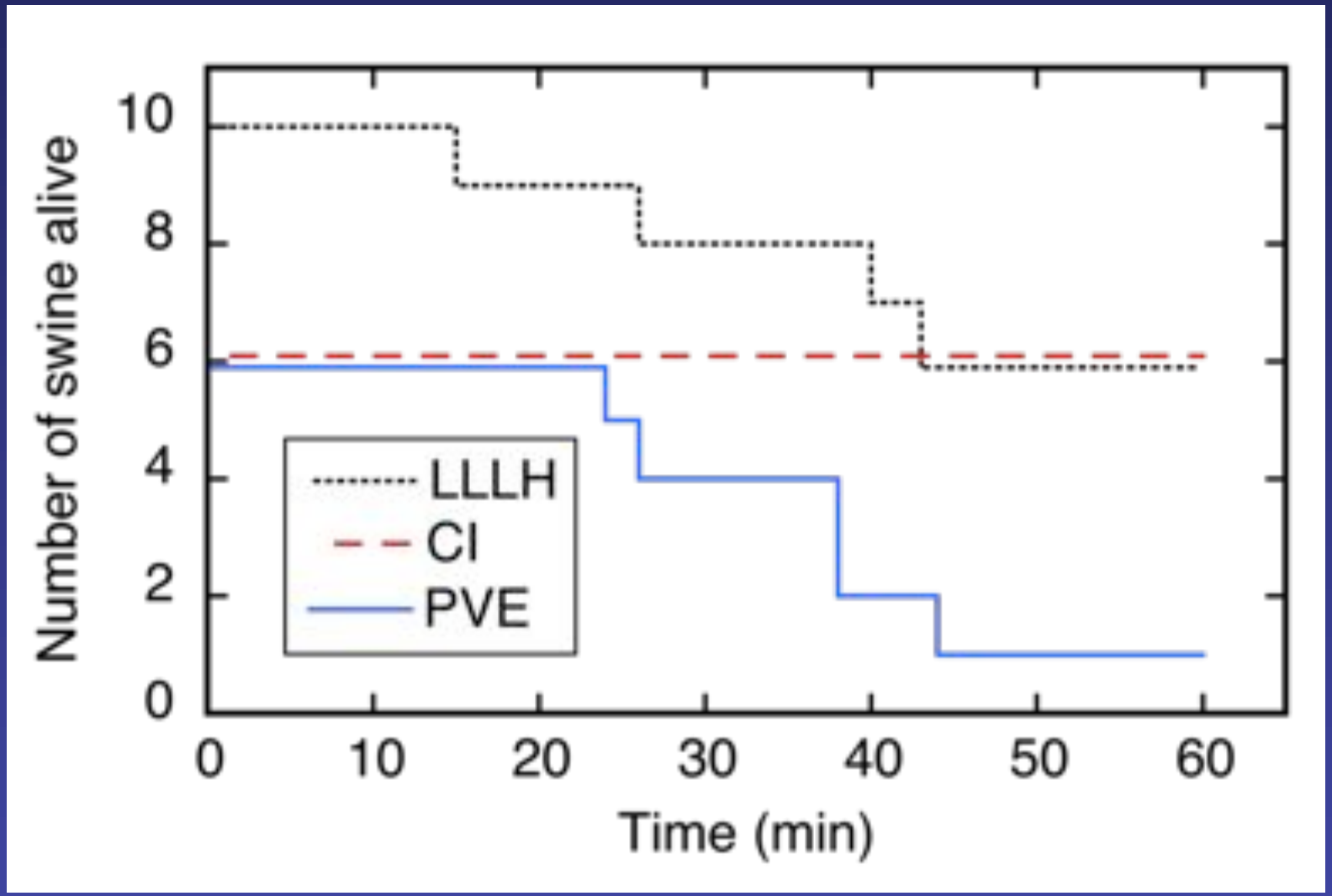


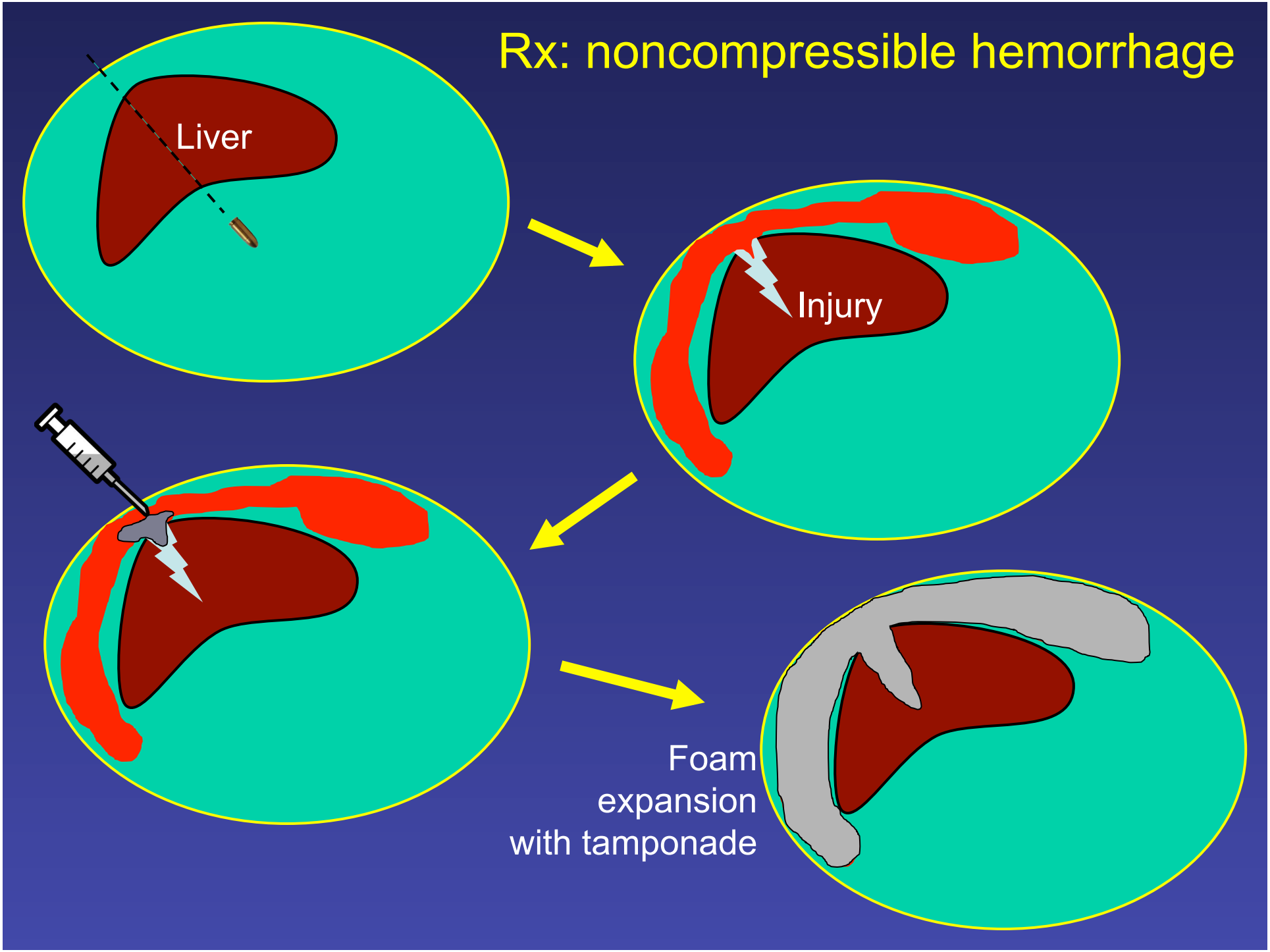
Liver

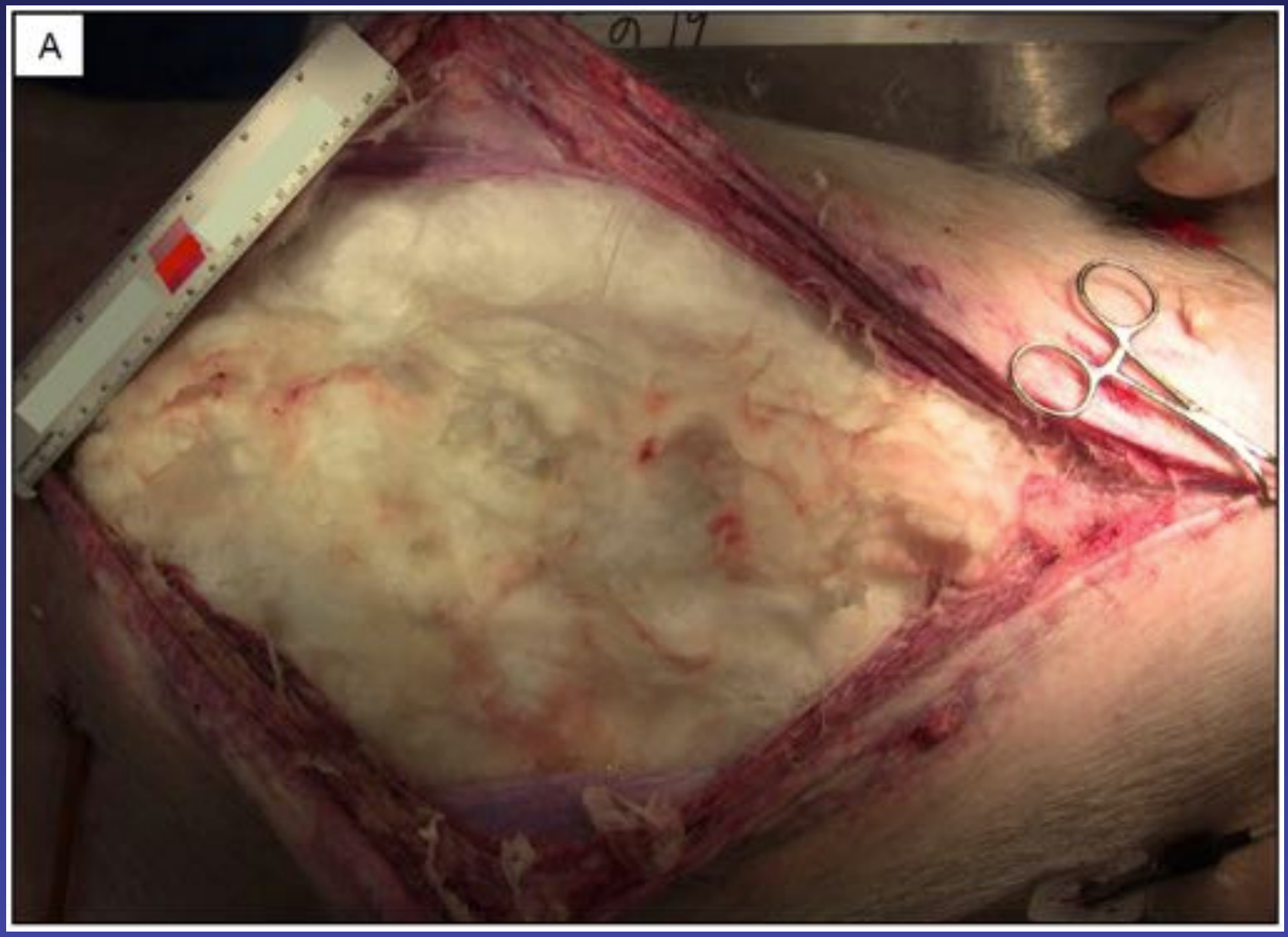

A
H
LM
S
H
B
D
H
RM
Gb
LM
H
C
RL
LL
LM
RM
D
RL
RM
LL


Gb
RM
Q
LM
7
5
6
3
LL
4
2
RL
1
Wound Measuring Guide
Discard after single use
Date
Patient Initials/Number

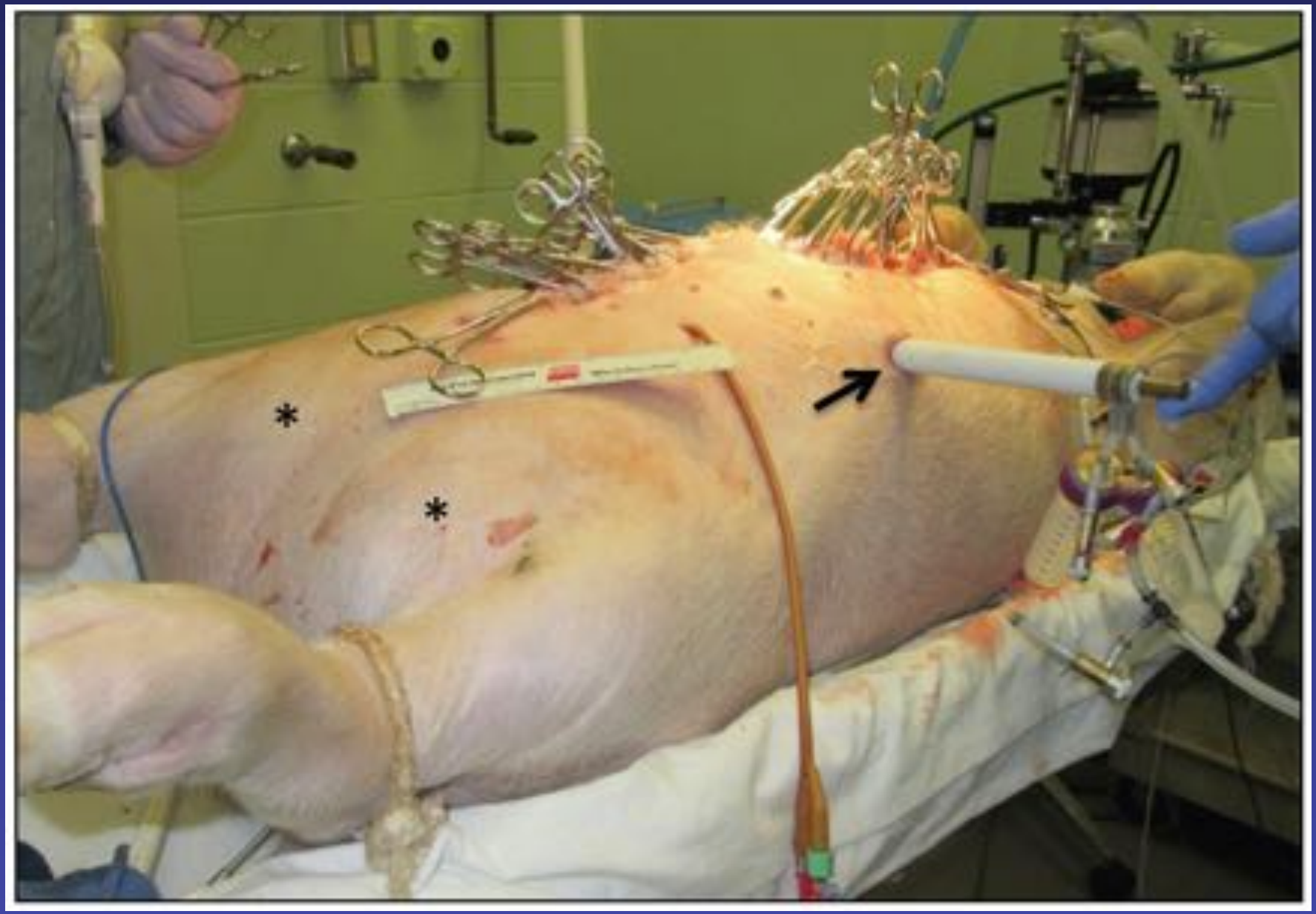

A
Gb
LM
RM
LL
Q
1
2
4
RL
3
C
B
RL
RM
4
C
Q
Gb
1
LM
LL

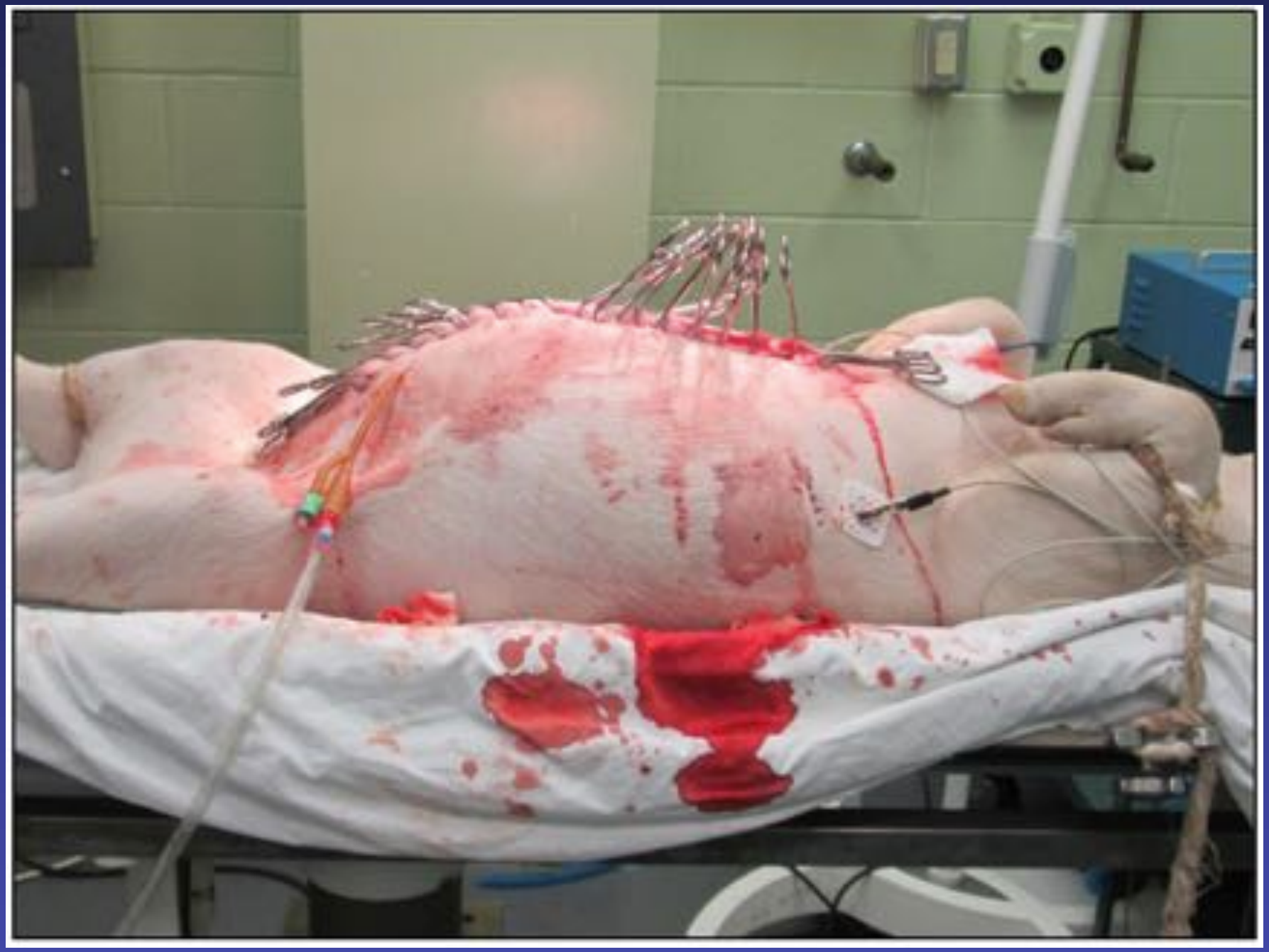

Number of swine alive
10
8
6
4
2
0
0
10
20
30
40
50
60
Time (min)
LLLH
CI
PVE

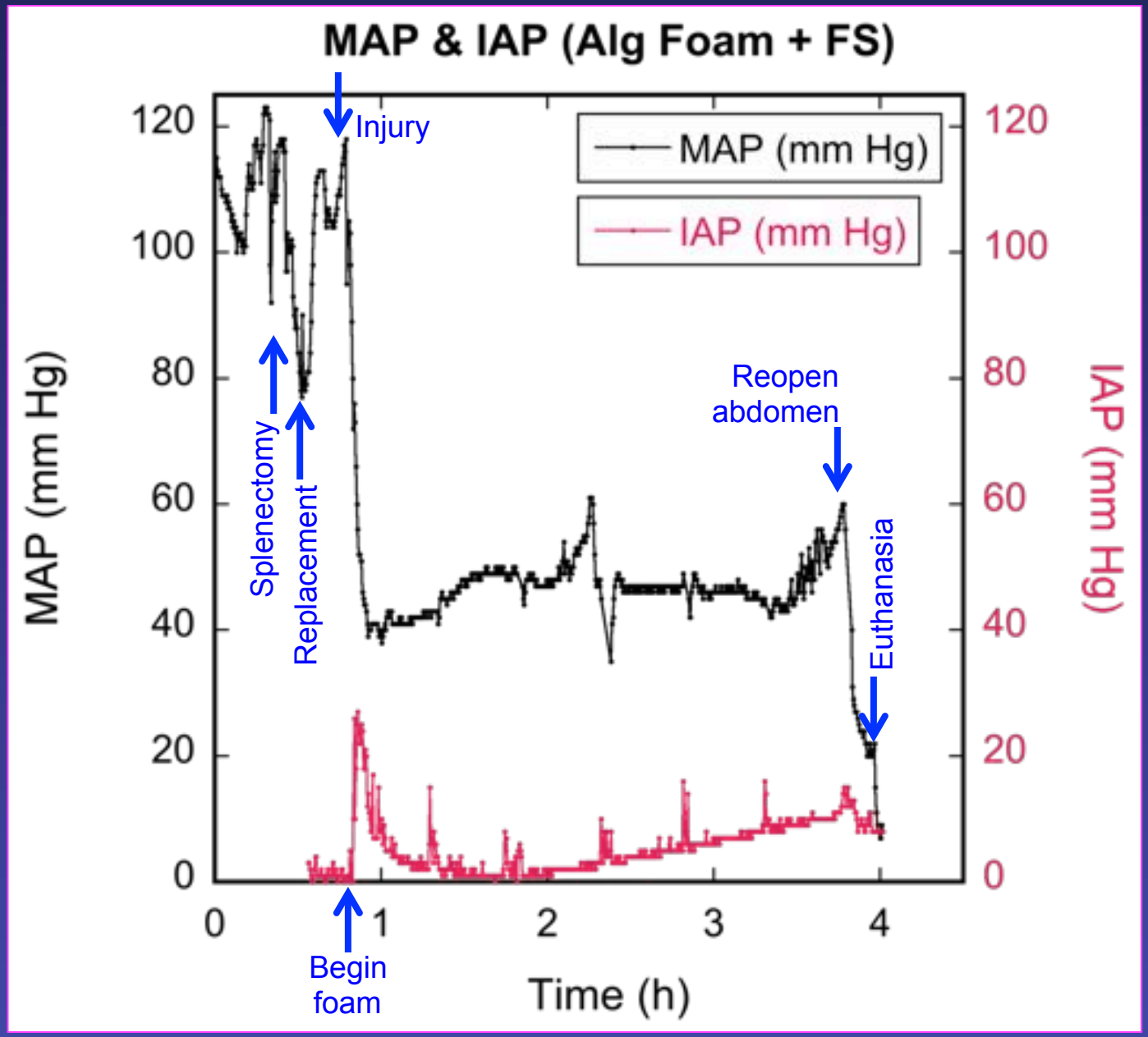

Rx: noncompressible hemorrhage
Liver
Injury
Foam
expansion
with tamponade



Calcium Alginate Foam At Necropsy






MAP & IAP (Alg Foam + FS)
MAP (mm Hg)
IAP (mm Hg)
Injury
Splenectomy
Replacement
Reopen abdomen
Euthanasia
Begin foam
Time (h)
MAP (mm Hg)
IAP (mm Hg)
0
20
40
60
80
100
120
0
1
2
3
4

Injury Mechanism

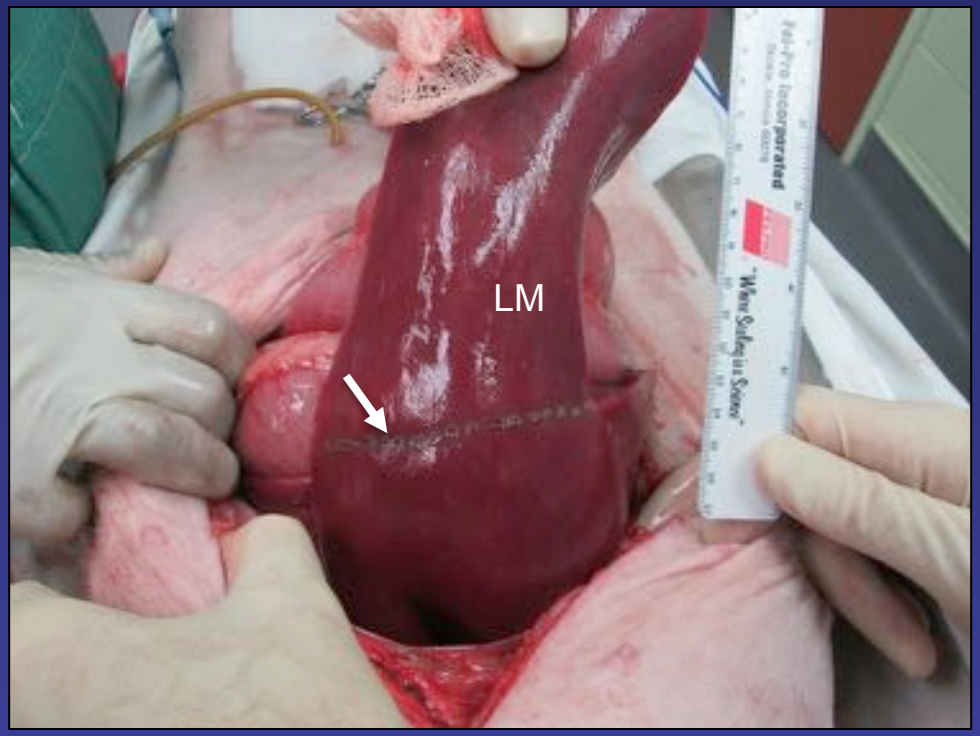


Figure 1, swine 241. Site of injury, prior to making the cut. The left medial liver lobe (LM) has been exteriorized out of the midline incision. The planned line of lobectomy has been marked with a cautery score on the liver capsule (arrow). View from the head down to the hindlimbs (superior view).

Injury Mechanism

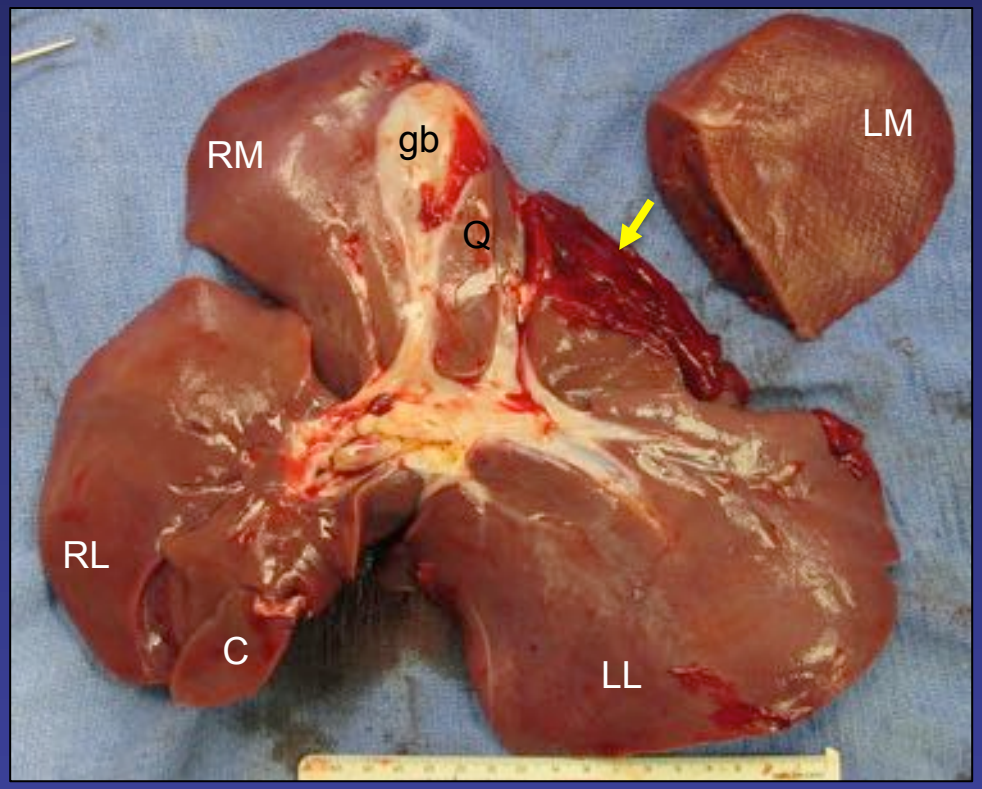


Figure 4, swine 246. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate. gb = gallbladder. Arrow = large clot overlying surface of injury. Anterior is at top of image.

Injury Mechanism

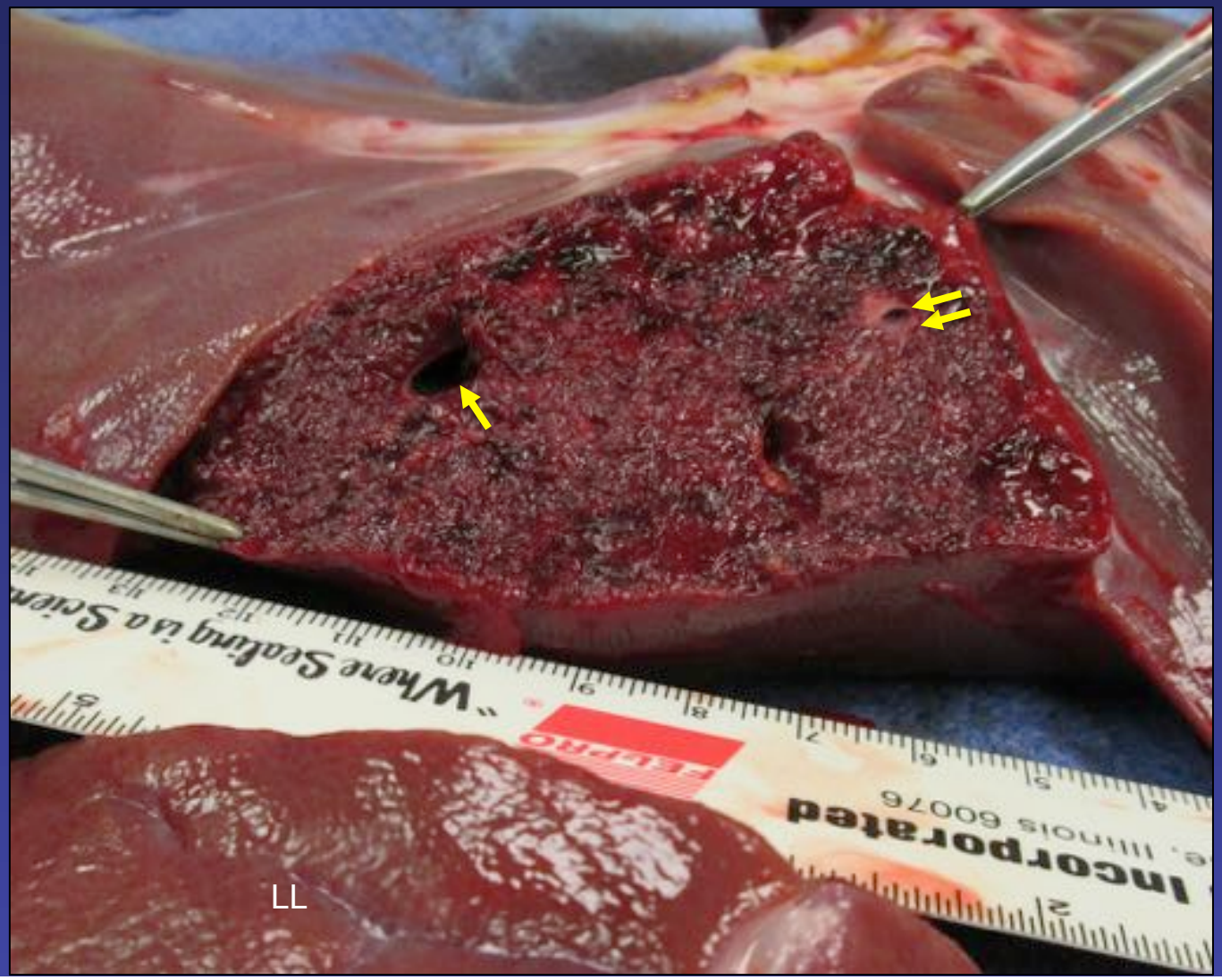
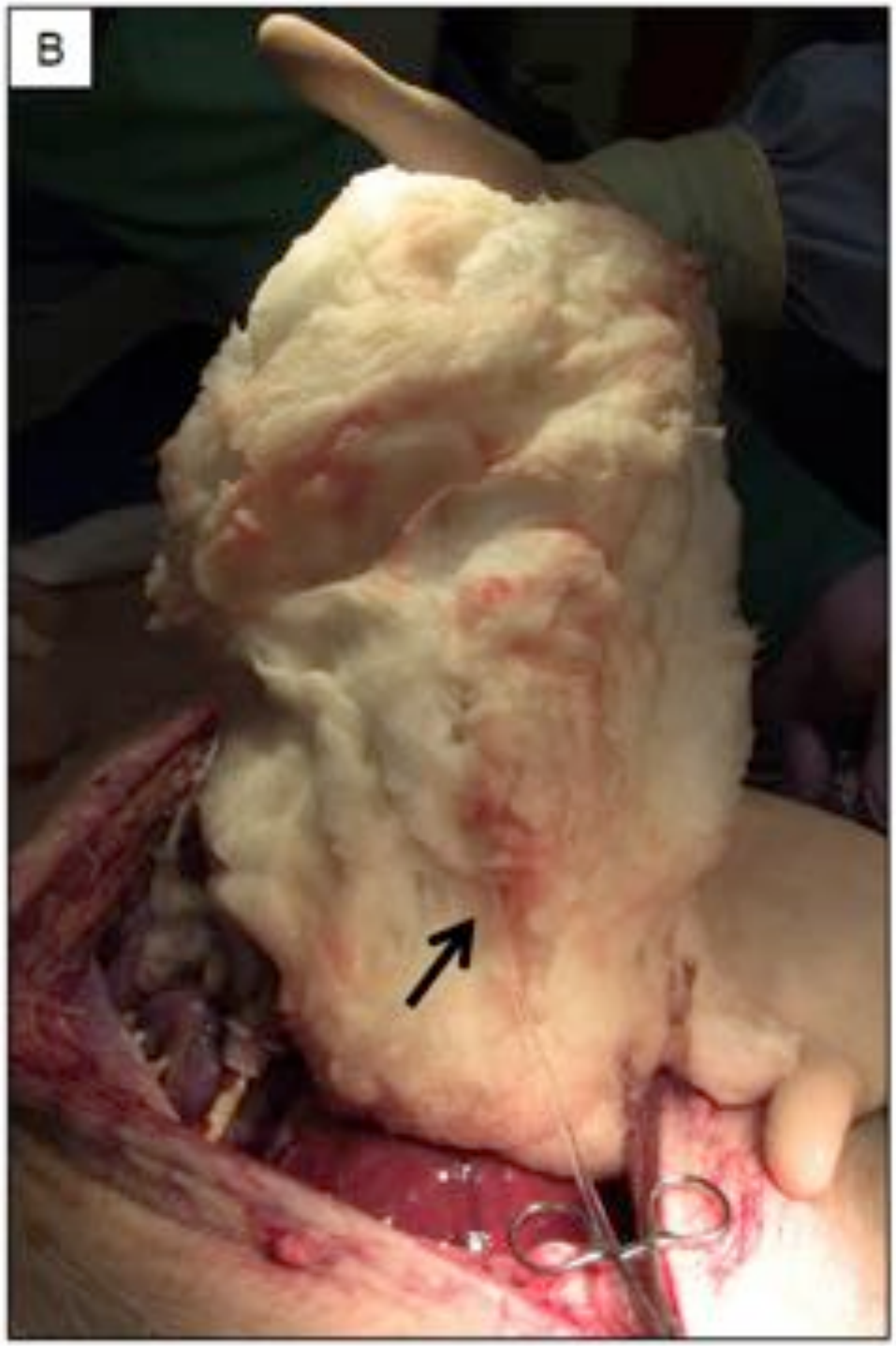


Figure 5, swine 246. Liver *ex vivo*, close-up of injury/lobectomy site (left lateral aspect, inferior is toward top of image). The clot has been removed from the cut liver surface. Injury mechanism was complete transection of LM lobe near its base. Single arrow indicates orifice of transected hepatic vein to LM lobe; double arrow indicates orifice of transected PV branch to LM lobe. These transected veins were all occluded by clot at the time of necropsy.


B

Liver ex vivo

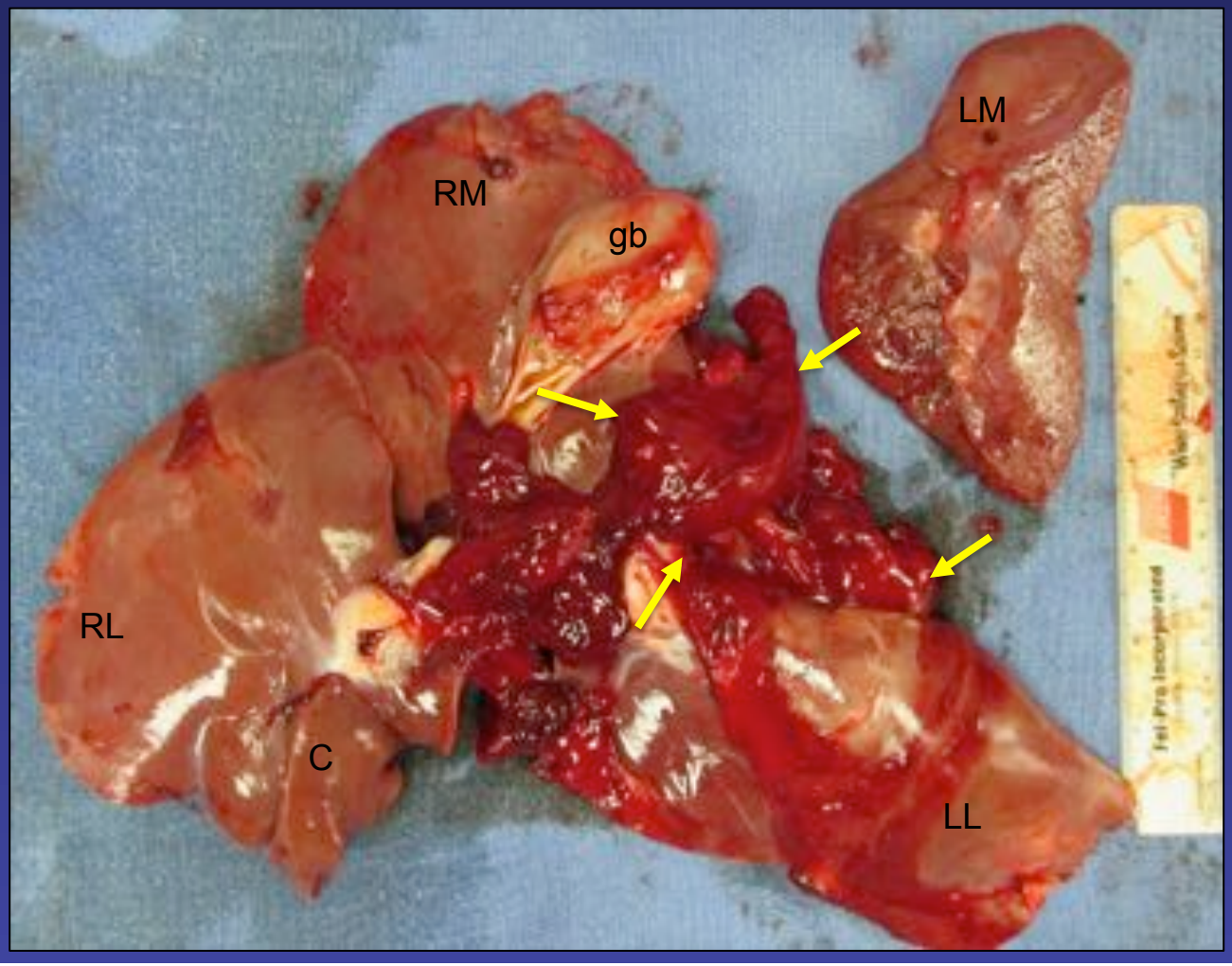
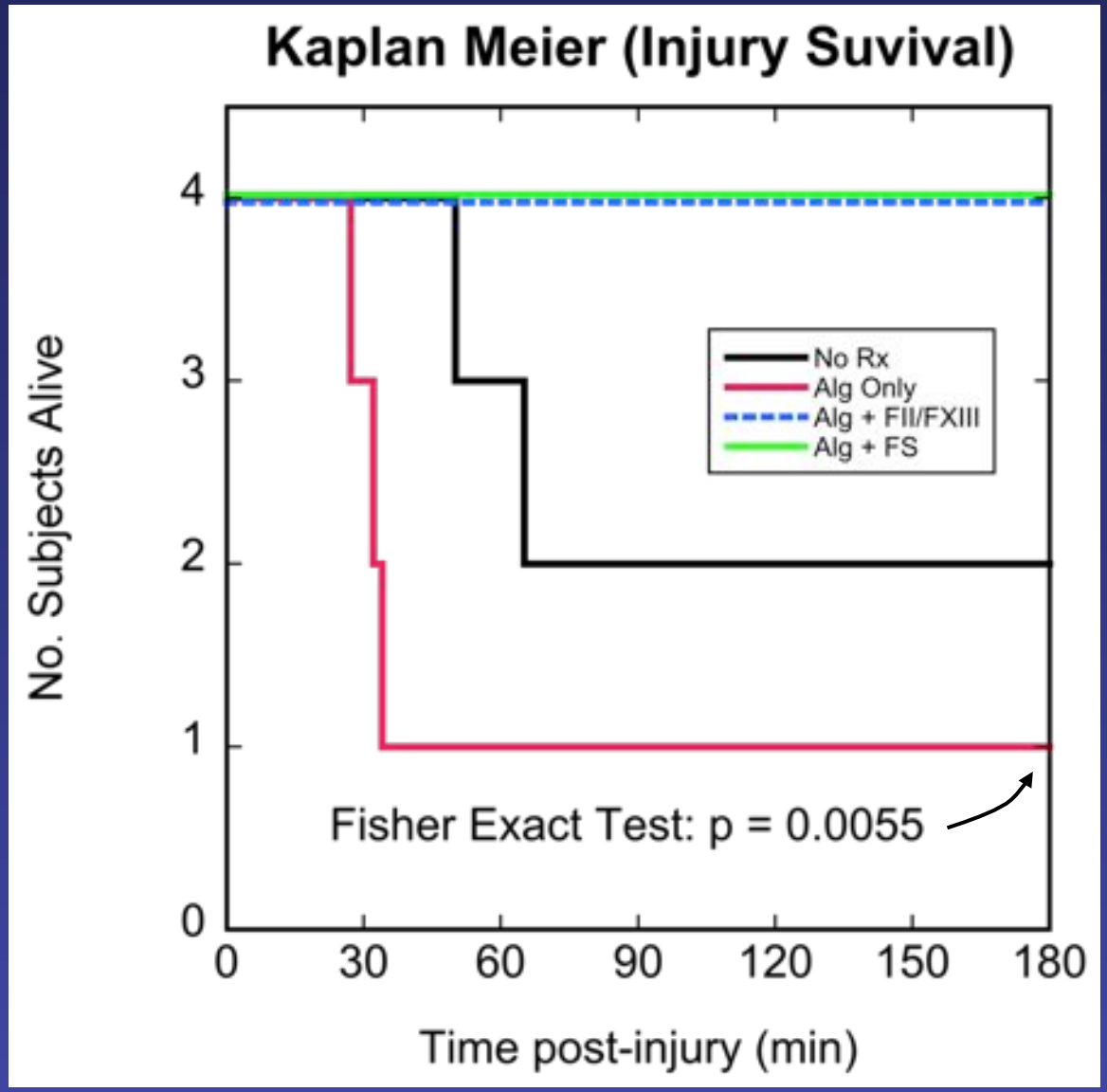
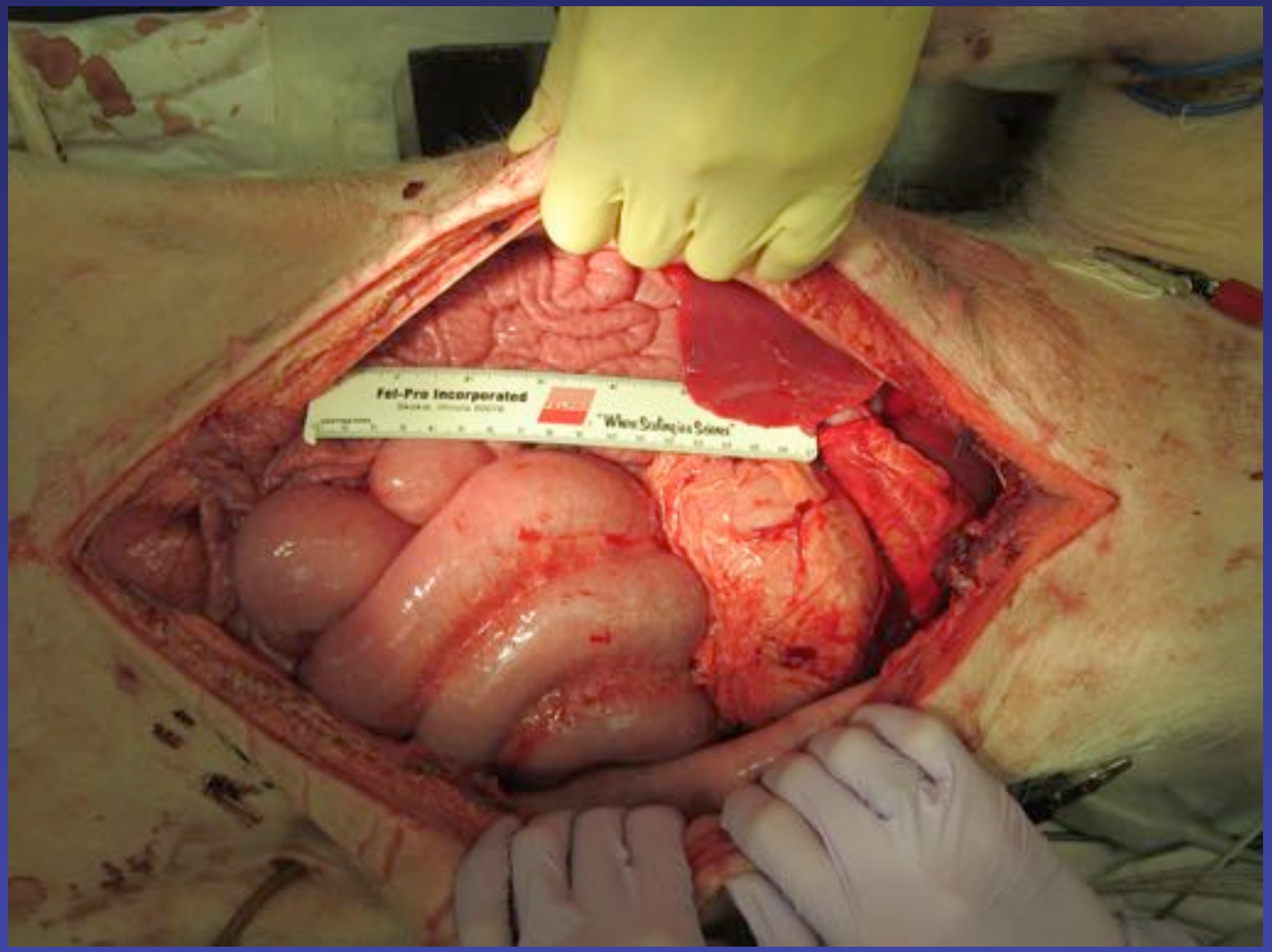


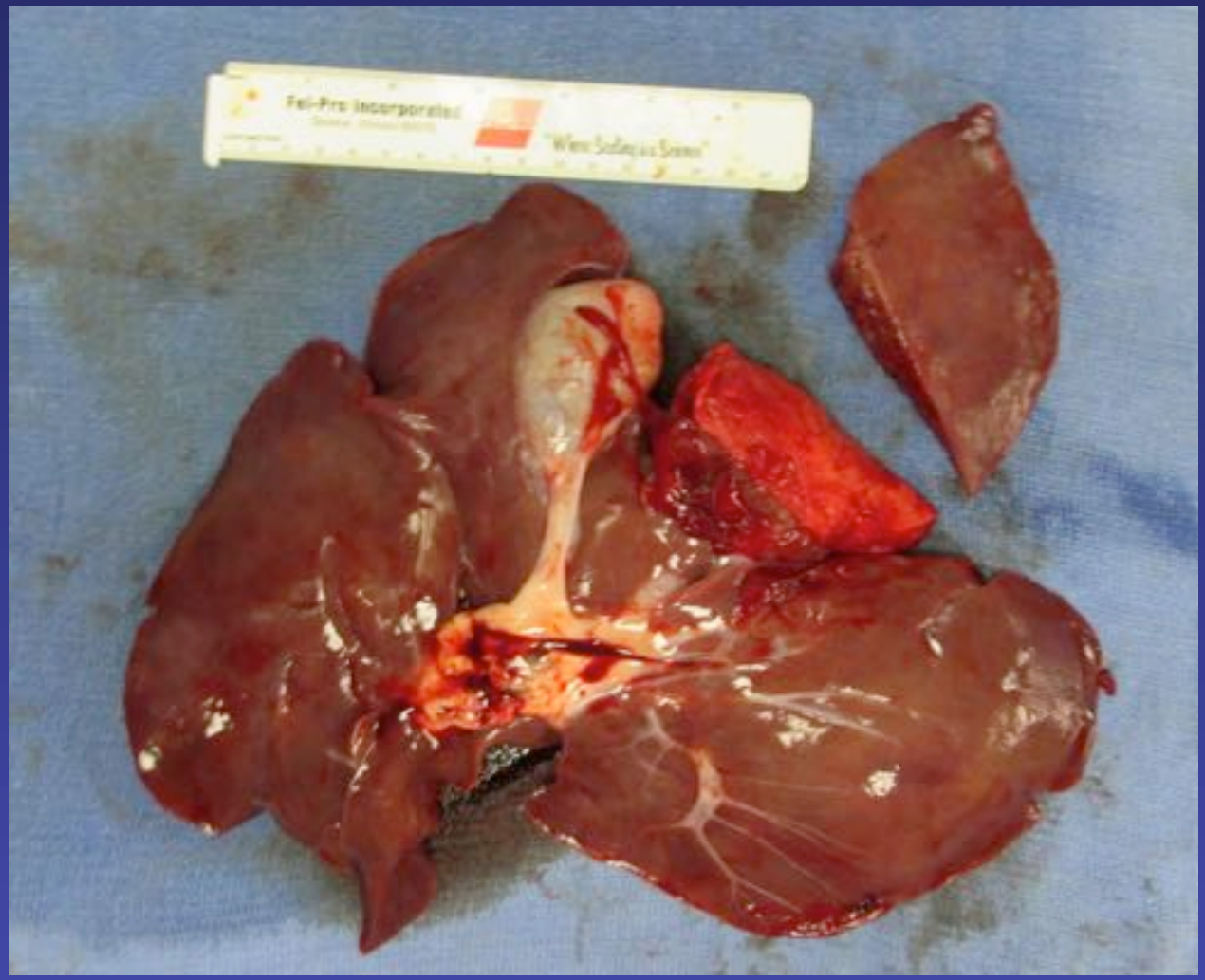
Figure 5, swine 263. Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Arrows = large clot overlying surface of injury. Anterior is at top of image. Color of liver appears good (contrast #262).


Kaplan Meier (Injury Suvival)
No. Subjects Alive
4
3
2
1
0
0
30
60
90
120
150
180
Time post-injury (min)
No Rx
Alg Only
Alg + FII/FXIII
Alg + FS
Fisher Exact Test: p = 0.0055

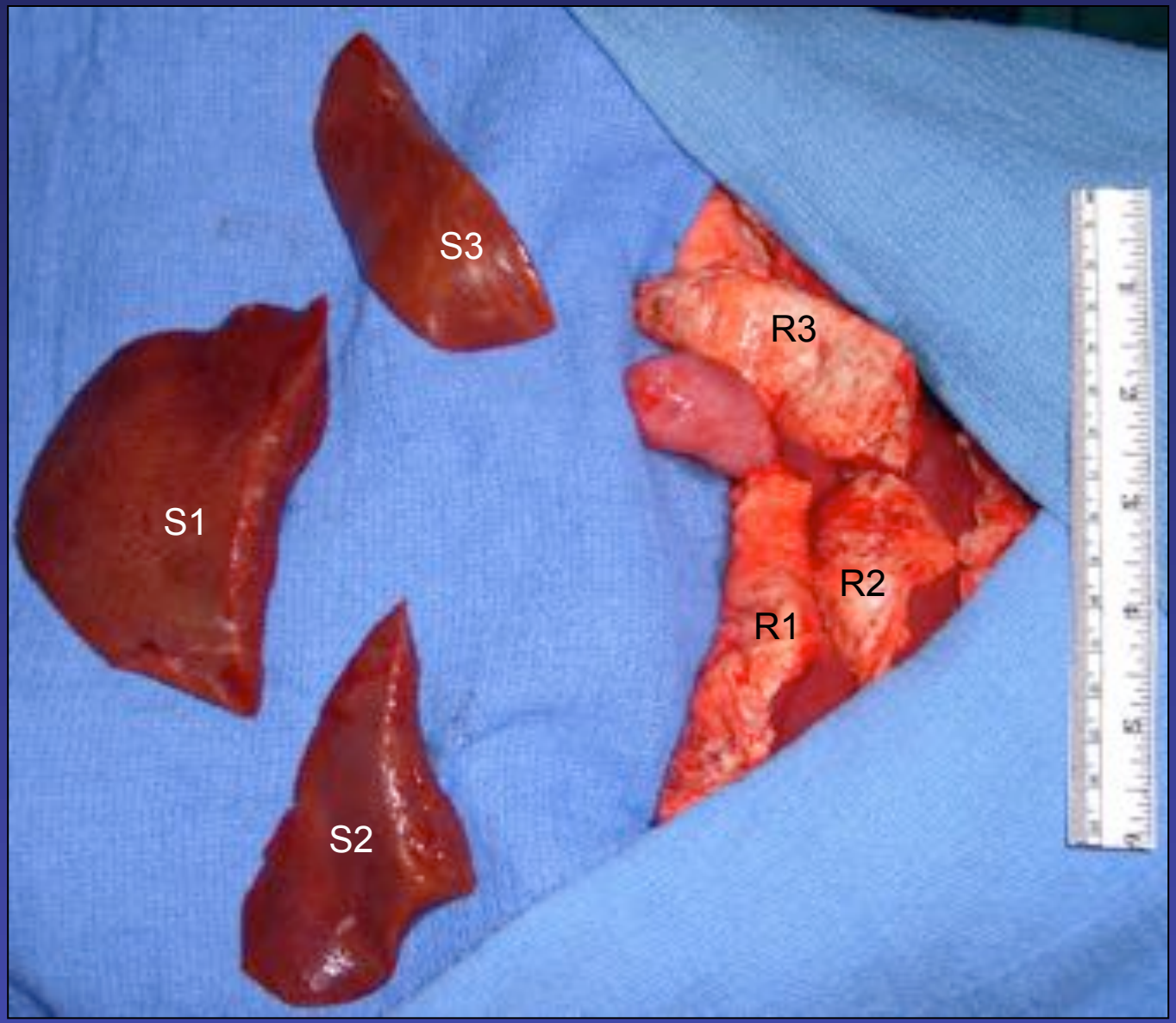
PCL bandage + FS (NEDED)



PCL bandage + FS (NEDED)



Major liver resection treated with PLA embedded with recombinant fibrinogen, thrombin, and XIIIa



Current TCCC Guidelines: Fluid Resuscitation

- In the absence of colloids, use crystalloids
- Crystalloids: 500 mL bolus infusions until one of the following is achieved:
  1. Palpable radial pulse
  2. Systolic blood pressure of 80-90 mm Hg
  3. Improved mental status.

Table 1. Blood loss, fluid input, liver weight, and lacerated veins.

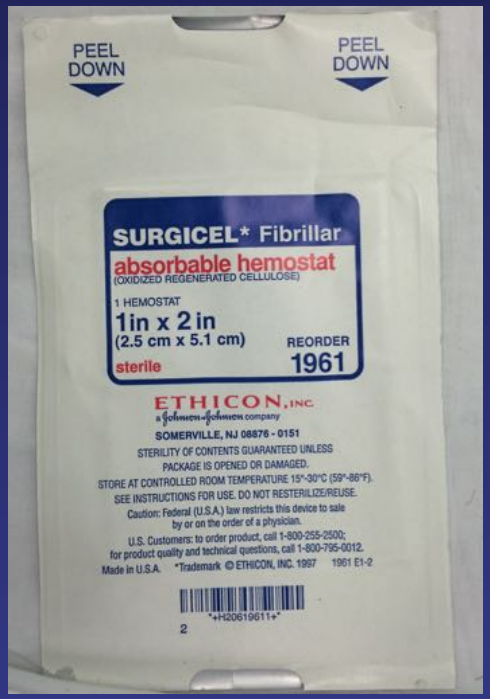
	Blood loss (mL)		**Fluid input (mL)**		**Liver wt (g)**	**Veins Lacerated**	
Infusion Rate	Pre injury	Post injury	Pre injury	Post injury		Hepatic	Portal
Rapid	430 ± 151	2738 ± 693	1288 ± 458	3644 ± 523	852.9 ± 114.8	0.9 ± 0.3	1.3 ± 0.4
Slow	414 ± 48	1600 ± 360	1138 ± 181	3082 ± 1720	941.0 ± 127.3	1.0 ± 0.0	1.4 ± 0.5
P value*	0.297	**0.0001**	0.6861	0.8174	0.0833	0.4884	0.7075

All values are mean ± sd; time points are with respect to injury; *Kruskal-Wallis test; significant tests are **bolded**.

Conclusions: Fluid Resuscitation

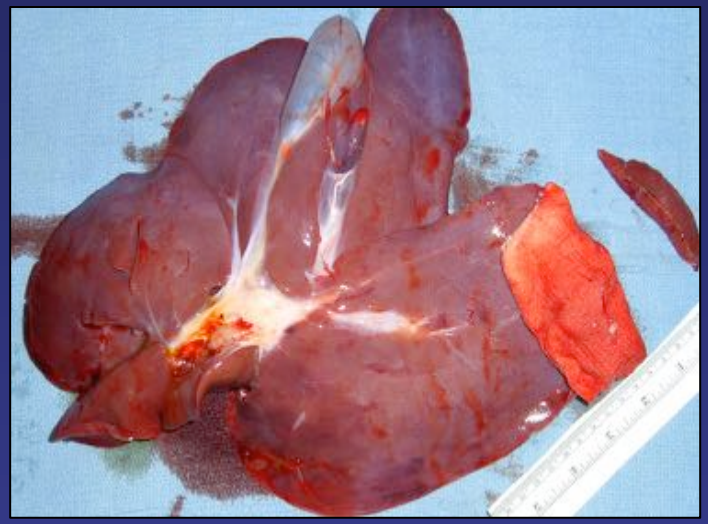
- Slow infusion rate: improved outcome (blood loss, Hb, coagulation)
- Study not powered to detect a difference in survival
- Crystalloid infusion rate may be another consideration in the field resuscitation of a patient in hemorrhagic shock

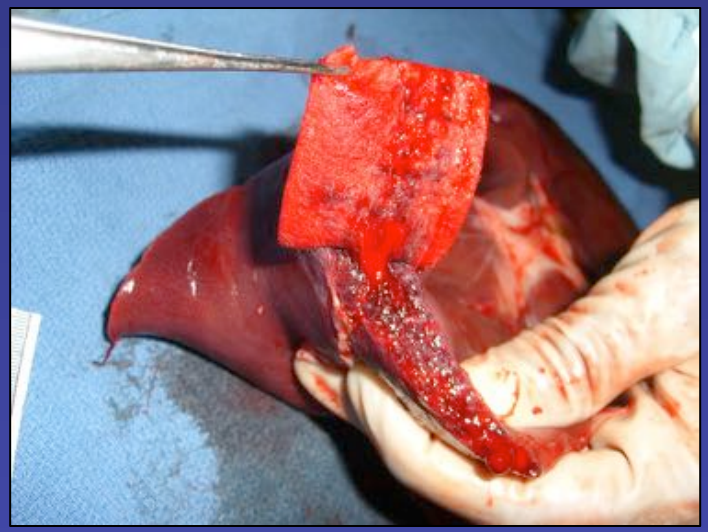
Surgicel® Cost

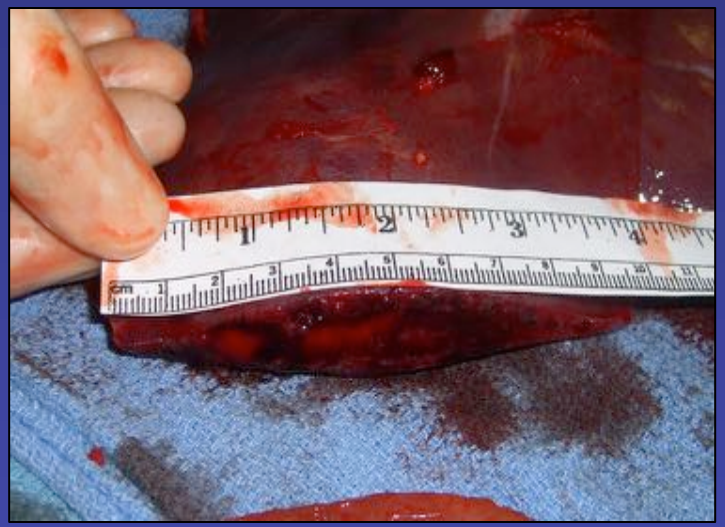


Source	Cost per box	No.	Size	sq in/unit	cm2/unit	total cm2	$/cm2
Omaha VAMC	$960.00	12	2x14in	28	180.6	2167.2	0.44
HomeHealthMedical.com	$2,906.99	24	4x8in	32	206.4	4953.6	0.59
Omaha VAMC	$1,272.00	12	4x8in	28	180.6	2167.2	0.59
Just For Medical Supplies	$1,103.99	12	3x4in	12	77.4	928.8	1.19
4MD Medical Solutions	$188.08	24	0.5x2in	1	6.5	154.8	1.21
4MD Medical Solutions	$2,277.26	24	3x4in	12	77.4	1857.6	1.23
Save Rite Medical	$653.90	12	2x3in	6	38.7	464.4	1.41
Just For Medical Supplies	$653.90	12	2x3in	6	38.7	464.4	1.41
Healthcare Supply Pros	$1,783.28	24	2x3in	6	38.7	928.8	1.92
DealMed Medical Supplies	$344.10	12	0.5x2in	1	6.5	77.4	4.45
4MD Medical Solutions	$692.15	24	0.5x2in	1	6.5	154.8	4.47
Healthcare Supply Pros	$419.97	12	0.5x2in	1	6.5	77.4	5.43

PCL Bandage: Inexpensive Surgicel® Mimic







Cancer

The Discovery of Angiogenesis Inhibitors in the 1990's

Judah Folkman, MD
1933-2008

"Judah [Folkman] is going to cure cancer in two years."

— James D. Watson, 1998

"I do know this: if you have cancer and you are a mouse, we can take good care of you."

— Judah Folkman, 1998

Mouse:

Reasonable model for *homo sapiens*, but…

Table 1. Murine *vs.* human phenotype for select mutations.

Mutated gene	Murine phenotype	Human phenotype
APC	Small intestine polyps	Colorectal cancer
CFTR	Intestinal disease	Cystic fibrosis
RB1	pituitary tumors	Retinoblastoma
FANC	low weight	Fanconi anemia

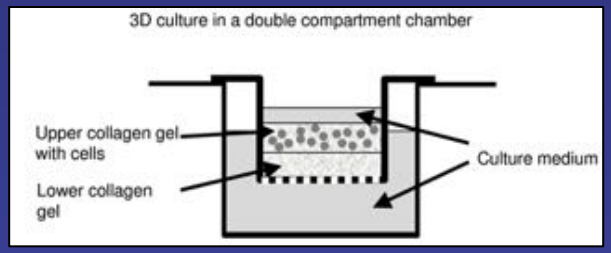
Cancer Research

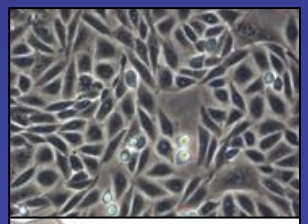
Clinical studies

Mouse models

Large Animal?

3D cell culture models





Monolayer cell culture

… a continuum

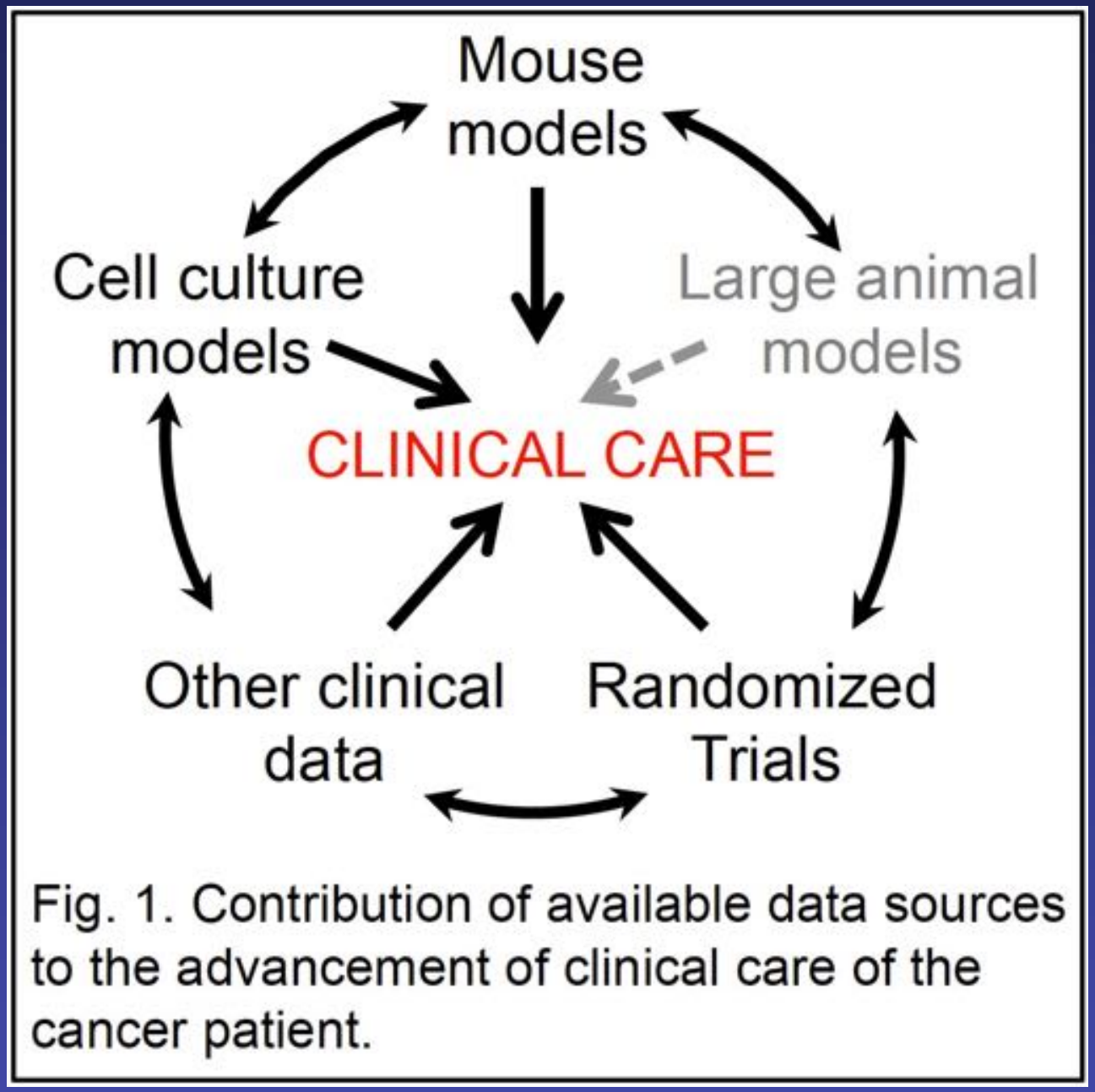


Fig. 1. Contribution of available data sources to the advancement of clinical care of the cancer patient.

With a good large animal model of cancer, one might:

- Screen & develop new therapeutics
- Discover new cancer markers
- Study early disease phenomena
- Study metastatic phenomena
- Research & develop noninvasive imaging
- Test & optimize surgical devices
- Advance minimally invasive oncologic surgery
- Teach surgical trainees
- And so forth…

Why swine?

- Established model in multiple areas
- Porcine genome, transgenics
- We are in Nebraska


Abdalla
C. Are
M. Are
Armitage
H. Band
V. Band
Batra
Bessho
Bierman
Bociek
Borgstahl
Brattain
Bronich
Cavalieri
Chan
Cheng
Christman
Cohen
Cowan
Datta
Davis
Dong
Edney
Enke
Eudy
Faber
Foster
Ganti
Garrison
Gould
Greiner
Grem
Guda
Hemstreet
Hinrichs
Hollingsworth
Huang
Iqbal
Jain
Johannson
D. Johnson
K. Johnson
Joshi
Karpf
Kessinger
Lackner
Lawson
Lewis
Lin
Loberiza
Lu
Luo
Ly
Lyubchenko
MacDonald
Maness
Maurer
Mehta
Mirvish
Mott
Naramura
Natarajan
Nawshad
Oakley
Opavsky
Ouellette
Oupicky
Pavlov
Peng
Pour
Reed
Rizzino
Sasson
Schwarz
Sharp
Shcherbakova
Sherman
Shonka
Silva
Singh
Solheim
Tahirov
Teoh-Fitzgerald
Trujillo
Tsai
Vetro
Vinogradov
Vose
Wagner
A. Wahl
J. Wahl
Jenny Wang
Jue Wang
S. Wang
Zhou

Table 3. Possible targets for mutation in the transformations of Aim 2.

Organ	Gene target	Reference
Breast	*PIK3CA*, *TP53*, *GATA3*, *ERBB2*, *CDH1*, *MAP3K*	[27-29]
Esophagus	*TP53*, *PIK3CA*, *NOTCH1*, *BRAF*	[30-32]
Pancreas	*KRAS*, *TP53*, *INK4A*, *SMAD4*	[9,33]
Rectum	*APC*, *CTNNB1*, *BRAF*, *SMAD4*, *KRAS*, *PRKDC*	[27,34]

Analogous KRAS/p53 model in the mouse

Trp53^{R172H} and *Kras*^{G12D} cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice

Sunil R. Hingorani,[1,2,*] Lifu Wang,[2] Asha S. Multani,[4] Chelsea Combs,[2] Therese B. Deramaudt,[1,3] Ralph H. Hruban,[5] Anil K. Rustgi,[1,3] Sandy Chang,[4] and David A. Tuveson[1,2,*]

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[2]Department of Cancer Biology, Abramson Family Cancer Research Institute, Abramson Cancer Center at the University of Pennsylvania, Philadelphia, Pennsylvania 19104
[3]Gastroenterology Division, University of Pennsylvania, Philadelphia, Pennsylvania 19104
[4]Department of Molecular Genetics, M.D. Anderson Cancer Center, Houston, Texas 77030
[5]Departments of Pathology and Oncology, Sol Goldman Pancreatic Cancer Research Center, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287
*Correspondence: shingo@mail.med.upenn.edu (S.R.H.); tuveson@mail.med.upenn.edu (D.A.T.)

Summary

To define the genetic requirements for pancreatic ductal adenocarcinoma (PDA), we have targeted concomitant endogenous expression of *Trp53*^{R172H} and *Kras*^{G12D} to the mouse pancreas, revealing the cooperative development of invasive and widely metastatic carcinoma that recapitulates the human disease. The primary carcinomas and metastases demonstrate a high degree of genomic instability manifested by nonreciprocal translocations without obvious telomere erosion—hallmarks of human carcinomas not typically observed in mice. No mutations were discovered in other cardinal tumor suppressor gene pathways, which, together with previous results, suggests that there are distinct genetic pathways to PDA with different biological behaviors. These findings have clear implications for understanding mechanisms of disease pathogenesis, and for the development of detection and targeted treatment strategies.

Introduction

Ductal adenocarcinoma of the pancreas (PDA) is an almost uniformly lethal disease, largely because it eludes diagnosis until very advanced stages. Indeed, greater than 80% of patients with PDA have locally unresectable or frankly metastatic disease at the time of presentation (Warshaw and Fernandez-del Castillo, 1992; Yeo et al., 2002b). PDA is also unusually resistant to all forms of cytotoxic chemotherapies and ionizing radiation, and as a result, as many people die of PDA each year as are newly diagnosed with it. Now the fourth leading cause of cancer-related mortality among men and women in the United States, approximately 31,000 new cases and deaths were expected from PDA in 2004 (Jemal et al., 2004).

The current standard of care for advanced PDA is infusional gemcitabine, a deoxycytidine analog and inhibitor of nucleic acid synthesis, which prolongs survival by only a few weeks and provides symptomatic improvement in a minority of patients (Burris et al., 1997). For those rare patients able to undergo complete surgical resection of their primary tumor, five-year survival can be as high as 20%–40%; however, even these highly selected patients eventually succumb to both locally recurrent and metastatic disease (Allison et al., 1998; Yeo et al., 2002a). Thus, in addition to methods to detect preinvasive disease, therapies that can kill invasive and metastatic pancreatic cancer cells are needed.

Considerable insight into potential mechanisms of disease pathogenesis has been gleaned from static analyses of resected pancreatic tumor specimens giving rise to histologic (Brat et al., 1998; Hruban et al., 2001a; Klimstra and Longnecker, 1994) and molecular (Hruban et al., 2000; Hruban et al., 2001b) frameworks for disease progression. These studies of sporadic pancreatic cancers have suggested a model of disease evolution through a preinvasive state, termed pancreatic intraepithelial neoplasia (PanIN), involving progressive cellular and architectural atypia accompanied by increasingly frequent mutations in a key oncogene and select tumor suppressor

SIGNIFICANCE

Cancer is a genetic disease driven by the stochastic acquisition of mutations and shaped by natural selection. Genomic instability, a hallmark of human epithelial cancers, propagates these mutations, allowing cells to overcome critical barriers to unregulated growth, and may therefore herald a defining event in malignant transformation. How and when during the course of tumor progression significant genomic instability arises, and whether a cancer can be cured or even contained after that point, represent pivotal and largely unanswered questions. We describe here a murine model of pancreatic ductal adenocarcinoma, characterized by the development of widespread and complex chromosomal instability, which may prove useful in investigating these issues.

Method #1:
Germline manipulation (transgenic swine)

KRAS/p53 “Onco-Pig” (NSRRC, Columbia MO)

- New, unpublished transgenic swine model
- *KRAS*^{G12D} & *p53*^{R167H} cassette
- Floxed termination signal
- Local treatment with AdCre (e.g., lung, pancreas)
- Local KRAS activation and p53 inhibition
- Monitor for tumor formation

WT

APC^{WT}/APC^{1311}

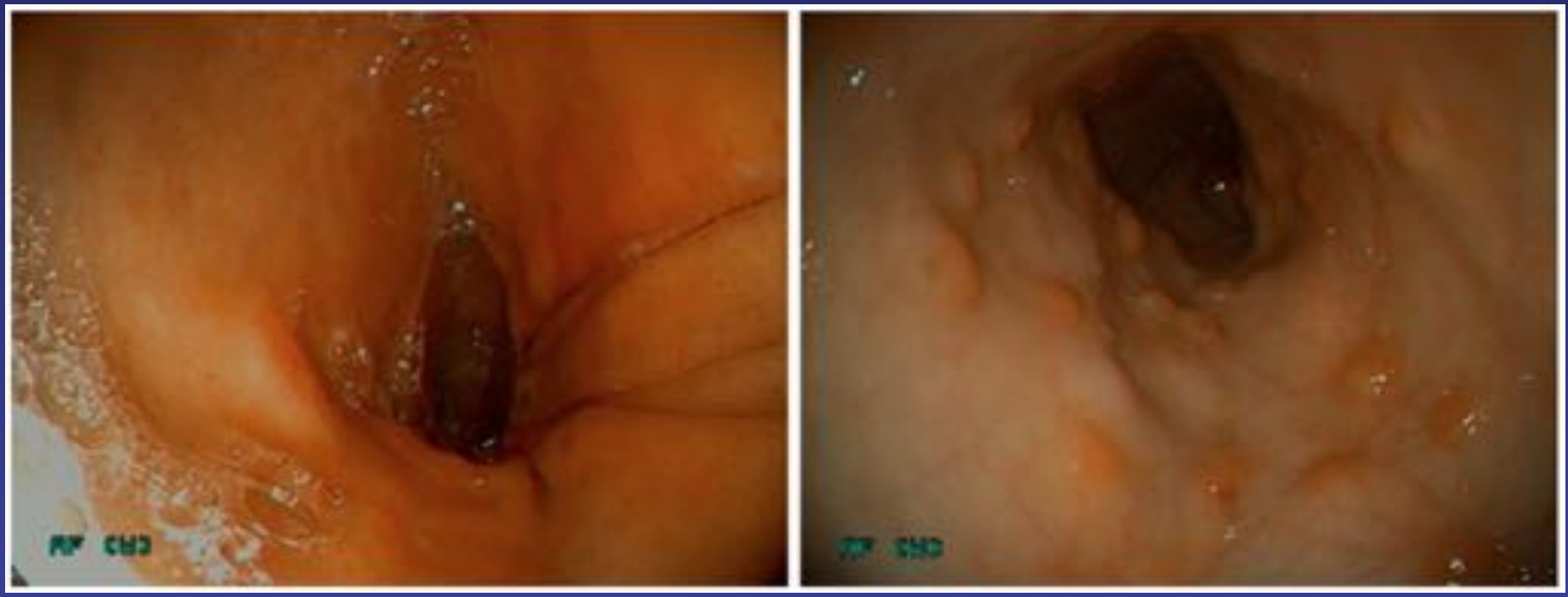
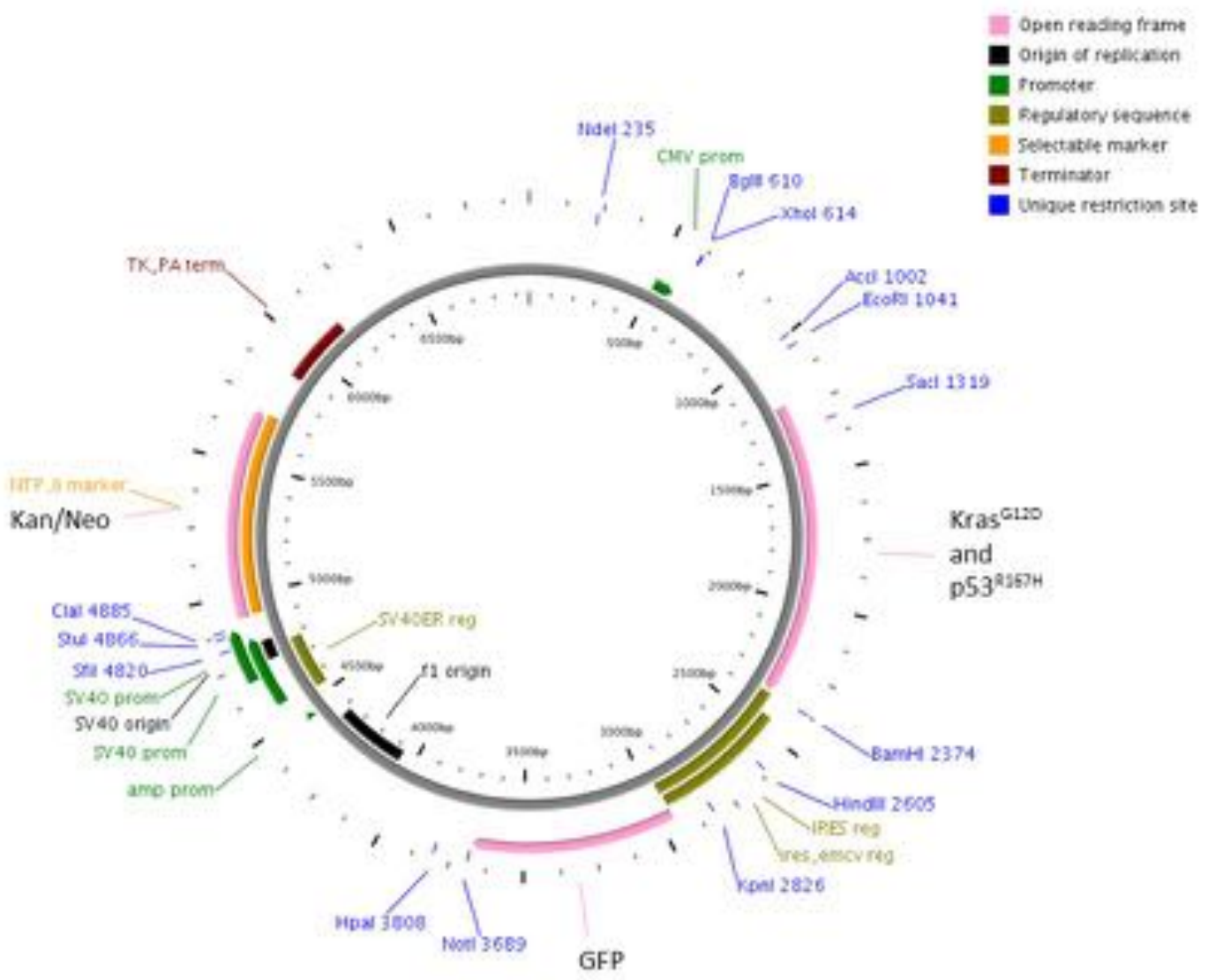


Fig. 1 Endoscopic imaging of the rectums of two sibling F1 generation male pigs at 7 months old. The animal on the *left* is wild type, the animal on the *right* carries the APC^{1311} mutation in heterozygous form

Flisikowska et al., *Transgen Res* 2013;22:673.

Method #2:
Ex vivo transformation, orthotopic transplantation

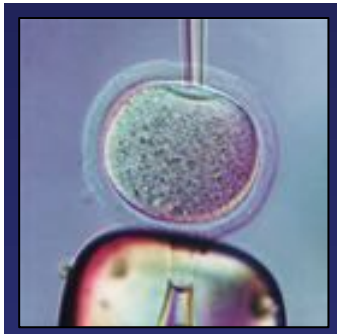
- Primary cells cultured from tissue explants
- Immortalize primary cells as necessary
- Transfect/transduce with cancer-inducing genes specific to organ/primary cell type
- Select & expand
- Orthotopic allogeneic implantation
- Monitor for tumor formation



Appendix A. The oncoplasmid was created by inserting mutated Kras (KrasG12D) and mutated p53 (p53R167H) genes into the backbone vector “pIRES2-AcGFP1” obtained from Clontech Inc. The vector map was updated using the sequence information provdied by Clontech to show where the Kras and p53 genes were inserted. The KrasG12D and p53R167H genes were PCR amplified from a plasmid obtained from Dr. Lawrence Schook’s laboratory using primers that contained cut sites found in the MCS of the pIRES2-AcGFP1 vector then ligated into the vector using standard cloning techniques. The resulting lentiviral vector was then sequence verified to ensure proper ligation of the two genes into the vector backbone.

Method #3:
In vivo transformation

- Organ infection with lentivirus carrying cancer-inducing genes specific to site/organ
- Wild-type/immunocompetent subjects
- Monitor for tumor formation



Targeted gene therapy

- Knock-in, knock-out, transgenic = germline mutation (++precision)
- Transplantation of autologous altered cells
- Local application viral vector with oncogene
- Autologous BM transplant with altered marrow

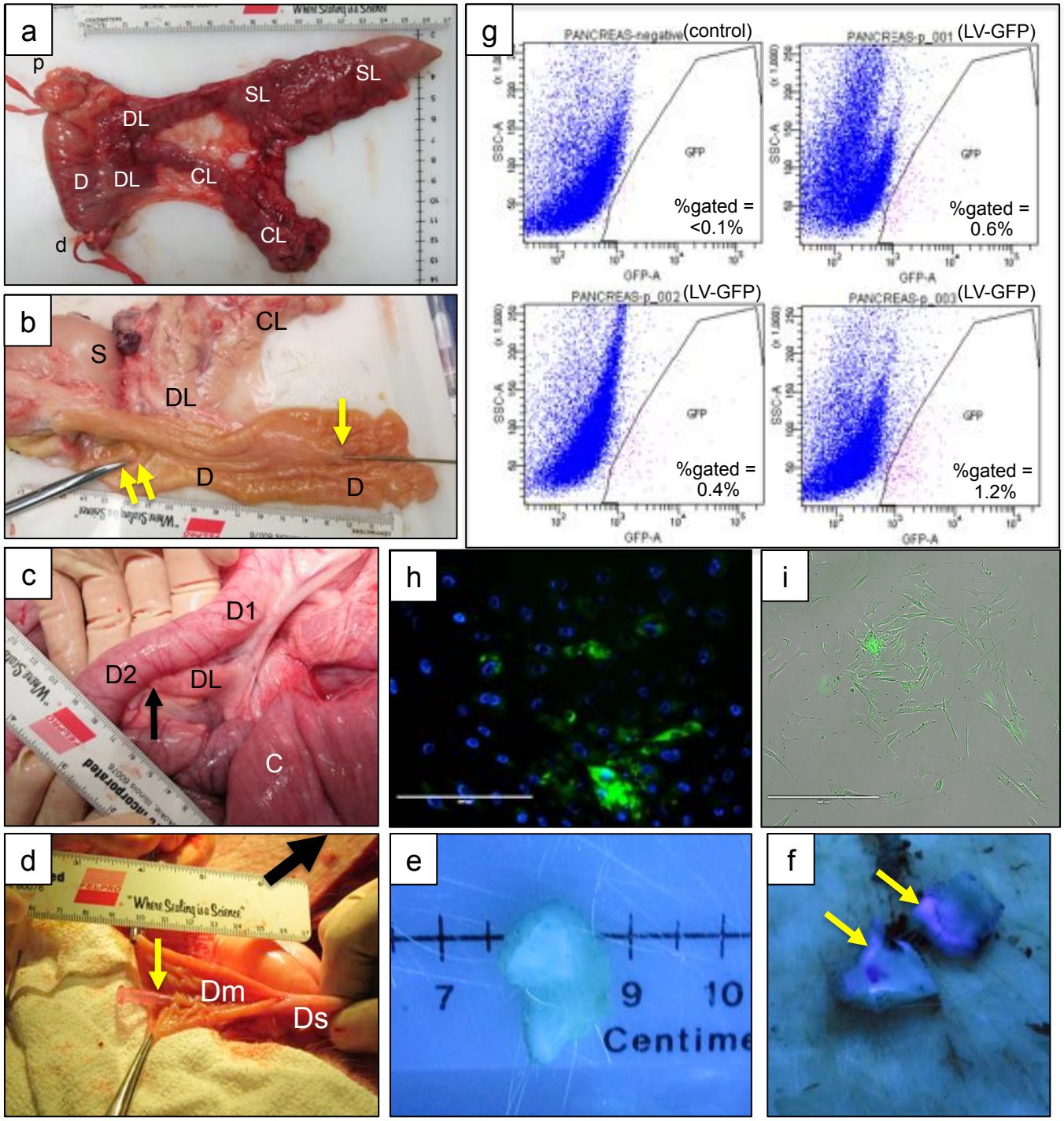


Fig. 1. Anatomy of porcine pancreas and preliminary *in vitro* work. (a) Explanted pancreas + duodenum, anterior surface. D = duodenum; p = proximal; d = distal; DL = duodenal lobe of pancreas; SL = splenic lobe of pancreas; CL = connecting lobe of pancreas. (b) *Ex vivo* dissection, anterior surface of panel *a*. Duodenum (D) has been opened along its antimesenteric border. Double arrow and scissors tip converge on the biliary papilla; single arrow indicates pancreatic papilla with probe inserted into the orifice, located about 10 cm distal to the biliary papilla. S = stomach. (c) *In vivo* pancreas exposure. Approximate location of pancreatic duct orifice entering duodenal wall indicated with arrow. Cephalad at top of image. D1 = 1st portion of duodenum; D2 = 2nd portion of duodenum; DL = duodenal lobe of pancreas; C = colon. (d) Duodenum in panel *c* has been opened along its antimesenteric border; a catheter (yellow arrow) has been inserted into the orifice of the pancreatic duct. Large arrow = cephalad; Dm = duodenal mucosa; Ds = duodenal serosa. (e & f) Raw images of UV-illuminated porcine nipple specimens, bivalved, showing subcutaneous side. (e) Control (non-injected) nipple. (f) Nipple injected 3 wk prior with LV-GFP vector; fluorescence indicated by arrows. (g) Flow cytometry of cells isolated from porcine pancreas injected 3 wk prior with LV-GFP; non-injected pancreas used as negative control. (h) Cytokeratin 18/19 immunohistochemistry of primary porcine pancreatic ductal cells in monolayer, DAPI counterstain, bar = 200 µm. (i) Fluorescent/ phase microscopy of porcine fibroblasts stably transfected with LV-GFP, bar = 400 µm.

UNL transgenic swine

Harvest zygotes, micro-inject with genetic material, reimplant zygote

Table 3. Possible targets for mutation in the transformations of Aim 2.

Organ	Gene target	Reference
Breast	*PIK3CA*, *TP53*, *GATA3*, *ERBB2*, *CDH1*, *MAP3K*	[27-29]
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Peripheral Arterial Disease

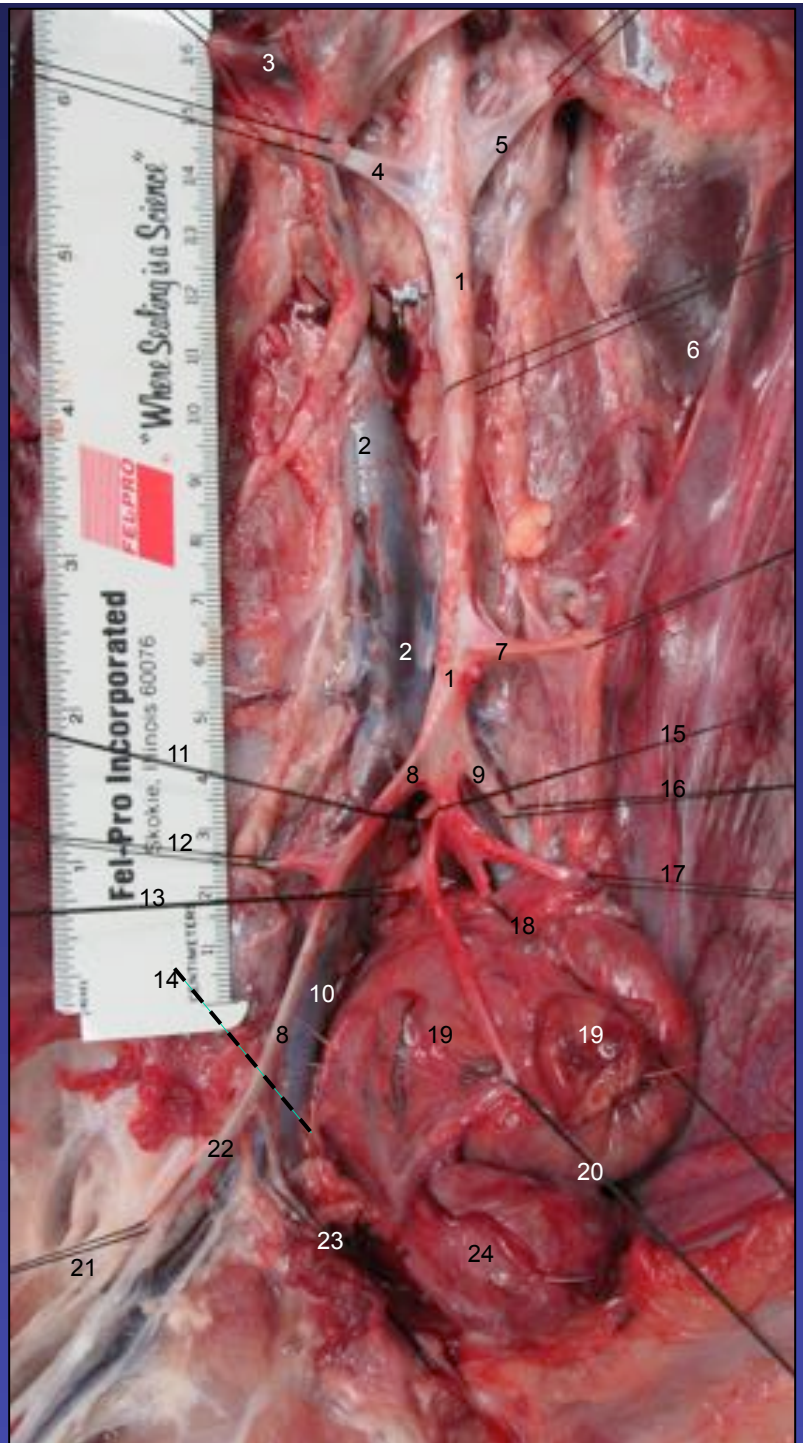
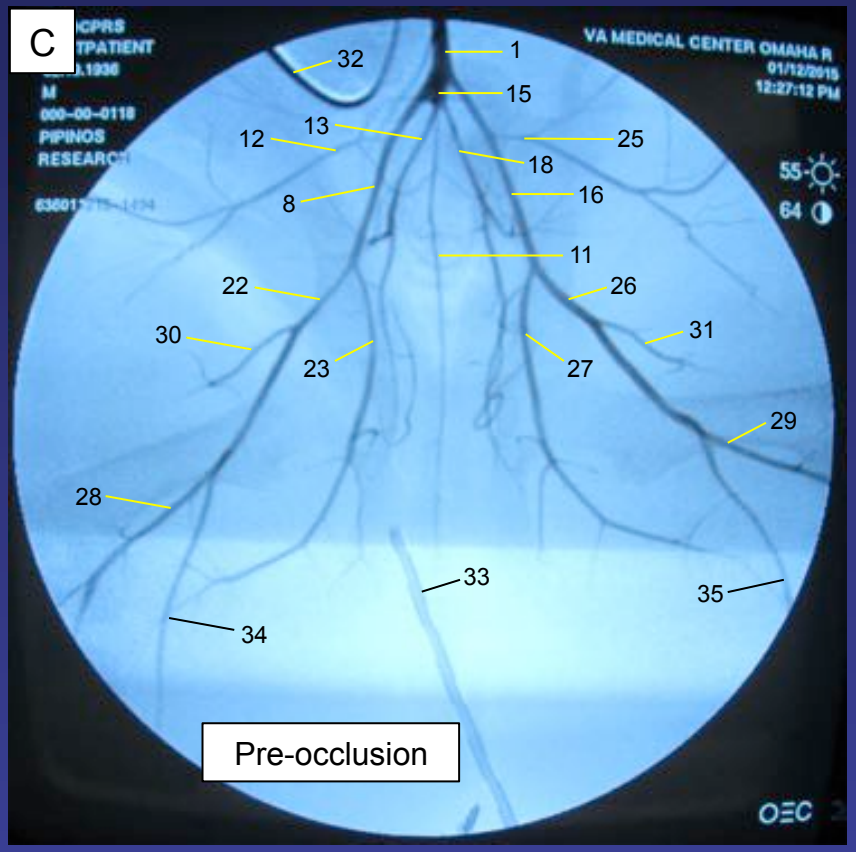


Figure 1. View of lower porcine abdomen in a postmortem subject from IACUC protocol #00760. Subject underwent euthanasia prior to commencing this dissection. Cephalad is at top; viscera have been removed in order to better visualize arterial anatomy. Dissection was performed to expose infrarenal vasculature.

Key to structures:

1. Infrarenal aorta
2. Inferior vena cava
3. Right renal vein
4. Right renal artery
5. Left renal artery
6. Lower pole left kidney
7. Inferior mesenteric artery
8. Right external iliac artery
9. Left external iliac artery
10. Right external iliac vein
11. Unknown on silk stay
12. Circumflex artery on silk stay
13. Right internal iliac artery on silk stay
14. Approximate level of inguinal ligament
15. Sacral artery on silk stay
16. Left external iliac artery on silk stay
17. Rectal branch on silk stay
18. Left internal iliac artery on silk stay
19. Rectal stump
20. Vesicular branch on silk stay
21. Lateral circumflex branch silk stay
22. Superficial femoral artery
23. Profunda femoris on silk stay
24. Urinary bladder

PAD Model


C
VA MEDICAL CENTER OMAHA R
01/12/2015
12:27:12 PM
32
1
15
12
13
25
18
8
16
11
22
26
30
31
23
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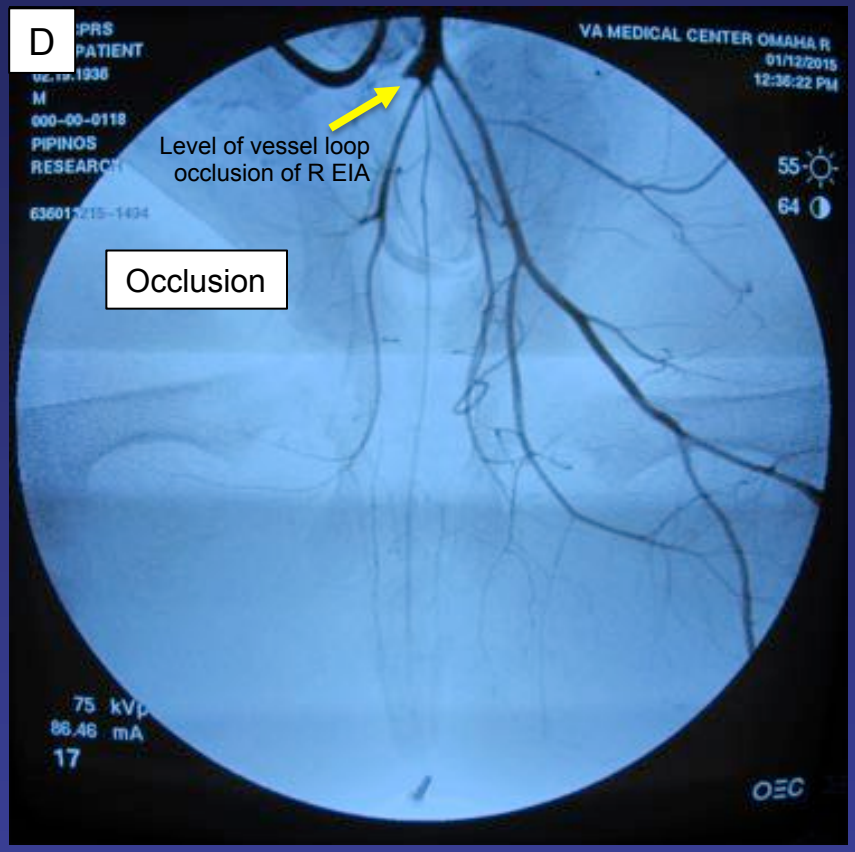
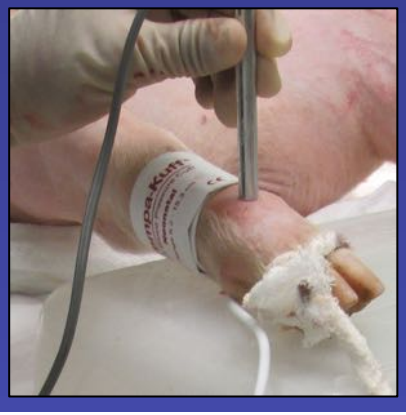
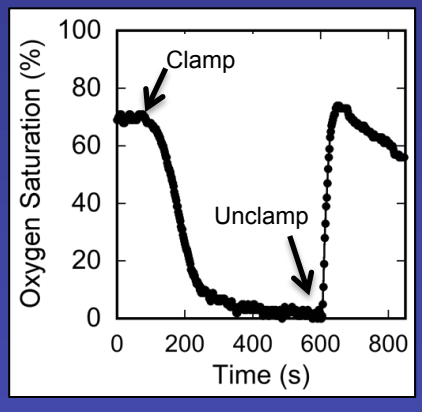
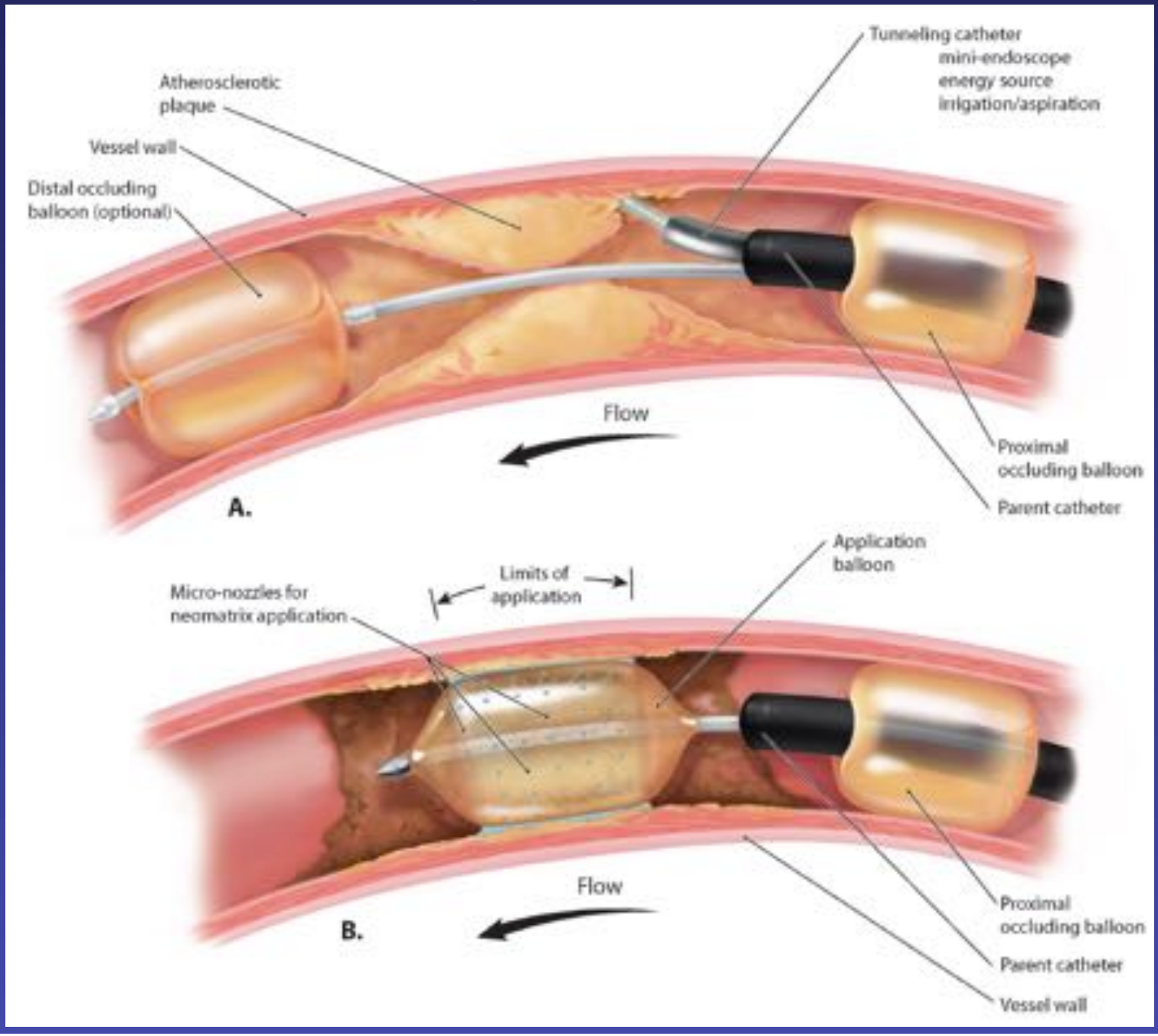
29
28
33
35
34
Pre-occlusion
OEC


D
VA MEDICAL CENTER OMAHA R
01/12/2015
12:36:22 PM
Level of vessel loop occlusion of R EIA
Occlusion
75 kVp
86.46 mA
17
OEC

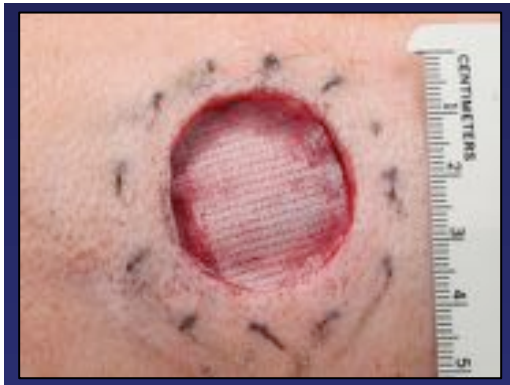


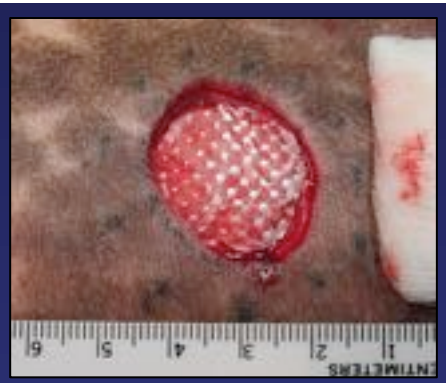

Oxygen Saturation (%)
100
80
60
40
20
0
Clamp
Unclamp
0
200
400
600
800
Time (s)

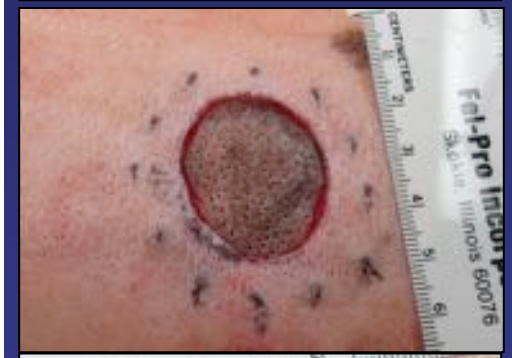
Endovascular resurfacing catheter for atherosclerotic disease

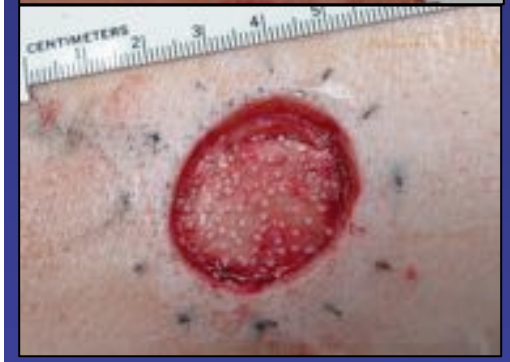


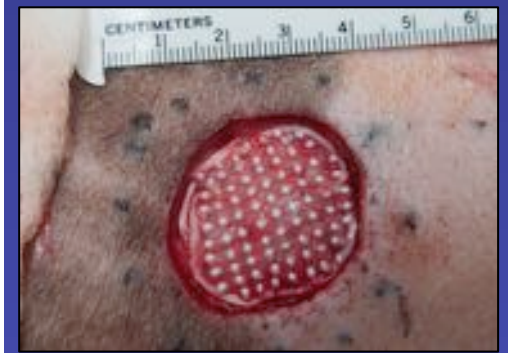
Skin Substitute/Engineering



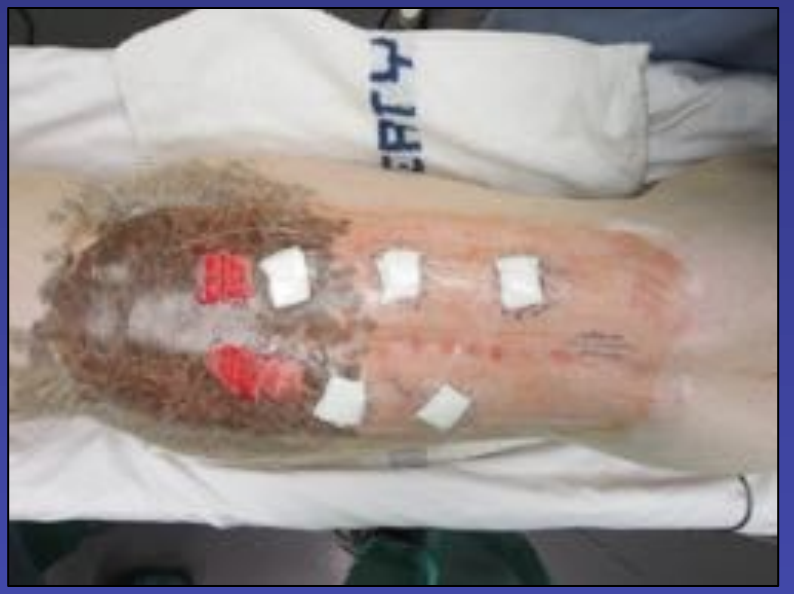








Nanoengineered synthetic matrices embedded with autologous microskin grafts for treatment of full-thickness skin defects



Hemophilia A

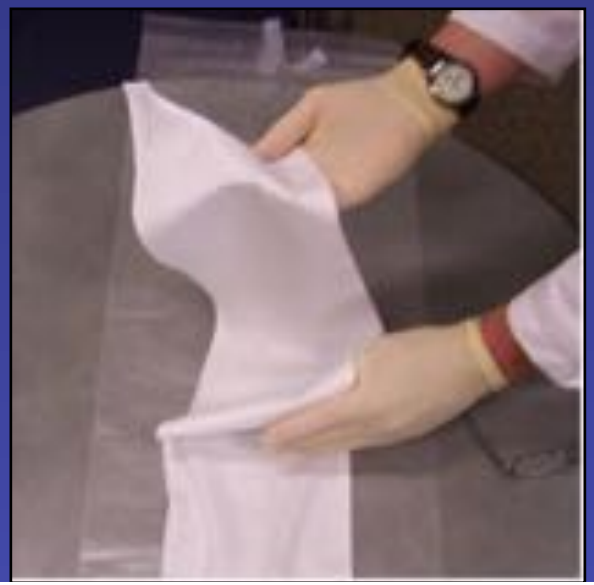
(Factor VIII deficiency)

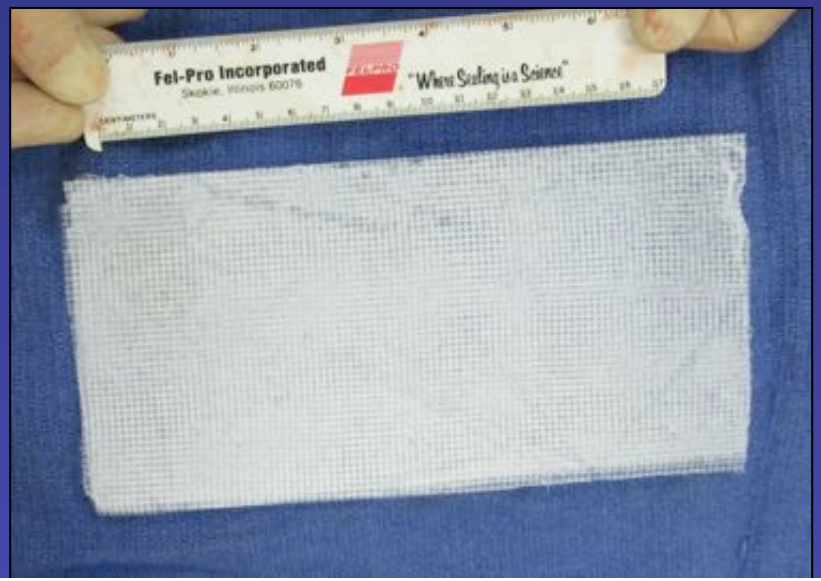
Rationale: Hemophiliac Pig

- Improvement of current FVIII therapies (a >$5B annual market)
- Amelioration of immune intolerance to FVIII replacement therapy
- Development of safe gene therapy to correct FVIII deficiency

Resorbable Hernia Mesh

Mesh	Dimension (cm)	Cost per unit ($)	Relative cost ($/cm^2)
Ultrapro™ (coated polypropylene)	30 x 30	75	0.08
Surgipro™ (polypropylene)	15 x 15	33	0.15
Vicryl (polyglactin 910)	15 x 15	402	1.79
C-QUR™ (coated polyester)	15 x 20	765	2.55
Parietex™ (coated polyester)	30 x 20	1,728	2.88
DualMesh® (polytetrafluoroethylene)	15 x 19	1,203	4.22
Strattice® (acellular porcine dermis)	10 x 20	10,361	51.80





Miscellaneous Projects

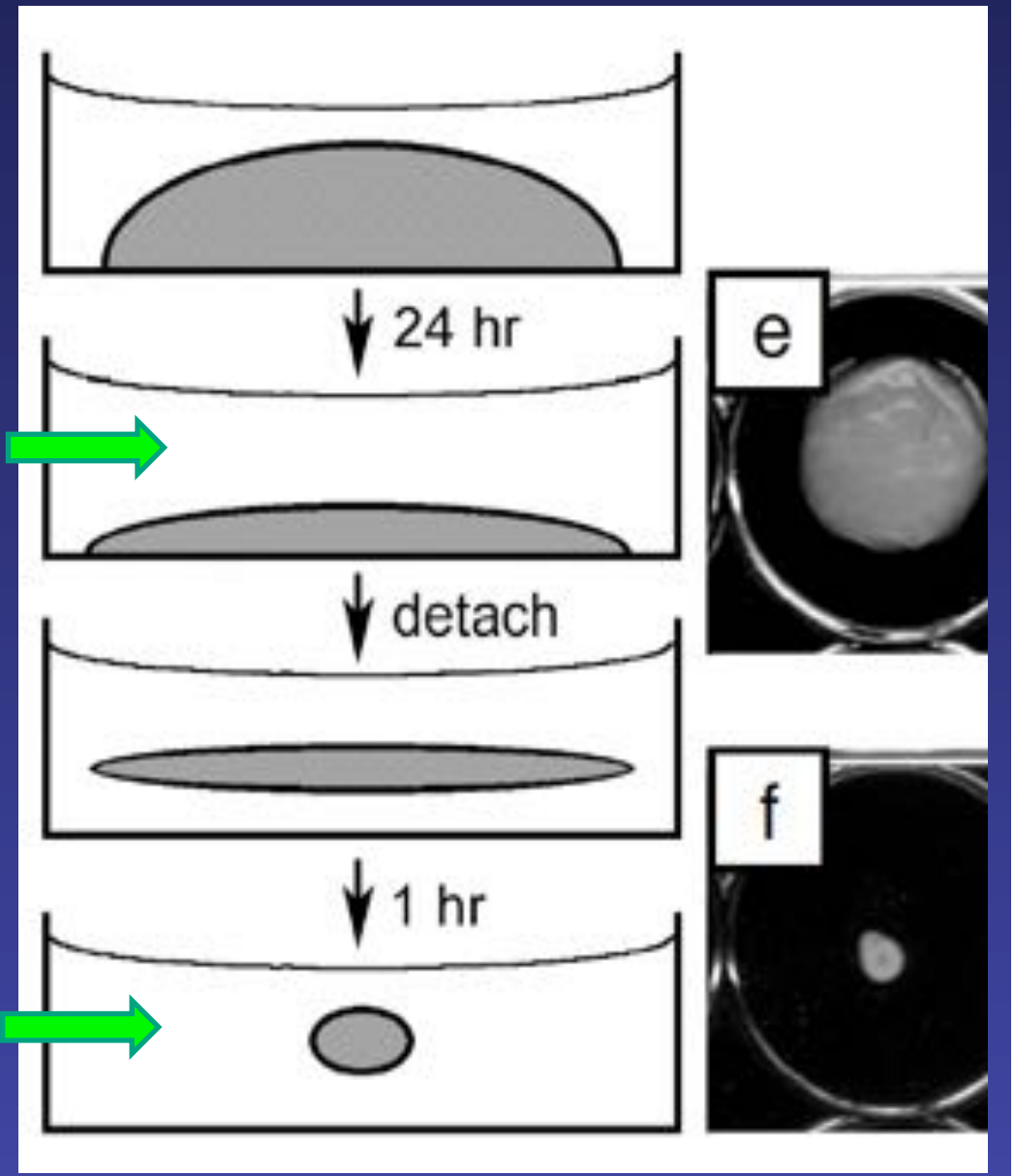
Fibroblast Populated Collagen Matrix Model (FPCM)

"attached" = mechanically stressed (survival and cell cycle both upregulated ➔ matrix cell population increases)

"detached "= stress-released

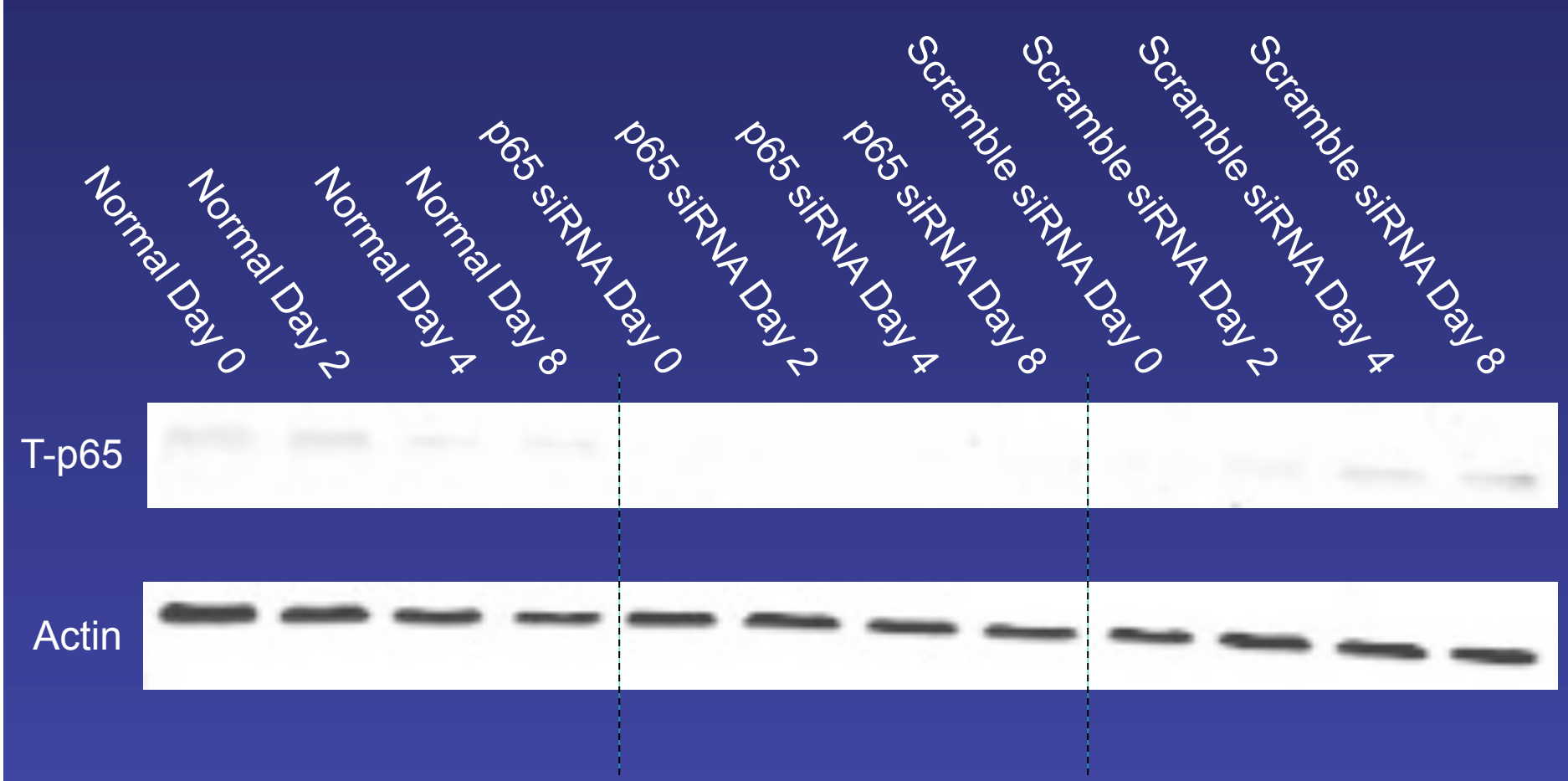
(survival and cell cycle both downregulated ➔

matrix cell population decreases)

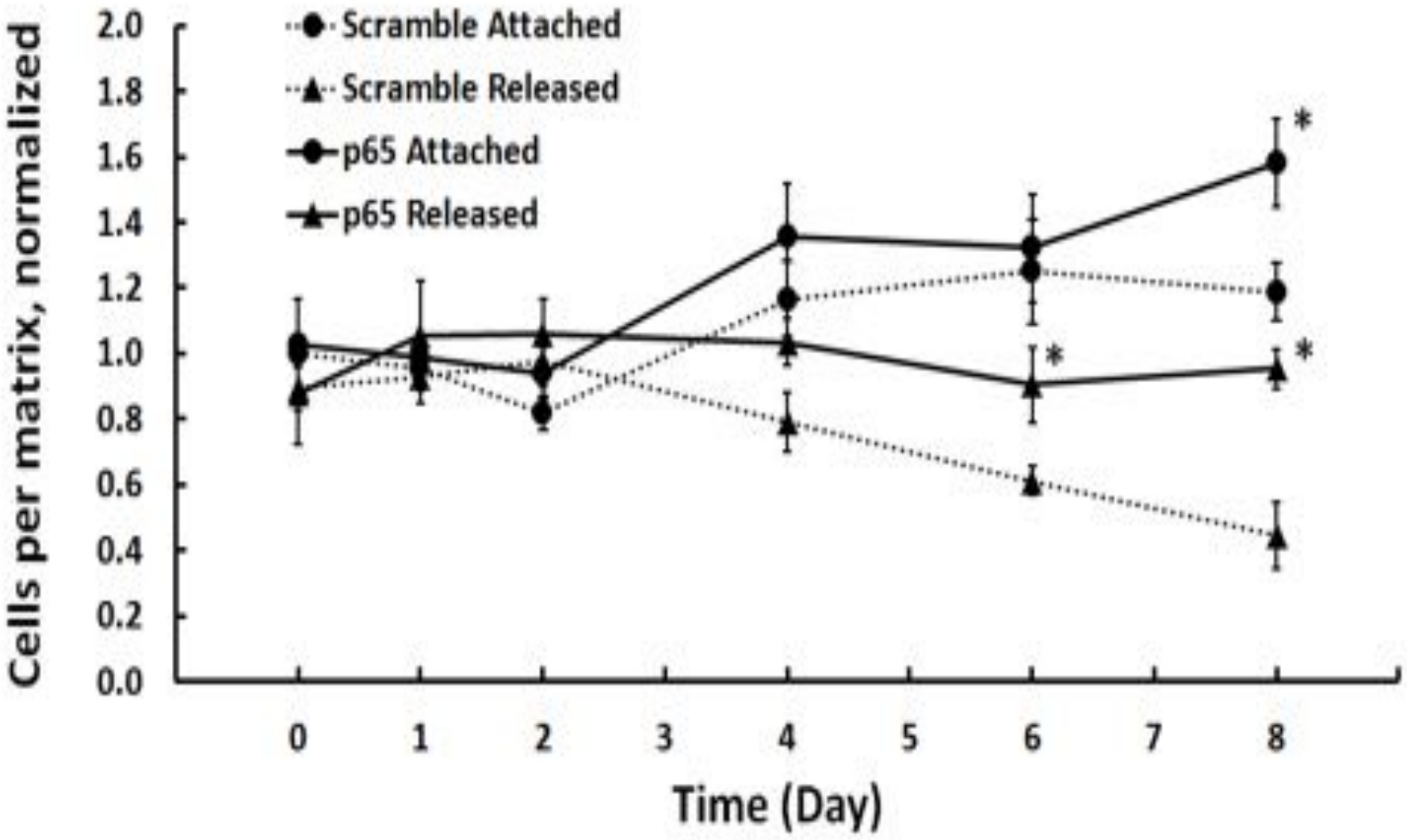


Microarray data has shown that the NF-kB pathway become activated after detachment

p65 RNAi Efficiency



Effect of NF-kB inhibition (p65 RNAi) on matrix cell population



Counts normalized to scramble attached day 0; each data point represents the mean ± SEM of three experiments on different strains; *p < 0.05 compared to respective scrambled attached or released, ANOVA and unpaired t-test.

In vitro aging model: Neonatal foreskin fibroblasts

Decrease in cellular DNA mass with increased passage number?

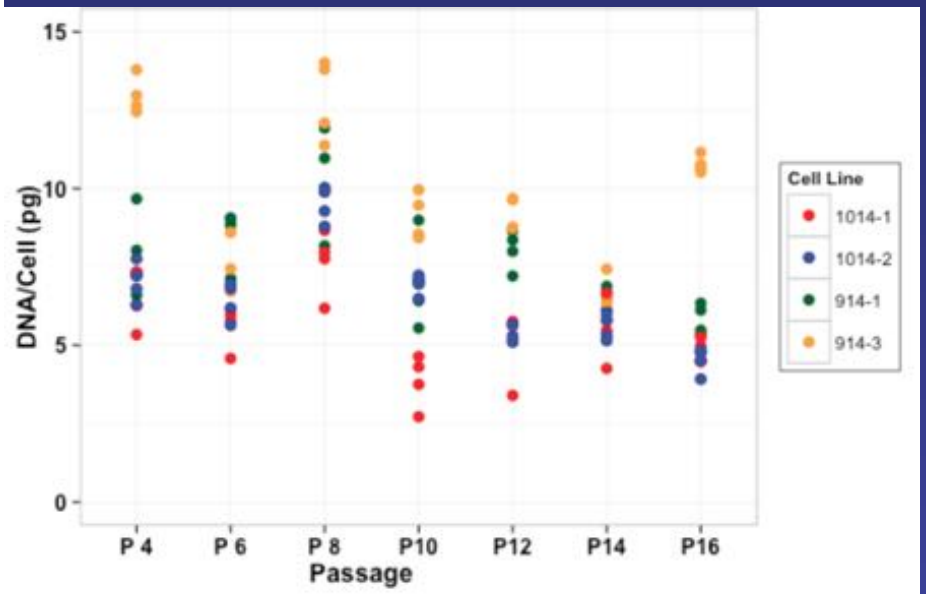


Figure 1. Scatterplot illustrating the amount of DNA/cell as observed in each passage for all cell counts of all four cell lines.

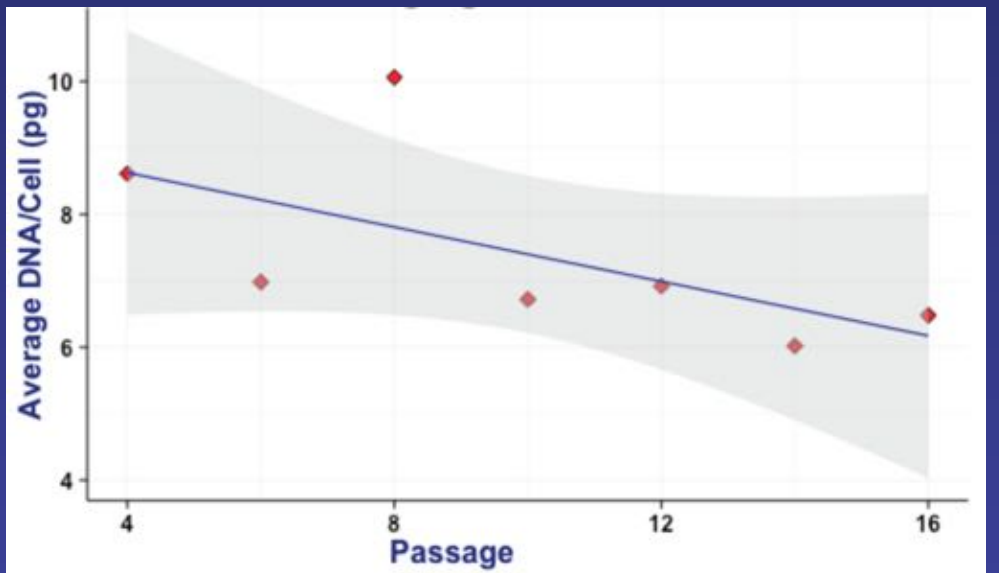
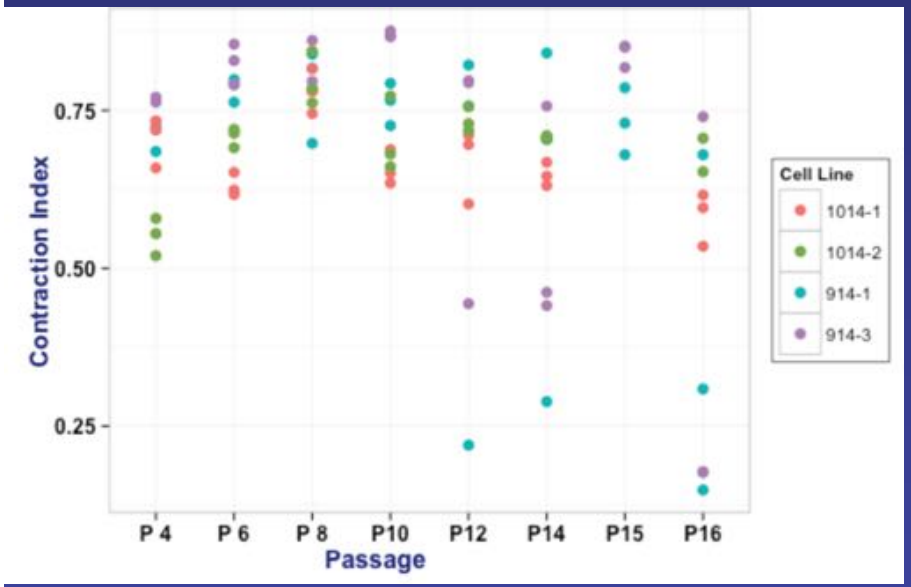
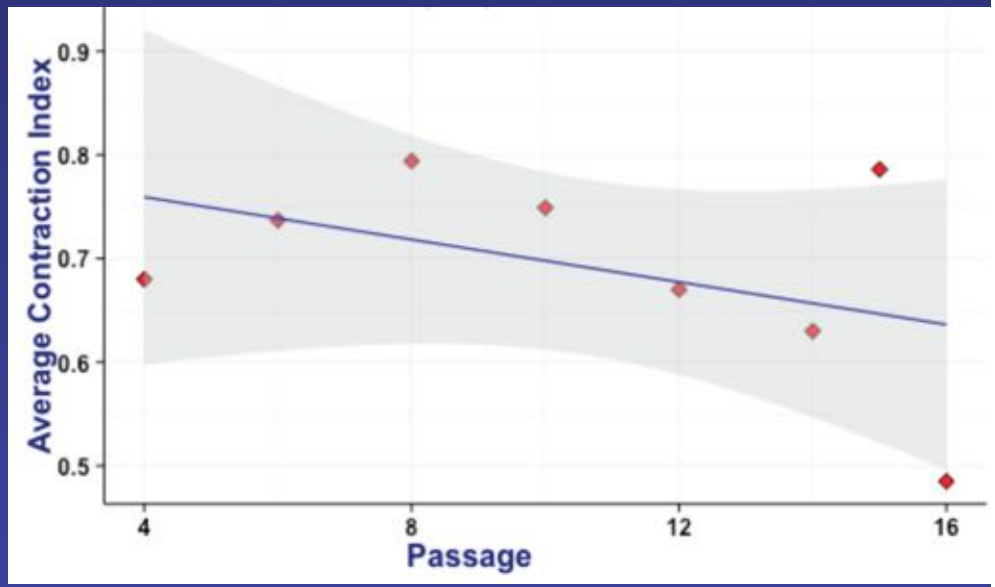


Figure 4. Scatterplot showing the average amount of DNA/cell for each passage, from all cell counts of every cell line. A regression line is shown with the data points, and the gray band represents the 95% confidence interval for the data.

In vitro aging model: Neonatal foreskin fibroblasts

Decrease in 3D matrix contraction with increased passage number?





Questions?

UNMC

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University of Nebraska Medical Center

Journal of Trauma and Acute Care Surgery

Fluid resuscitation rate for uncontrolled intraabdominal hemorrhage in pigs

--Manuscript Draft--

Manuscript Number:	
Full Title:	Fluid resuscitation rate for uncontrolled intraabdominal hemorrhage in pigs
Article Type:	Original Article
Section/Category:	
Keywords:	Noncompressible hemorrhage; swine; damage control resuscitation; prehospital management; crystalloid
Corresponding Author:	Mark A. Carlson, MD University of Nebraska Medical Center Omaha, NE UNITED STATES
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Nebraska Medical Center
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To: Editors, *Journal of Trauma and Acute Care Surgery*
Re: manuscript submission, "Fluid resuscitation rate for uncontrolled intraabdominal hemorrhage in pigs"

On behalf of my co-authors, I would like to submit the above manuscript to you for consideration of publication in the *Journal of Trauma and Acute Care Surgery*. The manuscript type is Original Article. Some data in this manuscript were presented at the Annual Meeting of the Central Surgical Association (March 5-7, 2015 in Chicago, IL) and at the Military Health Systems Research Symposium (August 17-20, 2015 in Ft. Lauderdale, FL). Other than the programs of the above meetings, the data of this study have not been published. This manuscript is not under consideration for publication at another journal.

The principal finding of this study was that a restricted rate of crystalloid infusion produced improved outcomes in a porcine model of uncontrolled noncompressible intraabdominal hemorrhage.

My co-authors and I have no relevant conflicts of interest to report. The work reported herein was funded by an award from the United States Army.

Thank you and all best regards,

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Manuscript Title:
Fluid resuscitation rate for uncontrolled intraabdominal hemorrhage in pigs

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ABSTRACT

Background. We hypothesized that a slow rate of crystalloid resuscitation would improve blood loss and hemoglobin level compared to a rapid rate in a porcine model of uncontrolled intraabdominal hemorrhage.

Methods. Non-randomized domestic swine (N = 24, 33-48 kg) were anesthetized, splenectomized through a midline incision, and underwent hemitransection of the left lateral liver lobe; one minute later, warm Lactated Ringers solution was begun at 150 or 20 mL/min IV (rapid *vs*. slow group, respectively, N = 12 per group). The rapid and slow groups underwent vital sign monitoring with periodic blood testing for a maximum of 60 or 180 min, respectively, to achieve infusion of a total of 100 mL/kg of crystalloid, and then necropsy.

Results. Pre-injury parameters did not differ between the two groups. Survival after one hour in the rapid *vs*. slow groups was 7 and 8 out of 12 subjects, respectively ($p>0.05$). The volume of post-injury resuscitation fluid was not different between the two groups. The slow group had a better outcome compared to the rapid group in terms of blood loss (1.6 *vs*. 2.7 L, respectively) and final hemoglobin concentration (3.4 *vs*. 6.0 g/dL). Postmortem dissection demonstrated that injury severity was not different between groups.

Conclusions. The data of this study suggested that crystalloid resuscitation with a slow IV infusion rate produced less blood loss and a higher hemoglobin level compared to a rapid infusion rate

in a porcine model of uncontrolled intraabdominal hemorrhage. These findings are relevant to the prehospital management of casualties who are in hemorrhagic shock but without immediate access to blood products.

Level of Evidence. Level III (case-control study without negative criteria); study type = Therapeutic/Care Management.

Keywords

Noncompressible hemorrhage; swine; damage control resuscitation; prehospital management; crystalloid

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Short title:
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Conclusions. The data of this study suggested that crystalloid resuscitation with a slow IV infusion rate produced less blood loss and a higher hemoglobin level compared to a rapid infusion rate in a porcine model of uncontrolled intraabdominal hemorrhage. These findings are relevant to the prehospital management of casualties who are in hemorrhagic shock but without immediate access to blood products.

Level of Evidence. Level III (case-control study without negative criteria); study type = Therapeutic/Care Management.

Keywords

Noncompressible hemorrhage; swine; damage control resuscitation; prehospital management; crystalloid

BACKGROUND

Massive hemorrhage and traumatic brain injury each account for about half of early mortality on the modern battlefield.[1-3] Preclinical and clinical research directed at these difficult scenarios has promoted management strategies, such as such as rapid evacuation and damage control resuscitation, which have improved outcomes for critically injured personnel.[4-6] A central tenant of damage control resuscitation has been the resuscitation of a casualty in hemorrhagic shock with whole blood (preferred) or with blood products using a 1:1:1 ratio of plasma, red blood cells, and platelets.[6, 7] If blood products are not available, then colloid and/or crystalloid fluids are given in 500 mL boluses "until a palpable radial pulse, improved mental status or systolic BP of 80-90 mmHg is present."[7] This latter aspect of damage control resuscitation, in which crystalloid administration is minimized during the evacuation phase of a subject with hemorrhagic shock until the subject reaches a forward surgical unit, also has been described as hypotensive resuscitation.[6, 8, 9]

Since the 1990's, animal[8, 10] and clinical data[5, 9, 11, 12] have indicated that hypotensive resuscitation improves survival after severe hemorrhagic injury. As a result, the basic tenants of hypotensive resuscitation have been adopted[6] into the Tactical Combat Casualty Care (TCCC) guidelines[7] of the United States Army. The current TCCC guidelines[7] do not explicitly state parameters for either a fluid maximum or an optimal fluid administration rate with respect to the prehospital management of a victim with uncontrolled hemorrhage and shock. In the present non-randomized case-control study, we hypothesized that a reduced rate of intravenous crystalloid fluid resuscitation would improve outcomes (primarily blood loss and hemoglobin

level) compared to a more rapid rate in a porcine model of uncontrolled intraabdominal hemorrhage.

METHODS

Animal Welfare

Refer to the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments[13]) information in Supplementary Table S1. This animal research study was carried out in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (8th ed.) from the National Research Council and the National Institutes of Health,[14] and also in accordance with the Animal Welfare Act of the United States (U.S. Code 7, Sections 2131 – 2159).[15] The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the VA Nebraska-Western Iowa Health Care System (protocol number 00760), by the IACUC of the University of Nebraska Medical Center (protocol number 11-064-07-ET), and by the Animal Care and Use Review Office (ACURO) of the United States Army Medical Research and Materiel Command (award number W81XWH-11-1-0836). All procedures were performed in animal facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC; www.aaalac.org) and by the Office of Laboratory Animal Welfare of the Public Health Service (http://grants.nih.gov/grants/olaw/olaw.htm). All surgical procedures were performed under isoflurane anesthesia, and all efforts were made to minimize suffering. Euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals.[16]

Study Design and Determination of Subject Numbers

The study design of this report was a non-randomized case-control type. The minimum number of swine (n = 12) utilized in each group was determined with a statistical power

analysis[17] using Δ/σ (Cohen's *d*, in which Δ is the desired difference in means of numerical data set by the observer, and σ is the estimated standard deviation) = 1.25, false positive rate (α) = 0.05, false negative rate (β) = 0.2, and power ($1 - \beta$) = 0.8. The endpoints targeted for the power analysis were blood loss and final hemoglobin level.

Animal Preparation

Refer to the flow diagram in Fig. 1. Domestic swine (castrated males, 3 months) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska–Lincoln and acclimatized for 3-5 days under veterinary supervision. Subjects were fed *ad libitum* with corn-soybean meal and water. Subjects were fasted for 12 h prior to surgery, but with no water restriction. Immediately prior to the procedure, animals were premedicated[18] with a single 3 mL IM injection containing 150 mg Telazol® (tiletamine hydrochloride and zolazepam hydrochloride, 1:1 by weight; Fort Dodge Animal Health, New York, NY), 90 mg ketamine, and 90 mg xylazine.

Sedated subjects then were weighed, intravenous access was established via an ear vein, endotracheal intubation was performed, and general anesthesia was maintained with 0.5-1.5% isoflurane throughout the procedure using a Matrx™ Model 3000 Veterinary Anesthesia Ventilator (Midmark Corp., Versailles, OH). Central arterial and venous lines were placed through a cutdown in the right neck for pressure monitoring, blood sampling and fluid resuscitation. MAP (mean arterial pressure), end-tidal pCO_2, rectal temperature, cardiac electrical activity, and pulse oximetry were continuously recorded.[19] Mechanical ventilation was maintained at 12-15 breaths per minute, with a tidal volume of 10-15 mL/kg, in order to keep the

end-tidal pCO_2 at 35-45 mm Hg. A water-circulated warming pad (set at 39˚C) was placed under each subject to support body temperature.

A ventral midline laparotomy incision then was made, splenectomy was performed,[8, 10, 20-24] and a transabdominal cystostomy tube was placed to control urine flow.[19] Per published protocols,[20-22, 24] the excised spleen was weighed and then a volume of warm Lactated Ringers (LR; 37˚C) solution equivalent to three-fold the splenic weight was administered through the jugular line, using a rapid infusion pump (Cole-Palmer Masterflex L/S; Vernon Hills, IL) set at 150 mL/min. Prior to injury, any blood loss incurred during the preparation was quantified by weighing tared surgical sponges that were used to absorb lost blood, and then a volume of LR equivalent to three-fold the pre-injury blood loss (typically <50 mL) was given using the infusion pump.

Injury Mechanism, Resuscitation, and Observation

Pre-injury vital signs were recorded, the lower half of the midline incision was closed with towel clips, and then the injury mechanism (hepatic left lower lobe hemitransection) was applied, as previously described (a 4 cm cut across the base of the left lateral lobe of the liver[19]). Immediately after injury, the laparotomy incision was closed with towel clips. All the subjects were allowed to bleed without any efforts at local hemostasis (compression, bandage, vessel clamping, etc.). This injury was intended to have no intraabdominal treatment, in order to model the course of hemorrhage in a battlefield from an uncontrolled noncompressible truncal injury.

The goal of the post-injury resuscitation was to give all subjects the same volume of fluid, but with different administration rates. At 60 s after injury, Lactated Ringers (LR) solution (stored at 37˚C) was begun at either 150 or 20 mL/min IV (rapid and slow group, respectively, N

= 12 per group) using the infusion pump. The maximum volume post-injury LR resuscitation volume was capped at 100 mL/kg; this limit (equivalent to 7 L of crystalloid in a 70 kg patient) was empirically chosen, based on the assumption that most trauma victims in hemorrhagic shock eventually will receive blood products, as opposed to unlimited crystalloid resuscitation. The resuscitation goal (i.e., target MAP) was defined as 80% of the pre-injury MAP, per published protocols with these subjects.[23]. As long as the MAP was below this target, resuscitation continued until the animal expired or the 100 mL/kg fluid maximum was reached.

The maximum post-injury observation time for the rapid *vs*. slow groups was 60 *vs*. 180 min, respectively. Subjects remained under general anesthesia, with continuous monitoring of vital signs and periodic blood draws for laboratory testing. If a subject was still alive at the end of the prescribed observation period, then it was euthanized by increasing the isoflurane fraction to 5% for 5 minutes, followed by bilateral diaphragm incision with transection of the supradiaphragmatic inferior vena cava (a method of intentional exsanguination approved by the AMVA[16]).

Endpoints

Heart rate, MAP, pulse oximetry, end-tidal pCO_2, and rectal temperature were continuously recorded, as described above. Arterial blood samples were drawn pre-injury, at 15 min post-injury, and then at the "final" time point, which was defined as the time of imminent death (for those subjects not surviving the prescribed observation period) or upon completion of the prescribed observation period. Imminent death was defined as MAP ≤20 mm Hg with an arterial pressure wave of ≤10 mm Hg. Death was defined as MAP ≤20 mm Hg with no

identifiable pressure wave on the monitor's arterial tracing, end-tidal pCO_2 <5 mm Hg, and absent corneal reflex.

Immediately after each subject was declared dead, the laparotomy incision was re-opened, and all clots and blood were rapidly evacuated using a combination of tared laparotomy pads, suction, and manual extraction. Tared buckets containing evacuated blood were weighed in order to calculate blood loss. Necropsy was performed immediately after expiration; the liver was explanted for inspection, dissection, photography, and documentation of the injury anatomy.

The CBC, PT/APTT, INR, fibrinogen, and ABG testing were contracted to the Clinical Laboratory of the VA Nebraska-Western Iowa Health Care System. This laboratory used the quantitative fibrinogen assay based on the von Clauss method.[25]

Statistical Analysis

Numerical data were reported as the mean ± standard deviation (SD). For a complete description of the statistical analysis, see the Supplementary Material. Unpaired continuous data were compared with ANOVA; significant results ($p < 0.05$) were confirmed with nonparametric Kruskal-Wallis analysis of variance. Groups of categorical data were compared with the Fisher exact test. If data for a given subject at a given time point were "missing" (e.g., no data captured secondary to lost blood tube, clotting of a blood specimen prior to running an assay, monitor malfunction, other miscellaneous events), then the respective cells in the data spreadsheet were kept empty (see Supplementary Material). If a coagulation test was reported as "failed" (meaning, no clot formation during the assay), then the respective data cells were filled as follows: QFA = 20 mg/dL; protime = 37 s; INR = 5 (critical values from contracting laboratory).

RESULTS

Pre-injury Data

All raw data from this study and a detailed statistical analysis thereof are contained in the Supplementary Material. Mean subject weights were 35.8 ± 1.8 and 37.9 ± 4.9 kg, rapid *vs*. slow groups, respectively ($p > 0.05$, unpaired t-test). Pre-injury blood loss (incurred during subject preparation, and including splenic mass) was not different between the two groups (Table 1); splenic mass was 360 ± 71 *vs*. 343 ± 75 g, rapid *vs*. slow groups, respectively ($p > 0.05$, unpaired t-test). Pre-injury fluid administration, which included splenic replacement per the 3x formula described in the Methods, also was not different between the two groups (Table 1). The pre-injury MAP, heart rate, temperature, hemoglobin concentration, platelet count, quantitative fibrinogen assay (QFA), protime (PT), and arterial blood gas data were not different between two groups (Tables 2-4; see also full statistical analysis in Supplementary Material). There was a small but significant difference in the International Normalized Ratios between the two groups (Table 4), but this did not seem physiologically relevant.

Injury Survival

For a full description of the typical response to this injury, including video, please refer to a previous publication.[19] The procedure survival of the rapid *vs*. slow infusion groups was 60 was 58% (7/12 subjects) *vs*. 67% (8/12 subjects); $p > 0.05$ (Fisher exact test; see Kaplan-Meier plot in Fig. 2). The five subjects in the rapid group that expired prior to the end of the prescribed observation period were pronounced dead at 15, 26, 38, 40, and 43 min after injury; the four subjects in the slow group that similarly expired died at 30, 34, 35, and 53 min after injury. All

deaths occurring prior to the prescribed observation period were preceded by terminal hypotension (i.e., no dysrhythmia, ventilatory inadequacy, or other contributing factors were noted).

Vital Sign Data

The MAP dropped precipitously (from >100 mm Hg to 30-60 mm Hg) within each group after injury, but there were no significant differences in post-injury MAP between the rapid and slow groups (Table 2) at the 15 min or final time points. Interestingly, the MAP in the rapid group trended higher (nonsignificant) at the 15 min time point with respect to the slow group, but the reverse nonsignificant trend was present at the final time point (i.e., the MAP of the slow group trended higher). With the exception of a small significant difference (~1˚C) in temperature at the final time point, there were no post-injury differences between the two groups for heart rate and temperature (Table 2).

Blood Loss and Hematology Data

Post-injury blood loss was ~70% greater in the rapid group compared to the slow group (~2.7 *vs*. 1.6 L, respectively), but total post-injury crystalloid administered was not different (Table 1). The latter result was intentional per the experimental design; i.e., the total maximum crystalloid volume was set at 100 mL/kg for a subject in either group. Post-injury serum hemoglobin in the rapid group was about half the value of the slow group at both the 15 min and final time points; platelet concentration was lower in the rapid group only at the 15 min time point (Table 3). The QFA, PT, and INR were all more negatively affected in the rapid group

compared to the slow group at the 15 time point (Table 4). Data on APTT were not analyzed secondary to an excessive number of missing values.

Blood Gas and Necropsy Results

With respect to arterial blood gas data, the within-group pH, pCO_2, bicarbonate, and base excess all trended down after injury, but there were no differences in these values comparing the rapid *vs*. slow groups at any time point (see Supplementary Material). End-tidal pCO_2 was monitored but the electronic readout was not reliably captured, so comparative analysis of these data were not possible. Liver mass at necropsy not different between the groups (Table 1). Postmortem dissection of the explanted liver demonstrated that there was no difference between the two groups with respect to the number of hepatic or portal veins that were lacerated (Table 1).

DISCUSSION

In this study of uncontrolled intraabdominal hemorrhage from a combined portohepatic venous injury in swine, it was found that a 20 mL/min rate ("slow") of intravenous crystalloid resuscitation resulted in a reduction in blood loss and an improvement in hemoglobin, platelet, and coagulation parameters, compared to a resuscitation rate of 150 mL/min ("rapid"). Of note, the prescribed resuscitation volume was limited in all subjects to 100 mL/kg; the actual volume of post-injury crystalloid given did not differ between the two groups. So what was really tested in this study was the effect of the pump rate for a fixed volume of fluid. Subjects with the slower pump rate did better.

Countering the improved endpoints in the slow group was the observation that the final mean temperature was about 1˚C greater in the rapid infusion group. This result likely was secondary to the fact that rapid group received the fixed volume of pre-warmed LR resuscitation fluid over a shorter time period. It is not clear whether the difference in final rectal temperature (36 *vs*. 35˚C) was biologically relevant.

This study had a non-randomized case-control design (the fast group data was accrued first, followed by the slow group data). The slow resuscitation protocol required extending the post-injury observation period to 180 min, so that the total resuscitation volume between groups would be equal. So with respect to blood loss (measured at death or the end of the observation period), the two groups of swine were not strictly comparable. The fact that the slow group lost ~1 L less blood than the rapid group, despite the slow group having three times the duration of post-injury observation, emphasizes the conclusion that the slow resuscitation rate resulted in less blood loss.

We could have compared the two resuscitation protocols at one hour, thus eliminating the issue of differing durations of post-injury monitoring, but then the total fluid volume would not have been equal between groups. Alternatively, we could have redone the study in a randomized fashion, using a 3 h observation period in all subjects. But given the convincing outcomes obtained with the case-control subjects reported herein, the overseeing animal committees would not have permitted these experiments to be redone.

Pigs undergoing a 40% blood volume bleed through a carotid arterial catheter and not resuscitated had somewhat improved coagulation parameters after a 2 h observation period compared to pigs who were fluid resuscitated.[26] Pigs undergoing a grade V liver injury and not resuscitated shed less blood compared to fluid resuscitated pigs after a 2 h observation period,[27] though blood pressure was marginally less in the former pigs. No conclusive data are available in preclinical models on the effect of restricted resuscitation on survival or long-term complications. As indicated in the Introduction, data from clinical studies have suggested[5, 9, 11, 12] that if blood products are not available, then fluid restriction (i.e., hypotensive resuscitation) in the pre-hospital management of hemorrhagic shock may produce better outcomes. So the results of the present study are consistent with previously-published data.

A ready explanation for the apparent detrimental effect of rapid intravenous resuscitation for hemorrhagic shock in this study was hemodilution. Although both groups received the same fluid volume of crystalloid in the post-injury period, perhaps the slower infusion rate provided adequate time for that infused fluid to equilibrate into the extravascular space without causing hemodilution, while the rapid infusion rate overwhelmed the vascular space and diluted its contents. Furthermore, it has been demonstrated in animal models that saline infusion can

activate systemic inflammation;[28-30] what role this phenomenon may have played in the present study is not known.

With 12 subjects per group, this study was not powered to detect a difference in survival between the rapid *vs*. slow crystalloid infusion groups. If the survival of the rapid infusion group is held constant at 58%, then in order to have 80% power to detect a significant difference ($p < 0.05$) with the Fisher exact test, the survival of the slow infusion group would need to be 100% with 17 subjects per group. In order to detect a survival difference of 20% at 80% power, the study would have required 85 subjects per group. So with only 12 subjects per group, the ability of the present study to detect a difference in survival was limited.

In addition to the power issue and different post-injury observation times discussed above, limitations of this study include no long-term survival data, data acquisition only under general anesthesia, and no data on the systemic inflammatory response. Moreover, there are controversial several aspects of porcine modeling of severe hemorrhage (including recently-developed models of uncontrolled intraabdominal hemorrhage) which were not intended targets in this study, including the necessity of routine pre-injury splenectomy,[31] the type of resuscitation fluid to employ,[26, 32] and the relative advantages of open vs. closed injury mechanisms [23, 33-37].

Parameters describing a fluid maximum or fluid administration rate for prehospital crystalloid administration are not described in the current TCCC guidelines.[7] While this study cannot provide these parameters, the data contained in this report can contribute to the ongoing discussion on how this aspect of prehospital management might evolve. In particular, it may ultimately be determined that a relatively slow intravenous infusion rate (as opposed to bolus infusion described in the current TCCC guidelines[7]) may be preferred for prehospital fluid

resuscitation of the patient with hemorrhagic shock for which blood products are not immediately available.

Conclusions

In a porcine model of uncontrolled intraabdominal hemorrhage, a relatively slow rate (20 mL/min) of intravenous crystalloid resuscitation produced improved short-term outcomes (blood loss, hemoglobin level, and coagulation parameters) compared to a more rapid rate (150 mL/min). The study was not powered to determine if there was a difference in short-term survival.

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Author contributions: GL, WHV, and MAC designed the study; URY, JMJ, IIP, and MAC performed the experiments and collected the data; RRH performed the statistical analysis; URY and MAC wrote the manuscript; all authors read, commented on, and approved the final manuscript; MAC supervised the study.

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Figure Legends

Fig. 1. Experiment Flow Chart. Swine were acclimatized (acclim), then on procedure day underwent general endotracheal anesthesia (GETA) followed by access of the carotid and jugular vessels (Vasc Acc), and then other preparatory procedures. After injury, subjects underwent IV crystalloid resuscitation (100 mL/kg maximum volume allowed) for the indicated observation period (Obs). Black bar underneath each observation period indicates relative time required for crystalloid infusion.

Fig. 2. Kaplan Meier plot of injured porcine subjects treated with rapid *vs*. slow crystalloid resuscitation.

Table 1. Blood loss, fluid input, liver weight, and lacerated veins.

	Blood Loss (mL)*		Fluid input (mL)*			No. Veins Lacerated**	
Infusion Rate	Pre-injury	Post-injury	Pre-injury	Post-injury	Liver weight (g)*	Hepatic	Portal
Rapid	430 ± 151	2738 ± 693	1288 ± 458	3644 ± 523	852.9 ± 114.8	0.9 ± 0.3	1.3 ± 0.4
Slow	414 ± 48	1600 ± 360	1138 ± 181	3082 ± 1720	941.0 ± 127.3	1.0 ± 0.0	1.4 ± 0.5
p-value	0.72	**<0.001**	0.31	0.30	0.089	0.4884	0.7075

All values are mean ± SD; time points are with respect to injury; *unpaired t-test (significant values are bolded); **Kruskal-Wallis test.

Table 2. Vital sign data.

Infusion Rate	MAP (mm Hg)			Heart rate (beats/min)			Temperature (°C)		
	Initial	15 min	Final	Initial	15 min	Final	Initial	15 min	Final
Rapid	113 ± 14	55 ± 31	27 ± 16	107 ± 21	108 ± 22	100 ± 23	36.9 ± 0.6	36.5 ± 0.5	36.0 ± 0.6
Slow	105 ± 18	33 ± 17	39 ± 31	90 ± 19	110 ± 25	112 ± 23	36.3 ± 1.1	35.9 ± 1.0	35.1 ± 1.0
*p-value	0.52	0.11	0.48	0.09	0.99	0.69	0.23	0.15	**0.010**

MAP = Mean Arterial Pressure. All values are mean ± SD; time points are with respect to injury; *Kruskal-Wallis test; significant tests are bolded.

Table 3. Hematologic testing results.

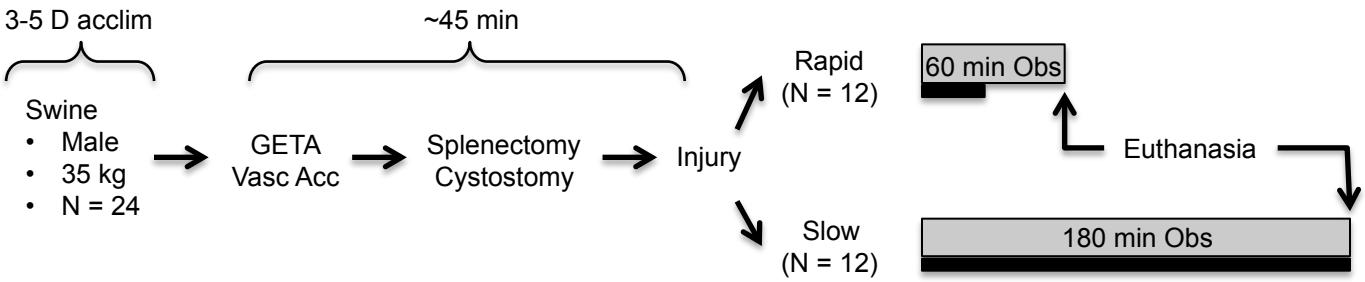
	Hemoglobin (g/dL)			Platelets (x 1,000/μL)		
Infusion Rate	0 min	15 min	Final	0 min	15 min	Final
Rapid	12.2 ± 0.9	4.6 ± 2.0	3.4 ± 2.3	306 ± 77	147 ± 66	122 ± 66
Slow	11.7 ± 0.9	8.4 ± 1.2	6.0 ± 2.2	315 ± 87	215 ± 55	171 ± 71
p-value*	0.39	**<0.001**	**0.025**	0.99	**0.029**	0.23

All values are mean ± SD; time points are with respect to injury; *unpaired t-test; significant values are bolded.

Table 4. Coagulation testing results.

	QFA (mg/dL)			Protime (s)			INR		
Infusion Rate	0 min	15 min	Final	0 min	15 min	Final	0 min	15 min	Final
Rapid	115 ± 13	41 ± 12	37 ± 13	11.5 ± 0.7	16.6 ± 4.7	18.9 ± 8.9	1.0 ± 0.1	1.5 ± 0.5	2.0 ± 1.3
Slow	109 ± 17	63 ± 9	47 ± 15	10.9 ± 0.6	11.3 ± 1.4	14.4 ± 9.5	0.9 ± 0.1	1.0 ± 0.1	1.4 ± 1.5
p-value*	0.58	**<0.001**	0.27	0.11	**0.003**	0.46	**<0.001**	**0.004**	0.69

QFA = Quantitative Fibrinogen Assay; INR = International Normalized Ratio. All values are mean ± SD; time points are with respect to injury; *unpaired t-test; significant values are bolded.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~45 min
Swine
• Male
• 35 kg
• N = 24
GETA
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Slow
(N = 12)
60 min Obs
Euthanasia
180 min Obs

Fig. 1

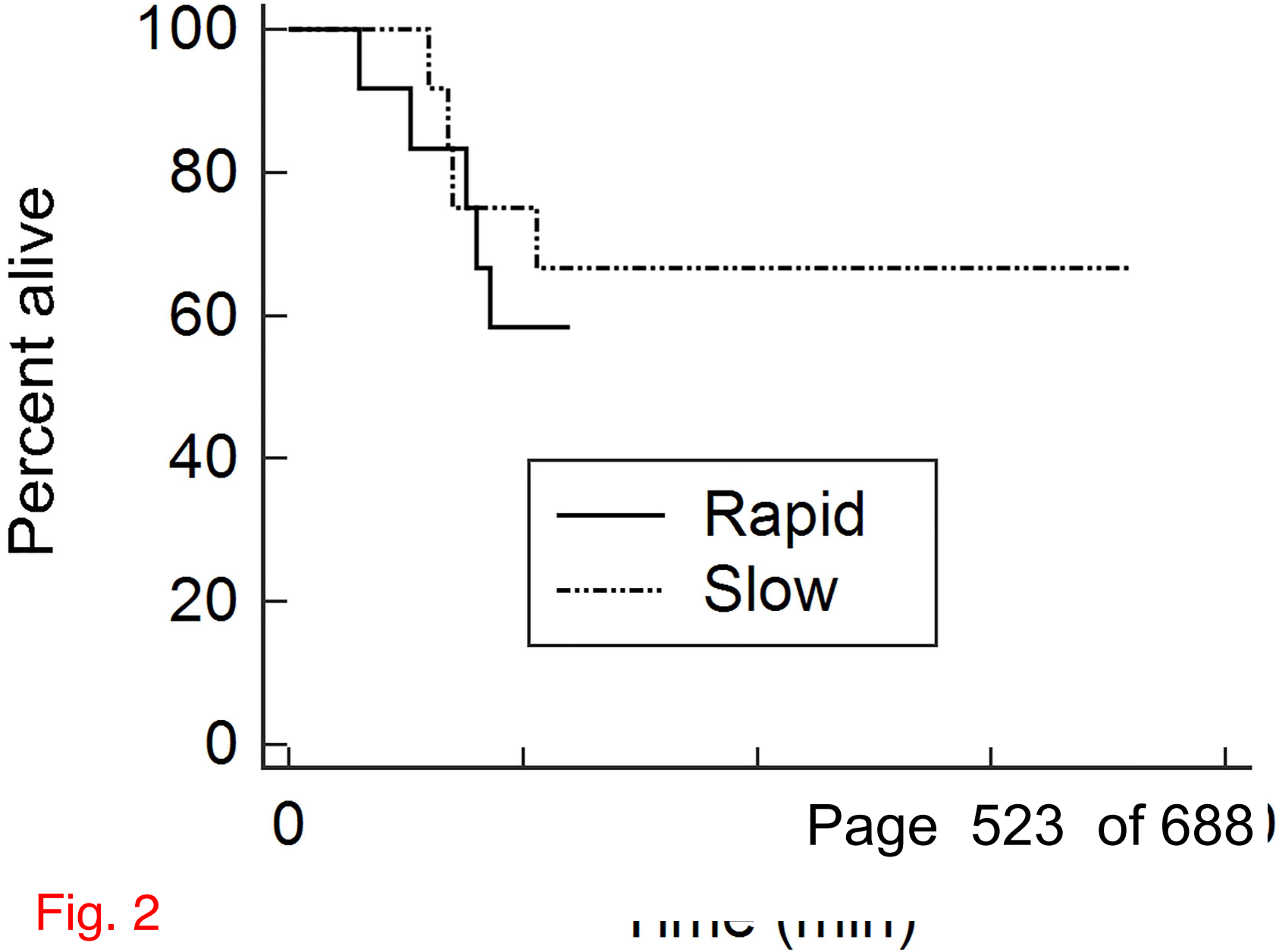

Fig. 2

Title

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Comparison of a Synthetic Resorbable Bandage vs. Oxidized Regenerated Cellulose for Treatment of Minor Surgical Hemorrhage in a Porcine Model

Short Title

[50 character limit for short title; current = 40, with spaces]

Bandage Comparison for Minor Hemorrhage

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Abstract [Unstructured, 300 word limit; current = 314]

Commercially-available topical hemostats for minor hemorrhage incurred during elective surgical procedures are relatively expensive. We believe that more economical synthetic hemostats could be produced. Our objective here was to compare the efficacy and toxicity of a synthetic resorbable hemostatic bandage *vs*. an analogous commercial product in a porcine model of minor hemorrhage. For the nonsurvival efficacy study, anesthetized domestic swine (boars, 3 months, 29-40 kg) underwent arterial/venous line placement and splenectomy. A 1 x 8 cm section of liver was resected from the edge of the left lateral lobe, and test bandage (macroporous polycaprolactone mesh, PCL; N = 10) or oxidized regenerated cellulose (ORC; Surgicel®, Ethicon®; N = 10) was applied with manual pressure for 5 minutes. Resuscitation then was performed with warm LR (target MAP = 80% of preinjury), and blood loss was measured 60 min after injury. For the survival toxicity study, a similar resection technique was employed (N = 6 for each material), and necropsy was performed at 30 days to evaluate for bandage toxicity (subject growth, serum chemistry, histology). Pre-injury weight, VS, and laboratory testing did not differ among groups. Resection mortality was zero. In the efficacy study, there were no differences between the PCL vs. ORC groups in blood loss or other post-injury variables (Table), except that the resuscitation fluid volume in the ORC group was greater. Other results from the efficacy study not shown in the Table include platelet counts and coagulation testing (no significant differences). Other than minor granuloma formation at the implantation site with both PCL and ORC, the survival study did not reveal any measurable toxicity. The efficacy and toxicity of the PCL test bandage vs. the ORC comparator were not different in a porcine model of minor hepatic hemorrhage. Based on projected costs of production (not shown), the PCL bandage could represent a lower-cost alternative to ORC for the treatment of minor surgical bleeding.

Introduction

Materials and Methods

2.1. Animal Welfare

This animal research study was carried out in accordance with recommendations in the *Guide for the Care and Use of Laboratory Animals* (8th ed.) from the National Research Council and the National Institutes of Health {Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011 #38}, and also in accordance with the Animal Welfare Act of the United States (U.S. Code 7, Sections 2131 – 2159) {United States Department of Agriculture, 2013 #39}. The animal protocol was approved by the Institutional Animal Care and Use Committee of the VA Nebraska-Western Iowa Health Care System (protocol number 00760), by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center (protocol number 11-064-07-ET), and by the Animal Care and Use Review Office of the United States Army Medical Research and Materiel Command (award number W81XWH-11-1-0836). All procedures were performed in animal facilities approved by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC; www.aaalac.org) and by the Office of Laboratory Animal Welfare of the Public Health Service (http://grants.nih.gov/grants/olaw/olaw.htm). All surgical procedures were performed under isoflurane anesthesia, and all efforts were made to minimize suffering. Euthanasia was performed in accordance with the AVMA Guidelines for the Euthanasia of Animals {American Veterinary Medical Association Panel on Euthanasia, 2013 #40}.

2.2. Determination of Subject Numbers

The minimum number of swine (n = 12) utilized in each group was determined with a statistical power analysis {Neter, 1990 #41} using Δ/σ (Cohen's *d*, in which Δ is the desired difference in means of

numerical data set by the observer, and σ is the estimated standard deviation) = 2.0, false positive rate (α) = 0.05, false negative rate (β) = 0.2, and power ($1 - \beta$) = 0.8. The endpoints targeted for the power analysis were blood loss and final hemoglobin level.

2.3. Animal Preparation

Domestic swine (castrated males, 3 months) were purchased from the Agricultural Research and Development Center (Mead, NE) of the University of Nebraska–Lincoln and acclimatized for 3-4 days under veterinary supervision. Subjects were fed ad lib with corn-soybean meal and had access to water. Prior to surgery, subjects were fasted for 12 hours, but with no water restriction. On the day of procedure, animals were premedicated {Carlson, 2014 #42} with a single 3 mL IM injection containing 150 mg Telazol® (tiletamine hydrochloride and zolazepam hydrochloride, 1:1 by weight; Fort Dodge Animal Health, New York, NY), 90 mg ketamine, and 90 mg xylazine.

Sedated subjects then were weighed, intravenous access was established via an ear vein, endotracheal intubation was performed, and anesthesia was maintained with 0.5-1.5% isoflurane throughout the procedure using a Matrx™ Model 3000 Veterinary Anesthesia Ventilator (Midmark Corp., Versailles, OH). Central arterial and venous lines were placed through a cutdown in the right neck for pressure monitoring, blood sampling and fluid resuscitation. MAP (mean arterial pressure), end-tidal pCO_2, rectal temperature, cardiac electrical activity, and pulse oximetry were continuously recorded. Mechanical ventilation was maintained at 12-15 breaths per minute, with a tidal volume of 10-15 mL/kg, in order to keep the end-tidal pCO_2 at 30-40 mm Hg. A heating pad was placed under each subject to support body temperature.

Subject preparation followed a previous descriptions {Yanala, 2014 #35}. A ventral midline laparotomy incision then was made, splenectomy was performed, a transabdominal cystostomy tube was

placed. The excised spleen was weighed, and then a volume of warm Lactated Ringers (LR; 37˚C) solution equivalent to three-fold the splenic weight was administered through the jugular line, using a rapid infusion pump (Cole-Palmer Masterflex L/S; Vernon Hills, IL) set at 150 mL/min. Prior to injury, any blood loss incurred during the preparation was quantified by weighing tared surgical sponges that were used to absorb lost blood, and then a volume of LR equivalent to three-fold the pre-injury blood loss (typically <50 mL) was given using the infusion pump.

2.4. Injury Mechanism and Resuscitation

Pre-injury vital signs were recorded, the lower half of the midline incision was closed with towel clips, and then the injury mechanism (hepatic left lower lobe hemitransection) was applied, as previously described {Yanala, 2014 #35}. In brief, liver injury was performed by making a 4 cm cut across the base of the left lateral lobe of the liver. Immediately after injury, the laparotomy incision was closed with towel clips. All the subjects were allowed to bleed without any efforts for local hemostasis (compression, bandage, vessel clamping, etc.). All injuries were intended to have no intraabdominal treatment models in order to depict the course of hemorrhage in a battlefield from an uncontrolled noncompressible truncal injury.

At 60 s after injury, Lactated Ringers (LR) solution (stored at 37˚C) was begun at either 150 or 20 mL/min IV (rapid and slow group, respectively, N = 12 per group) using the infusion pump. The maximum volume post-injury LR resuscitation volume was capped at 100 mL/kg; this limit (equivalent to 7 L fluid in a 70 kg person) was empirically chosen, based on the assumption that most patients in hemorrhagic shock eventually will receive blood products, as opposed to unlimited crystalloid resuscitation. The resuscitation goal (i.e., target MAP) was defined as 80% of the pre-injury MAP; as

long as the MAP was below this target, resuscitation continued until the animal expired or the 100 mL/kg fluid maximum was reached.

The rapid and slow groups were monitored for 60 or 180 min, respectively, while under general anesthesia, with continuous monitoring of vital signs and periodic blood draws for laboratory testing (see Discussion for explanation of the different post-injury observation periods). If a subject was still alive at the end of observation period, then it was euthanized by transection of the inferior vena cava and intentional exsanguination, while under deep general anesthesia (method approved by the AMVA {American Veterinary Medical Association Panel on Euthanasia, 2013 #40}).

2.5. Endpoints

Heart rate, MAP, pulse oximetry, end-tidal pCO_2, and rectal temperature were continuously recorded, as described above. Blood samples were drawn pre-injury, at 15 min post-injury, and then at 60 min post-injury or death ("final" time point), whichever came first. Death was defined as MAP ≤20 mm Hg with no identifiable pressure wave on the monitor's arterial tracing, end-tidal pCO_2 <5 mm Hg, and absent corneal reflex.

Immediately after the planned observation period or after the animal expired (whichever came first), the laparotomy incision was re-opened, and all clots and blood were rapidly evacuated into tared buckets with a combination of tared laparotomy pads, suction, and manual extraction. The buckets were weighed in order to calculate blood loss. Necropsy was performed immediately after expiration; the liver was explanted for inspection, dissection, photography, and documentation of the injury anatomy.

2.6. Laboratory Testing and Statistical Analysis

The CBC, PT/PTT, INR, fibrinogen, and ABG testing were contracted to the Clinical Laboratory of the VA Nebraska-Western Iowa Health Care System. This laboratory used the quantitative fibrinogen assay based on the von Clauss method {Lowe, 2004 #37}. Numerical data was reported as the mean ± standard deviation (SD). Groups of unpaired numerical data were compared with the nonparametric Kruskal-Wallis analysis of variance. Groups of categorical data were compared with the Fisher exact test. Significance was defined as $p < 0.05$. If a data for a given subject at a given time point was empty secondary to the subject's expiration prior to the time point, then that data cell was filled with the lowest value (for MAP, hemoglobin, platelets, and fibrinogen) or highest value (for PT, INR, APTT) recorded among the surviving subjects at that time point.

Results

Discussion

Conclusions

Acknowledgements

This work also was supported in part with resources and the use of facilities at the VA Nebraska-Western Iowa Health Care System. The authors would like to acknowledge the anesthesia expertise of John Cavanaugh, and the technical assistance Chris Hansen, Dean Heimann, and Gerri Siford. Portions of this work were presented at the 8th Annual Academic Surgical Congress (February, 2015, Las Vegas, Nevada, USA).

References

Supporting Information Captions

Figure Legends

Fig. 1. Dissection demonstrating the anatomy of the porcine intrahepatic portal venous system. *Ex vivo* porcine liver, inferior aspect (scale in cm). The soft tissues overlying the portal venous system have been dissected and retracted with silk stay sutures. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; Gb = gallbladder; 1 = cut orifice of main portal vein; 2 = intrahepatic portal vein; 3 = RM lobe portal vein branch; 4 = 1st LL lobe portal vein branch; 5 = cut orifice of 2nd LL lobe portal vein branch (proximal end); 6 = distal end of structure

Table tt01.

Table tt02.

Table TT01. Hemoglobin and platelet count (acute efficacy study).

Material	Hemoglobin (g/dL)		Platelet (1,000/μL)	
	Pre-injury	60 min	Pre-injury	60 min
ORC	12.3 ± 0.8	12.9 ± 0.9	324 ± 88	327 ± 94
PCL	12.2 ± 0.7	12.8 ± 1.2	289 ± 72	266 ± 56
p, unpaired t-test	0.661	0.854	0.363	0.115
p, paired t-test (ORC)	0.099		0.276	
p, paired t-test (PCL)	0.082		0.136	

Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury.

Table TT02. Arterial blood gas data (acute efficacy study).

		pH		pCO2 (mm Hg)		HCO3 (mEq/L)		BE (mEq/L)		pO2 (mm Hg)	
Material	Term	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	Mean	7.53	7.59	31.6	27.1	26.5	25.5	4.4	4.5	446	429
	SD	0.05	0.06	2.5	4.5	2.2	1.4	2.5	1.6	37	92
PCL	Mean	7.50	7.51	35.4	28.6	26.9	25.7	4.1	4.1	423	448
	SD	0.05	0.18	5.7	4.3	1.3	1.6	1.2	0.8	28	54
p, unpaired t-test		0.1255	0.2117	0.0753	0.4468	0.6076	0.7562	0.7465	0.5361	0.1392	0.5808
p, paired t-test (ORC)		**0.0257**		**0.0129**		0.0846		0.8448		0.4557	
p, paired t-test (PCL)		0.8928		**0.0013**		0.1181		0.9282		0.1347	

BE = Base Excess; SD = standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT03. Protime, INR, APTT, and fibrinogen (acute efficacy study).

	Protime (s)		INR		APTT (s)		QFA (mg/dL)	
Material	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	10.7 ± 0.9	10.9 ± 0.4	1.0 ± 0.1	1 ± 0.0	21.6 ± 4.8	18.7 ± 3.1	113 ± 20	88 ± 22
PCL	10.7 ± 0.5	10.6 ± 0.7	1.0 ± 0.0	1.0 ± 0.1	20.1 ± 2.3	18.2 ± 1.9	112 ± 13	88 ± 15
p, unpaired t-test	0.880	0.292	0.514	0.143	0.419	0.732	0.968	0.972
p, paired t-test (ORC)	0.963		0.681		**0.026**		**<0.001**	
p, paired t-test (PCL)	0.456		0.193		**0.012**		**<0.001**	

INR = International Normalized Ratio; APTT = Activated Partial Thromboplastin Time; QFA = Quantitative Fibrinogen Assay. Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT04. Blood pressure, heart rate, and temperature (acute efficacy study).

	MAP (mm Hg)		HR		Temp (˚C)	
Material	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	116 ± 18	93 ± 11	108 ± 12	101 ± 6	37.6 ± 0.8	36.3 ± 0.5
PCL	115 ± 16	89 ± 8	102 ± 12	102 ± 15	37.5 ± 0.9	36.3 ± 1.3
p, unpaired t-test	0.885	0.298	0.304	0.890	0.834	0.932
p, paired t-test (ORC)	**<0.001**		0.106		**<0.001**	
p, paired t-test (PCL)	**<0.001**		1.000		**0.003**	

MAP = mean arterial pressure; HR = heart rate. Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury; **p < 0.05 are bolded**.

Table TT05. Weight of subject, liver, spleen, and resection specimen (acute efficacy study).

Material	Subject (kg)	Liver (g)	Spleen (g)	Resection specimen (g)
ORC	36.1 ± 2.5 (33.0 – 40.4)	934 ± 161 (699 – 1,242)	319 ± 55 (235 – 402)	6.7 ± 2.0 (2.9 – 9.5)
PCL	33.9 ± 2.3 (29.4 – 37.0)	939 ± 120 (726 – 1,143)	299 ± 42 (219 – 360)	7.6 ± 1.9 (4.8 – 11.6)
*p**	0.057	0.943	0.387	0.317

Data are mean ± standard deviation, with range in parentheses; *Unpaired t-test (two tailed), comparing ORC *vs*. PCL.

Table TT06. Intravenous fluid and blood loss (acute efficacy study).

Material	IV fluid (mL)		Blood loss (mL)	
	Pre-injury	Post-injury	Pre-injury	Post-injury
ORC	1,229 ± 342 (860 – 1,900)	1,952 ± 1,363 (220 – 3,715)	380 ± 61 (265 – 475)	111 ± 55 (58 – 237)
PCL	1,061 ± 219 (800 – 1,540)	594 ± 425 (110 – 1,180)	374 ± 78 (252 – 532)	93 ± 27 (56 – 140)
**p*, unpaired t-test	0.209	**0.012**	0.870	0.381
p, Kruskal-Wallis		**0.008**		

Data are mean ± standard deviation; *two tailed, comparing ORC *vs*. PCL; **p < 0.05 are bolded**.

Table TT07. Thromboelastography data (acute efficacy study).

Material	R (min)		K (min)		α (degree)		MA (mm)	
	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min	Pre-injury	60 min
ORC	3.74 ± 0.91	3.25 ± 0.50	1.99 ± 1.52	1.35 ± 0.48	64.7 ± 15.2	71.2 ± 5.5	60.3 ± 18.0	61.7 ± 11.1
PCL	4.31 ± 0.52	3.85 ± 0.65	1.17 ± 0.18	1.17 ± 0.15	73.2 ± 3.1	72.8 ± 2.2	68.7 ± 4.6	65.2 ± 3.3
p, unpaired t-test								
p, paired t-test (ORC)								
p, paired t-test (PCL)								

R = Reaction time (first evidence of clot formation); K = Achievement of 20 mm clot strength (amplitude); α = alpha angle, a measure of clot formation or kinetics of clot development; MA = Maximal amplitude (maximum clot strength). Data are mean ± standard deviation; unpaired t-test = two tailed, comparing ORC *vs*. PCL; paired t-test = two tailed, comparing pre-injury *vs.* 60 min post-injury.

Table TT08. Initial subject weight, resected liver specimen, and implanted bandage; bandage hold time; and procedural blood loss, all on day zero of 30-day toxicity study.

Material	Initial wt (kg)	Resection specimen wt (g)	Bandage wt (g)	Hold time (min)	Blood loss (mL)
ORC	37.9 ± 5.3 (30.6 – 44.8)	2.12 ± 0.75 (1.36 – 3.04)	0.64 ± 0.24 (0.34 – 0.87)	6.3 ± 4.7 (1.0 – 13.0)	8.7 ± 8.3 (1.0 – 24.0)
PCL	35.7 ± 2.5 (33.0 – 38.8)	1.79 ± 0.11 (1.62 – 1.89)	0.30 ± 0.08 (0.18 – 0.38)	5.8 ± 5.0 (1.0 – 15.0)	8.3 ± 5.5 (2.0 – 17.0)
None	34.8 ± 2.2 (31.0 – 37.6)	2.05 ± 0.85 (0.90 – 3.52)	NA	NA	12.8 ± 8.0 (4.2 – 25.3)
*p**	0.333	0.668	**0.016**	0.862	0.522

Data are mean ± standard deviation, with range in parentheses; *ANOVA (one way), comparing ORC *vs*. PCL *vs*. None; **p < 0.05 is bolded.**

Table TT09. Hemoglobin, platelet count, and WBC from the 30-day toxicity study.

Material	Hemoglobin (g/dL)		Platelets (10^3/μL)		WBC (10^3/μL)	
	Pre-Injury	Final[1]	Pre-Injury	Final	Pre-Injury	Final
ORC	11.4 ± 1.0 (10.4 – 12.5)	10.2 ± 0.9 (9.1 – 11.2)	374 ± 89 (282 – 494)	336 ± 29 (300 – 364)	18.9 ± 5.2 (13.1 – 23.9)	14.0 ± 2.7 (10.7 – 17.2)
PCL	11.5 ± 1.4 (9.4 – 13.8)	10.6 ± 1.3 (9.0 – 12.1)	340 ± 125 (161 – 502)	344 ± 132 (91 – 459)	14.5 ± 3.0 (11.7 – 20.1)	16.7 ± 3.0 (14.0 – 21.3)
None	11.8 ± 1.3 (9.8 – 13.2)	11.6 ± 1.1 (10.3 – 13.5)	314 ± 119 (215 – 445)	304 ± 51 (243 – 372)	16.7 ± 3.0 (12.9 – 20.3)	15.5 ± 2.9 (11.1 – 19.5)
p, one-way ANOVA[2]	0.880	0.152	0.743	0.722	0.214	0.396
p, paired t-test (ORC)	NA		0.204		**0.005**	
p, paired t-test (PCL)	**0.013**		0.473		0.039	
p, paired t-test (None)	0.463		0.491		0.171	

Data are mean ± standard deviation, with range in parentheses; [1]Final blood specimen drawn just prior to euthanasia on day 30; [2]comparing ORC *vs*. PCL *vs*. None; **p < 0.05 are bolded.**

Table TT10. Protime, APTT, INR, and QFA from the 30-day toxicity study.

Material	Protime (s)		APTT (s)		INR		QFA (mg/dL)	
	Pre-Injury	Final[1]	Pre-Injury	Final	Pre-Injury	Final	Pre-Injury	Final
ORC	10.3 ± 1.1 (9.0 – 11.6)	9.7 ± 0.4 9.0 – 10.1)	18.1 ± 2.1 (16.6 – 19.5)	17.6 ± 1.7 (16.6 – 19.6)	0.9 ± 0.1 (0.8 – 1.0)	0.9 ± 0.0 (0.8 – 0.9)	97 ± 5 (93 – 104)	93 ± 8 (84 – 102)
PCL	10.7 ± 0.8 (9.5 – 11.5)	9.4 ± 0.7 (8.3 – 9.9)	21.5 ± 0.6 (21.0 – 21.9)	17.2 ± 1.0 (16.6 ± 18.4	1.0 ± 0.1 (0.9 – 1.0)	0.8 ± 0.1 (0.7 – 0.9)	99 ± 27 (64 – 125)	91 ± 14 (74 – 113)
None	10.3 ± 1.0 (8.6 – 11.0)	9.9 ± 0.7 (9.2 – 10.6)	17.8 ± 1.0 (16.6 – 18.4)	16.6 ± NA (16.6 – 16.6)	0.9 ± 0.1 (0.7 – 0.9)	0.8 ± 0.1 (0.8 – 0.9)	113 ± 46 (78 – 190)	110 ± 6 (103 – 114)
p, one-way ANOVA[2]	0.777	0.578	0.968	0.598	0.185	0.565	0.716	**0.006**
p, paired t-test (ORC)	0.270		NA[3]		0.333		0.312	
p, paired t-test (PCL)	**0.040**		NA[3]		**0.035**		0.224	
p, paired t-test (None)	0.076		NA[3]		0.092		0.394	

APTT = Activated Partial Thromboplastin Time; INR = International Normalized Ratio; QFA = Quantitative Fibrinogen Assay. Data are mean ± standard deviation, with range in parentheses. [1]Final blood specimen drawn just prior to euthanasia on day 30; [2]comparing ORC *vs*. PCL *vs*. None; [3]Test not available because of missing data; **p < 0.05 are bolded.**

Table TT11. Selected serum tests from a comprehensive metabolic panel obtained during the 30 day toxicity study.

		Gluc (mg/dL)		Creat (mg/dL)		AG (mmol/L)		Ca (mg/dL)		Alb (g/dL)		AlkP (U/L)		AST (U/L)		GGT (U/L)		Amyl (U/L)	
Material	Value	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin	Pre	Fin
ORC	Mean	112	90	1.2	1.4	11	13	9.7	9.9	3.0	3.0	186	166	32	50	41	48	624	644
ORC	SD	23	15	0.2	0.1	3	2	0.2	0.4	0.3	0.3	40	39	9	14	8	8	120	107
ORC	Min	85	69	0.9	1.3	6	10	9.3	9.2	2.6	2.4	141	122	22	35	30	38	512	543
ORC	Max	152	108	1.5	1.5	14	14	9.8	10.3	3.4	3.3	241	211	47	75	51	59	817	827
ORC	*p*, t-test[1]	**0.003**		**0.034**		0.114		0.095		0.500		**0.005**		**0.020**		0.079		0.109	
PCL	Mean	107	87	1.1	1.3	11	14	10.1	9.9	3.0	3.0	185	161	38	56	24	34	575	696
PCL	SD	18	8	0.2	0.2	2	3	0.7	0.6	0.4	0.3	47	35	17	17	7	15	70	67
PCL	Min	89	72	0.8	1.1	8	9	9.0	9.4	2.4	2.6	134	117	23	41	11	15	482	586
PCL	Max	134	98	1.3	1.6	13	17	10.9	10.9	3.5	3.4	253	195	70	88	30	59	660	774
PCL	*p*, t-test	**0.010**		**0.016**		**0.001**		0.320		0.316		0.068		0.056		0.052		**<0.001**	
None	Mean	109	100	1.2	1.7	13	12	10.3	9.9	2.9	3.1	163	163	37	33	50	46	637	743
None	SD	15	8	0.3	0.4	2	1	0.2	0.2	0.2	0.2	44	44	11	5	23	17	165	166
None	Min	95	90	0.8	1.3	10	10	10.1	9.6	2.6	2.8	134	110	20	28	25	33	444	521
None	Max	127	107	1.5	2.0	15	13	10.5	10.1	3.1	3.3	241	204	48	39	85	74	866	946
None	*p*, t-test	0.122		**0.001**		0.081		**0.016**		0.128		0.414		0.238		0.395		**0.015**	
	p, ANOVA[2]	0.922	0.258	0.708	0.074	0.282	0.300	0.105	0.968	0.808	0.899	0.637	0.971	0.672	0.058	**0.020**	0.212	0.668	0.384

Pre = pre-injury value; Fin = final value (just prior to euthanasia); SD = standard deviation; Min = minimum value; Max = maximum value; Gluc = glucose; creat = creatinine; AG = anion gap; Ca = calcium; Alb = albumin; AlkP = alkaline phosphatase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase; Amy = amylase. [1]Paired one tail t-test, comparing pre-injury vs. final; [2]one-way ANOVA, comparing ORC vs. PCL vs. None; **p < 0.05 are bolded**. Note: all total bilirubin values were ≤ 0.2 mg/dL, so these data were not listed here.

Table TT15. Subject weight, 30-day weight gain, and organ weights, all at necropsy from the 30-day toxicity study.

Material	Subject wt (kg)	Wt gain (kg)	Liver (g)	Heart (g)	Lungs (g)	Kidneys (g)	Brain (g)
ORC	50.6 ± 5.6 (44.8 – 59.0)	12.7 ± 2.7 (7.6 – 15.2)	1,178 ± 130 (1,080 – 1,423)	247 ± 21 (219 – 280)	328 ± 22 (295 – 346)	179 ± 21 (158 – 220)	79.3 ± 6.3 (73.0 – 91.0)
PCL	47.8 ± 1.1 (46.0 – 49.0)	12.1 ± 3.0 (7.2 – 15.0)	1,140 ± 70 (1,060 – 1,218)	230 ± 21 (217 – 273)	342 ± 29 (295 – 374)	173 ± 18 (151 – 194)	69.0 ± 11.6 (50.0 – 81.0)
None	43.7 ± 4.0 (38.4 – 47.6)	8.9 ± 3.1 (3.4 – 12.0)	914 ± 117 (776 – 1,067)	204 ± 20 (168 – 223)	297 ± 16 (272 – 313)	129 ± 25 (95 – 161)	69.2 ± 9.1 (57.7 – 81.4)
p, ANOVA (one way)*	**0.029**	0.086	**0.002**	**0.009**	**0.012**	**0.002**	0.121
p, Kruskal-Wallis*	0.051	0.064			**0.034**		0.188

Data are mean ± standard deviation, with range in parentheses; *comparing ORC *vs*. PCL *vs*. None; **p < 0.05 are bolded.**

I. OVERVIEW
Date: August 21, 2015
Swine no: 306
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Fibrin Sealant
Personnel: Carlson, Hansen, Siford, Ismail, Fabian, Spretz, Aravind (observing)

II. PRE-RESECTION PHASE

Start time: 07:50 AM
Swine sex: male
Date swine received from UNL Mead: 08/18/2015
Pre-procedure wt: 34 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 78
- MAP: 144
- Temp: 37.9
- EtCO2: 39

Blood draw no. 1 (initial): 8:05 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:10 AM
Spleen wt: 306.1gm
LR (22°C) infused after splenectomy: 900 mL at 150 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 306.1+ 30 + 2.8 = 338.9 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 50= 950 mL

Pre-resection VS
- HR: 92
- MAP: 126
- Temp: 37.5

- EtCO2 : 41

III. RESECTION & TREATMENT PHASE

Time resection began: 08:40AM, closed 8:48AM (t=0; 8:48 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen, and then resected at the level of the junction with the right medial lobe using the short curved single scissors (4 cm blade). Immediately after resection, the PCL bandage (pre-wet with FS while the lobe was being cut) was applied onto the bleeding resection site, and fibrin sealant was sprayed on top of the PCL until all the FS was consumed. I held pressure for 5 min (max hold time I will use in this protocol) after completion of the FS spray. After this 5 min hold, there was some ongoing hemorrhage because the bandage did not completely cover the resection site. The resected liver lobe was removed from the abdomen, and the the incision was rapidly closed with towel clips.
Bandage treatment: PCL, soft open-pore mesh, folded into dimensions of 5 x 15 cm (previously folded into dimensions of 6.7 x 10 cm)
Clotting factors: pdFI, rFII, rFXIII (252 mg FI, 10.08 mg FXIII, 2956.8 U thrombin, 0.34 mL CaCl2)
Abdominal closure: “fully-closed” technique, as described above
Resuscitation target MAP: 100
Resuscitation fluid: warm LR given at constant rate of 9.4 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the entire 60 min observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 0 + 0 + 132.3 (suction + clots + sponges) = 132.3 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 09:05 AM

15 min post-resection VS
- HR: 107
- MAP: 69
- Temp: 37.1
- EtCO2: 44
- IAP: 0
-

Blood draw no. 3 (30 min post-resection): 09:20 AM

30 min post-resection VS
- HR: 100
- MAP: 64
- Temp: 37.0
- EtCO2: 45
- IAP: 0

Blood draw no. 4: (Final; 60 min post-resection): 9:50 AM

Final (60 min) VS
- HR: 91
- MAP: 68

- Temp: 36.6
- EtCO2: 42

Survival at 60 min? Yes
Target MAP attained? No
Time of death: 9:55 AM
Cause of death: intentional exsanguination from euthanasia (transect supradiaphragmatic IVC)
Interval from completion of resection to death: 65 min

Post-treatment fluid data:

- Blood loss: 295.2 mL (suction) + 35mL (clot + lap pads) + 90 mL (phlebotomies) = 420.2 mL
- IV fluid given: LR (37°C): 700 mL + 0 mL (ear) + 0 (incidental) = 700 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, some clotted and nonclotted blood seen directly beneath the injury site (see Figs). Small amount active hemorrhage from resection site (see Figs). Bandage adherent to injury site, but there was an area on the site (in region of transected PV branch) that was not covered by bandage, and presumably where excessive blood loss occurred.
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 105.6 (resected LM lobe) + 904.9 (remaining liver) = 1010.5 g
Tissue harvested: Liver section from injury site for histology (bandage/liver capsule interface)

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage + FS. Total blood loss attributable to the injury was ~460 mL (130 mL during injury/treatment phase, and 330 mL during observation). The sealant set up very well, but today we again had an issue with incomplete coverage of the injury site by the bandage. When the bandage was 10 cm in longest dimension, I had difficulty making sure that both ends of the injury were covered. Today we used a bandage that was 15 cm in longest dimension (see Figs), which covered both ends of the injury without difficulty. The bandage was not adequate, however, in covering the breadth of the injury, because the new folding technique produced a final bandage of 5 cm in breadth. To ensure that I can get the injury covered in the "heat of the battle," I will need 1-2 cm of bandage overlap on all sides of the injury. For this purpose, I would suggest a final folded bandage size of 15 x 8 cm. So a triple-ply bandage starting out at 15 x 16 cm could be folded once to obtain a final bandage size of 15 x 8 cm (see Figs), with still enough plies to be hemostatic. So I think that if future triple-ply bandage were 15 x 16 cm prior to any folding, then this would minimize the risk that I would leave an area of the injury uncovered.

VII. PLAN

Perform next subject in this series on Fri Sep 28th, using PCL + FS, with larger PCL bandage.

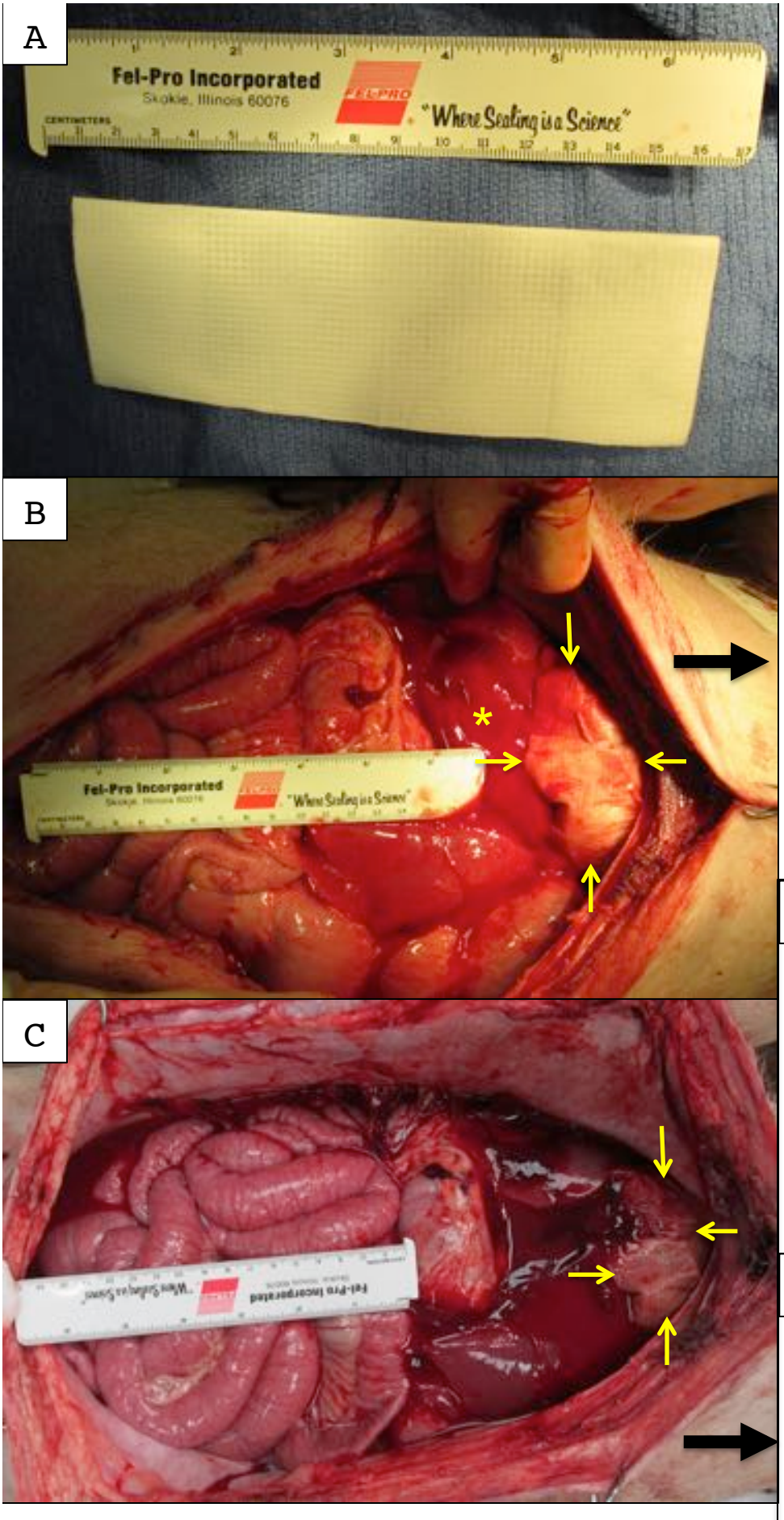


Figure 1, swine 306.
(A) PCL bandage prior to use. Bandage has been folded into dimensions of 5 x 15 cm (see panels D & E below).
(B) Injury site with bandage (yellow arrows) immediately after 5 min compressione time. Some limited hemorrhage (asterisk) still was evident after the 5 min compression. Black arrow = cephalad.
(C) View of upper abdomen & injury site (yellow arrows) immediately after 1 h observation period. Black arrow = cephalad.
(D) Prior schema to fold PCL bandage. Starts as a 20 x 10 cm triple-ply, folded into three layers of 6.7 x 10 cm.
(E) Bandage as folded & used in swine 306. Final folded dimensions = 5 x 15 cm.

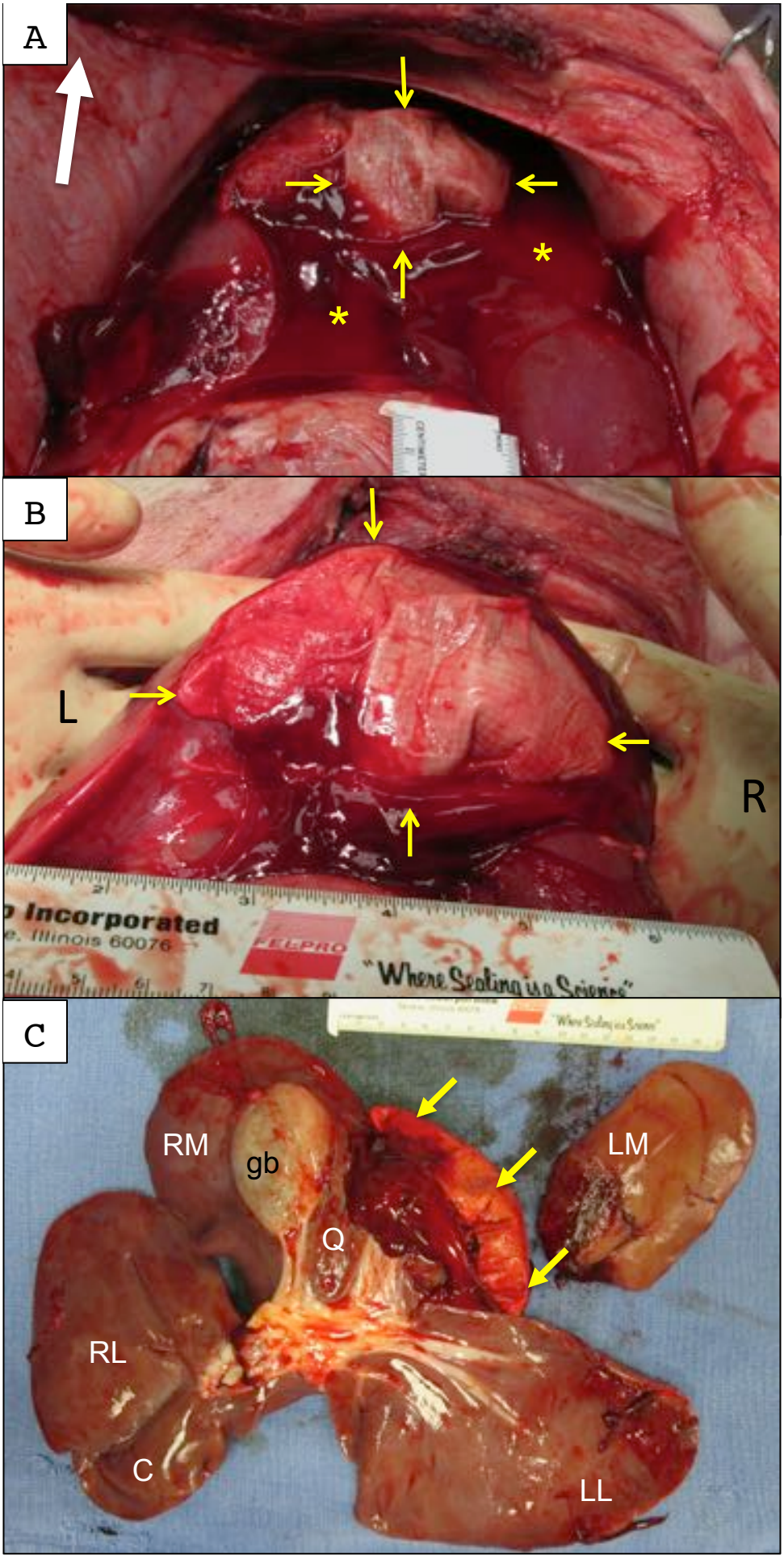


Figure 2, Swine 306.
(A) Another view of upper abdomen right after observation period. Blood has pooled inferior to injury (asterisk).
(B) Similar view as in panel A, except right (R) and left (L) hands are elevating liver up into wound to show injury site.
(C) Liver *ex vivo*, inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Intact bandage indicated by yellow arrows. Anterior is at top of image.

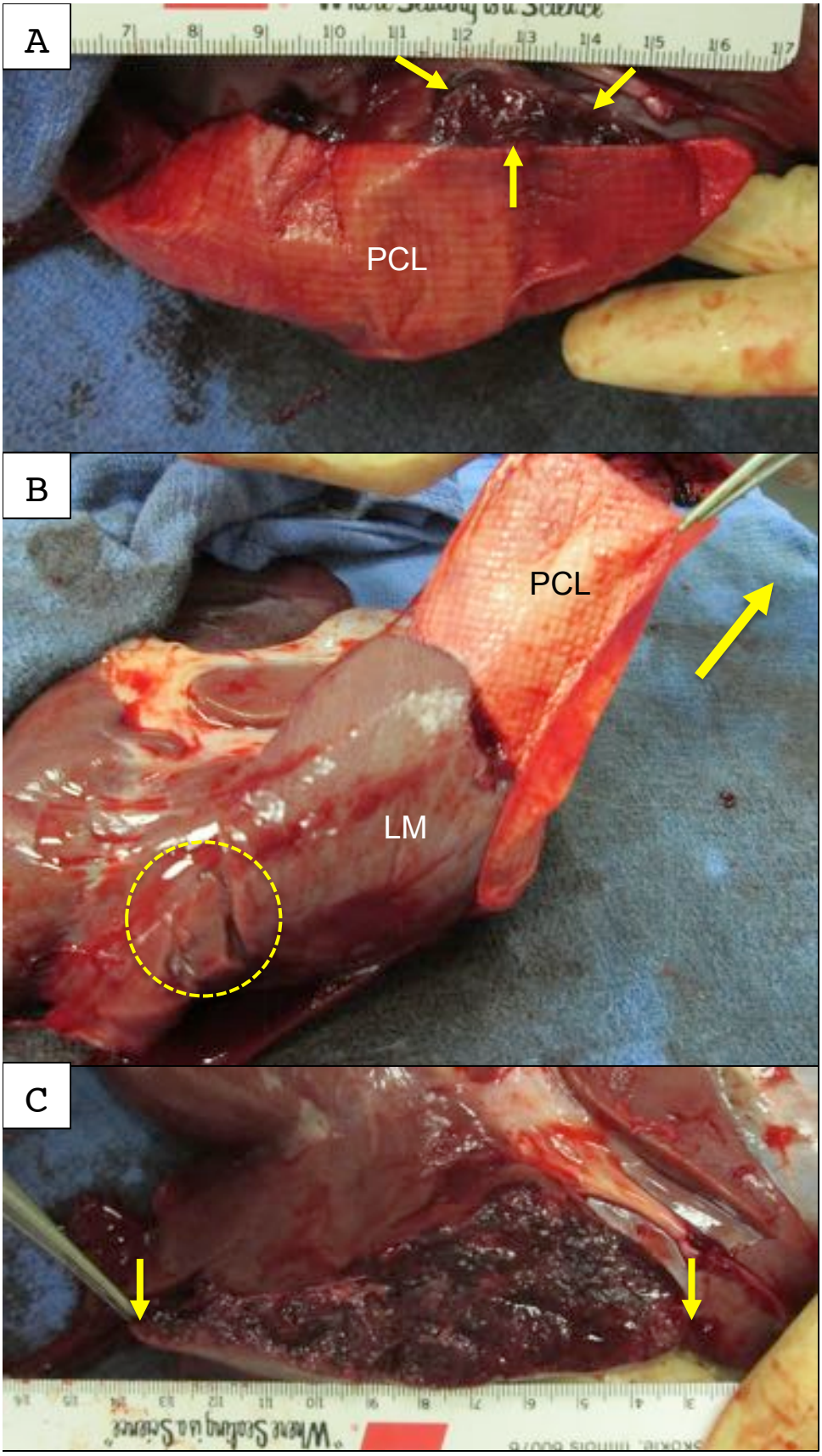


Figure 3, swine 306. Liver *ex vivo*.
(A) Close-up of PCL bandage on injury site. There was a section of the injury not covered by the bandage (yellow arrows).
(B) Peel-off of PCL bandage from LM injury site, in direction of yellow arrow. Only capsule exposed at this particular instant. Dashed circle: site of biopsy to illustrate PCL-capsule interface.
(C) Close-up of injury site (base of LM lobe) after removal of bandage and clot. Superior is at bottom of image. Length of injury site was ~11 cm (distance between two yellow arrows); breadth ~4 cm. Venous injuries not obvious, but HV x 1 and PV x 1.

I. OVERVIEW
Date: August 25, 2015
Swine no: 307
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate foam without clotting factors
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-INJURY PHASE

Start time: 07:48 AM
Swine sex: Male
Date swine received from UNL Mead: 8/18/2015
Pre-procedure wt: 34.0 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS
- HR: 87
- MAP:126
- Temp: 38.2
- EtCO2:50

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:08AM
Spleen wt: 320.7 gm
LR (22°C) infused after splenectomy: 950 mL at 150 mL/min

Pre-injury fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 320.7 + 30 + 5.9 = 356.6 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 60 (ear vein/jugular drip)= 1,010.0 mL

Pre-injury VS
- HR: 83
- MAP: 122

- Temp: 37.3
- EtCO2 : 48
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:27 AM

Injury type: Hepatic left medial lobectomy, nonanatomical (see Figures). The left medial lobe of the liver was transected at its base (at the level of the junction with the right medial lobe) with scissors, producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle (see Figs) was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the mid abdomen.

Treatment formulation: Sodium alginate foam 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: None.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision. The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. Gas was continually vented out of the abdomen during injection, to maximize the foam component of the injected material. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. IAP maxed at 25 mm Hg during the injection.

Total mass injected: 374.9g alginate foam; only 20 mL of 1 M $CaCl_2$ (should have been ~80 mL).

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: 8:45AM (~ 3 min after injury).

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 8:40 AM

10 min post-injury VS

- HR: 104
- MAP: 19
- Temp: 37.4
- EtCO2: 11
- IAP: 11

Survival at 180 min? No

Target MAP attained ? No

Time of death: 8:45AM

Cause of death: gas/foam embolism and exsanguination

Interval from injury to death: 18 min

Post-treatment fluid data:

- Blood loss: 523.7 mL (suction) + 0 mL (sponge/lap) + 620.6 mL (clots) + 0 mL (foam) + 60 mL (phlebotomies) = 1,204.3 mL
- IV fluid given: LR (37°C): 150 mL (infused) + 0 mL (ear vein) + 0 mL (jugular drip) = 150 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen distended; moderately tense (IAP ~ 11 mm Hg). Death occurred within 18 minutes post-injury. Upon re-opening abdomen, foam by itself was gelatinous and mostly in the superficial region; there was no stiffness to the foam. Large amounts of unclotted blood and clots were seen (see Figs). Injury site was not covered by anything except a very thin layer of fibrin. Subsequently chest was opened and explored to determine the cause of death. +++Foam and gas bubbles were seen in the right ventricle and vena cava (see Fig).

Volume foam recovered: Not measured
Heart: +++gas/foam in RV
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 114.9g (resected LM lobe) + 777.8 (remaining liver) = 892.7 g
Tissue harvested: none.

VI. COMMENTS

Noncompressible injury treated with calcium alginate foam without clotting factors. Survived 18 minutes; rapid death 2˚ to gas/foam embolism, but also hefty blood loss of 1,150 mL. We have not had a embolic death for a long time. My seat of pants impression is that foam is injecting too quickly, i.e., quicker than what I have experienced in the recent past. The abdomen fills up in what seems to be several seconds... that's a relatively vigorous, almost violent rate of abdominal filling. Makes me wonder if that is the cause of embolism. Also, foam stiffness was not adequate today, presumably from inadequate calcium… subject only received about 25% of the $CaCl_2$ that it should have received. Syringe pumps unable to keep up with rapid injection rate? I wonder if we would have better results if the injection could be slowed down so that it would occur during a 45-60 s interval.

VII. PLAN

Continue with this series (Ca alginate without clotting factors) on Thu Sep 27th.

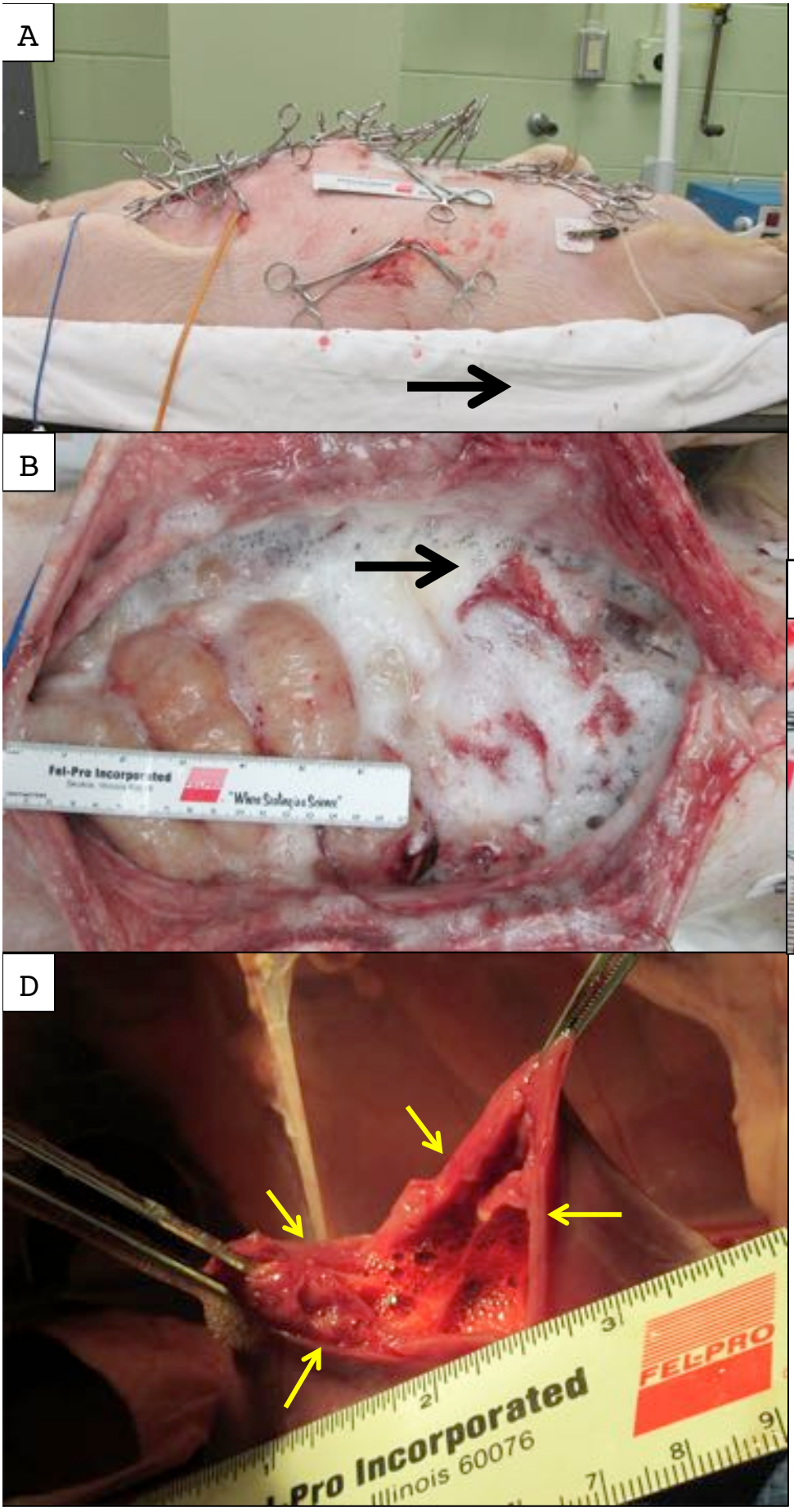


Figure 1, swine 307.
(A) Lateral view of swine, right after expiration at 18 min post-injury. Black arrow = cephalad.
(B) Abdomen re-opened after expiration. White foam with consistency of soap suds (no stiffness). No surface changes to viscera. Abdominal distension was secondary to butane carrier gas, and not foam.
(C) Close-up of foam removed from abdomen
(D) Thoracotomy and right ventriculotomy of heart at necropsy, demonstrating foam embolism (arrows) within the right ventricle.

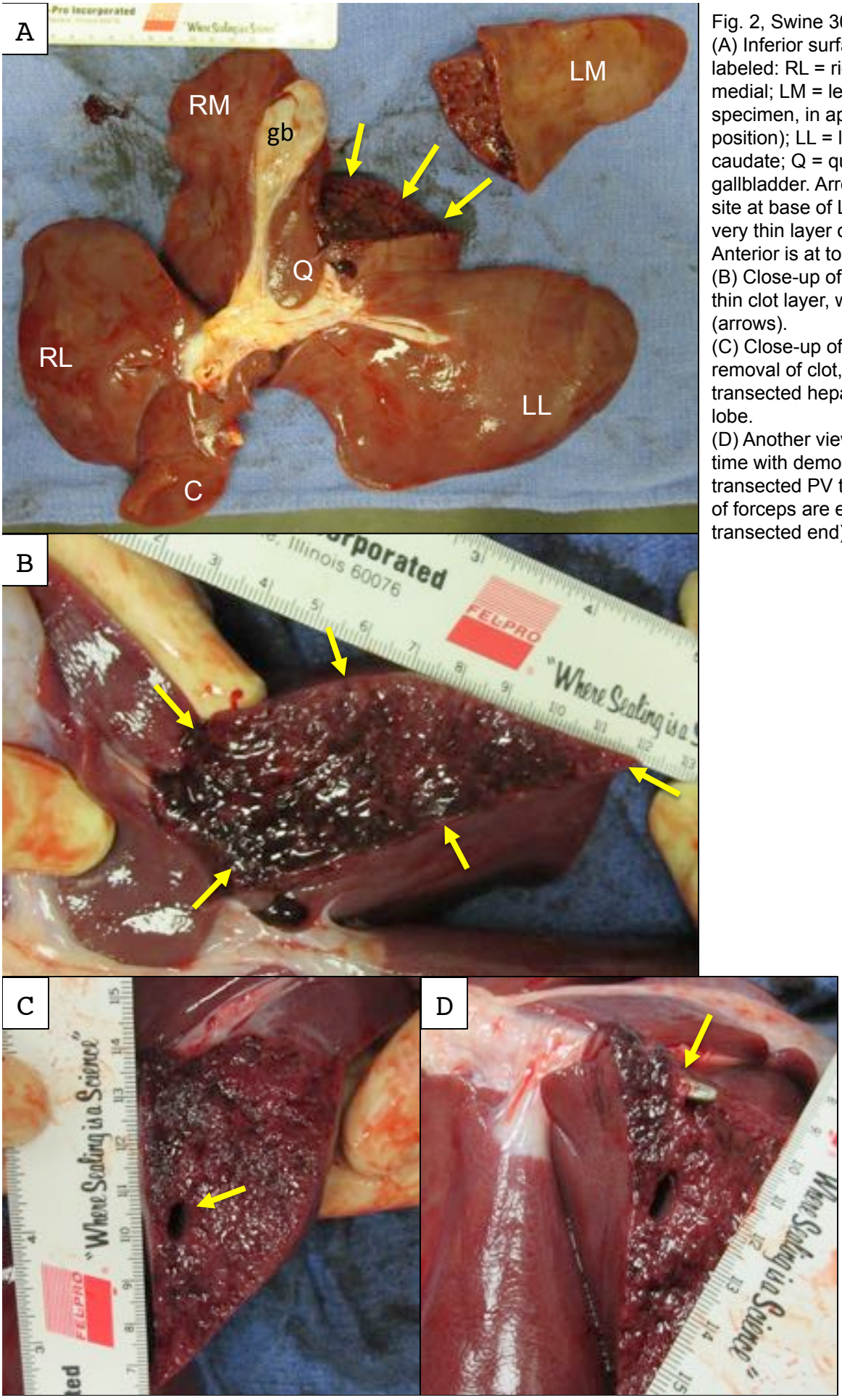


Fig. 2, Swine 307. Liver *ex vivo*.
(A) Inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, covered with very thin layer of fibrin, but no foam. Anterior is at top of image.
(B) Close-up of injury site with very thin clot layer, without adherent foam (arrows).
(C) Close-up of injury site after removal of clot, with demonstration of transected hepatic vein (arrow) to LM lobe.
(D) Another view of injury site, this time with demonstration of transected PV to LM lobe (arrow; tips of forceps are emerging from transected end).

I. OVERVIEW
Date: August 27, 2015
Swine no: 308
IACUC Protocol no. 00760
Model: swine, normothermic, normovolemic noncompressible hemorrhage; left medial lobe resection
Treatment: calcium alginate foam alone, no clotting factors
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-INJURY PHASE

Start time: 08:10 AM
Swine sex: Male
Date swine received from UNL Mead: 8/18/2015
Pre-procedure wt: 35.4 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject
10. Intraabdominal pressure monitor

Initial VS

- HR: 118
- MAP:99
- Temp: 36.9
- EtCO2:not recorded/wrongly recorded due to equipment failure

Blood draw no. 1 (initial): 8:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 8:35AM
Spleen wt: 219.6 gm
LR (22°C) infused after splenectomy: 650 mL at 150 mL/min

Pre-injury fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 219.6+ 30+ 0 = 249.6 mL
- LR (22°C) infused (spleen replacement + incidental): 650 + 35(ear vein/jugular drip)= 685.0 mL. Note: subject became hypotensive during 150 mL/min infusion of splenic replacement fluid, so infusion was temporarily stopped (1-2 min), and then restarted at 50 mL/min with no further incident.

Pre-injury VS

- HR: 81
- MAP: 106
- Temp: 35.9
- EtCO2 : 33
- IAP: 0

III. INJURY & TREATMENT PHASE

Time of injury: 8:50 AM

Injury type: Hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was transected at its base with scissors (at the level of the junction with the right middle lobe), producing a combined portal/hepatic venous injury. Prior to the injury, the lower half of the ventral midline incision was closed with towel clips. The line to the IAP monitor exited through the superior end of the midline incision. The single injector nozzle was inserted through the left lateral side of the abdomen to reduce air leak, with the tip initially directed into the mid abdomen.

Treatment formulation: Sodium alginate foam 3.8 %; no xanthan gum; Tween 20 = 0.6%; 1.14 M $CaCl_2$ (21 mL/min x 4 syringe injectors).

Clotting factors: none.

Technique: (see Figs) with the lower half of the incision closed with towel clips, the target liver lobe (left medial) was exteriorized through the upper half of the midline incision (see Figures). The injury then was created as described above. Immediately after injury, the resected liver lobe was removed from the abdomen, and the upper half of the incision was rapidly closed with towel clips. Injection of the foam + FS began 1 min after injury, after the abdomen had been closed with clips. The position of the nozzle was continually and slowly adjusted around to all four quadrants of the abdomen. Injection was quite vigorous, and abdomen visually distended within several seconds. IAP momentarily reach 32 mm Hg during injection, then decreased to <10 over the next several min.

Total mass injected: 274.7g alginate foam.

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 80

Resuscitation fluid: warm LR, 1.8 L preset maximum (50 mL/kg), given at constant rate of 10.3 mL/min, continuously during the entire 180 min observation period, or until animal expires. Formula for IVF rate in hypotensive resuscitation protocol = (Subject wt in kg) x (50 mL/kg) ÷ 180 min; begin at T + 1 min (T = time of injury) and continue for 180 min or until subject expires.

Time resuscitation fluid began: ~3 min post-injury

IV. POST-TREATMENT PHASE

Blood draw no. 2 (10 min post-injury): 9:00 AM

10 min post-injury VS

- HR: 107
- MAP: 32
- Temp: 35.7
- EtCO2: 14
- IAP: 08

Survival at 180 min? No
Target MAP attained ? No
Time of death: 9:05AM
Cause of death: gas/foam embolism
Interval from injury to death: 12 min

Post-treatment fluid data:

- Blood loss: 534.4 mL (suction) + 38.5 mL (sponge/lap) + 378.8 mL (clots) + 205.2 mL (foam) + 60 mL (phlebotomies) = 1,216.7mL (~950 mL from injury)
- IV fluid given: LR (37°C): 100 mL (infused) + 0 mL (ear vein) + 0mL (jugular drip) = 100 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

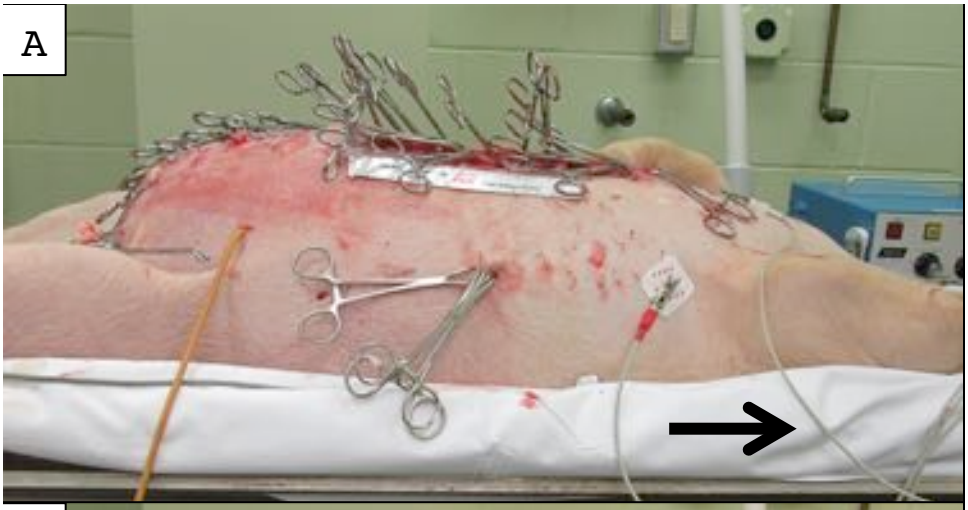
Findings upon abdominal/chest exploration: abdomen modestly distended; not very tense (IAP <10 mm Hg). Death occurred within 12 minutes post-injury. Upon re-opening abdomen, foam by itself was somewhat firm, not the best we have seen, but reasonable (see Figs), all in the superficial region of the abdomen. Moderate amounts of unclotted blood and clots were seen underneath. Injury site was not covered with any adherent clot or foam, except a very thin film of fibrin. Thoracotomy and ventriculotomy performed to determine the cause of death. Foamy gas bubbles were seen pouring out of the right ventricle (see Fig).
Volume foam recovered: ~500 mL
Heart: large amount gas/foam embolism in RV
Number of hepatic veins lacerated: 1, to LM lobe.
Portal vein injury: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 76.1g (resected LM lobe) + 714.5 (remaining liver) = 790.6 g
Tissue harvested: none.

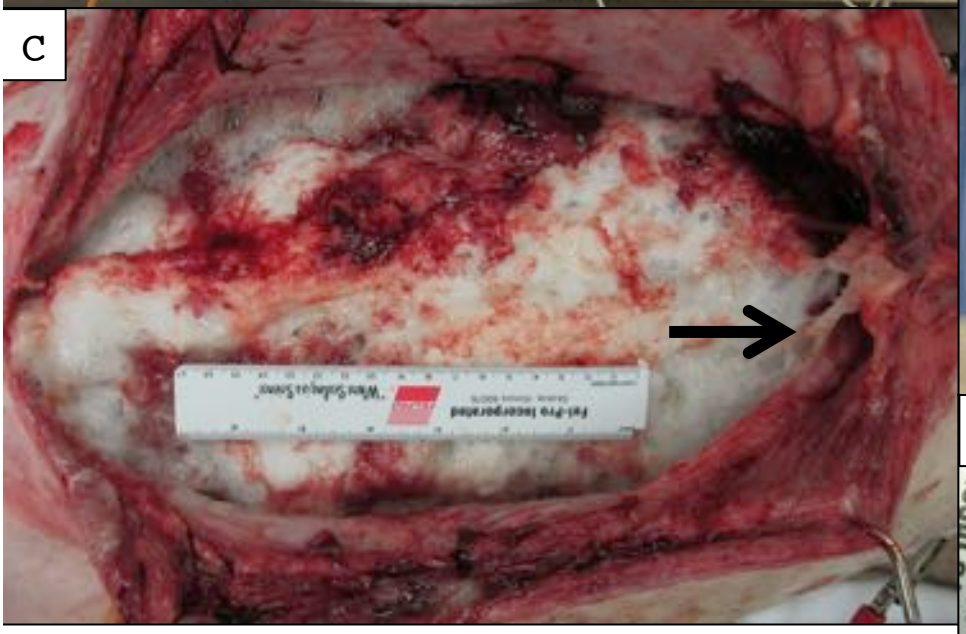
VI. COMMENTS

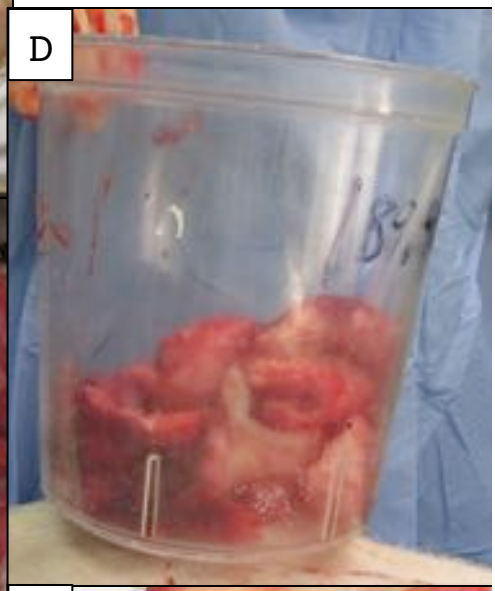
Noncompressible injury treated with calcium alginate foam without clotting factors. Foam was stiffer today, but not as stiff as we have seen previously. Dead at 12 minutes from gas/foam embolism. Last two subjects have died from gas/foam embolism… I doubt this is a coincidence. I am concerned that the rate of alginate injection is too fast, putting subject at risk for gas/foam embolism, and also not allowing calcium alginate reaction to produce stiff foam. Note that IAP drops rapidly as soon as injection stops… this suggests that foam is not adequately supporting IAP, that brief IAP elevation mostly due to injected butane. When we opened, there was only small (500 mL) volume of foam… not really enough to have for tamponade effect.

VII. PLAN

I think we need to put a hold on the foam experiments until we have a discussion regarding the foam delivery system, recent results, etc. While we have obtained some very interesting results recently, none of the foam subjects in the past month can be used as data points for our current study secondary to various technical issues.







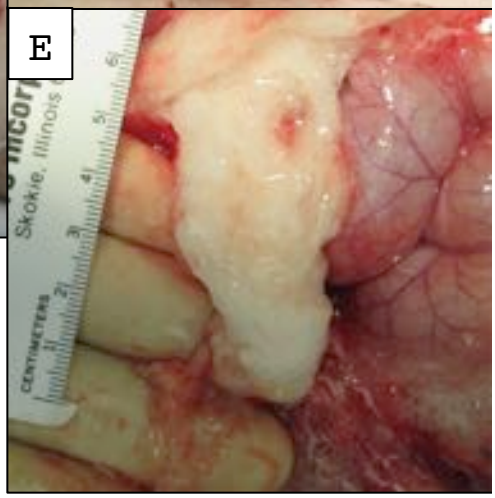


Figure 1, swine 308.
(A) Lateral view of swine, right after injection, 3 min post-injury. Black arrow = cephalad.
(B) Lateral view, at time of death (12 (12 min after injury).
(C) Overhead view of abdomen reopened after expiration.
(D) Intraabdominal foam retrieved in a 2 L canister (~500 mL foam).
(E) Close-up of foam. This iteration was stiffer than in subject #307.

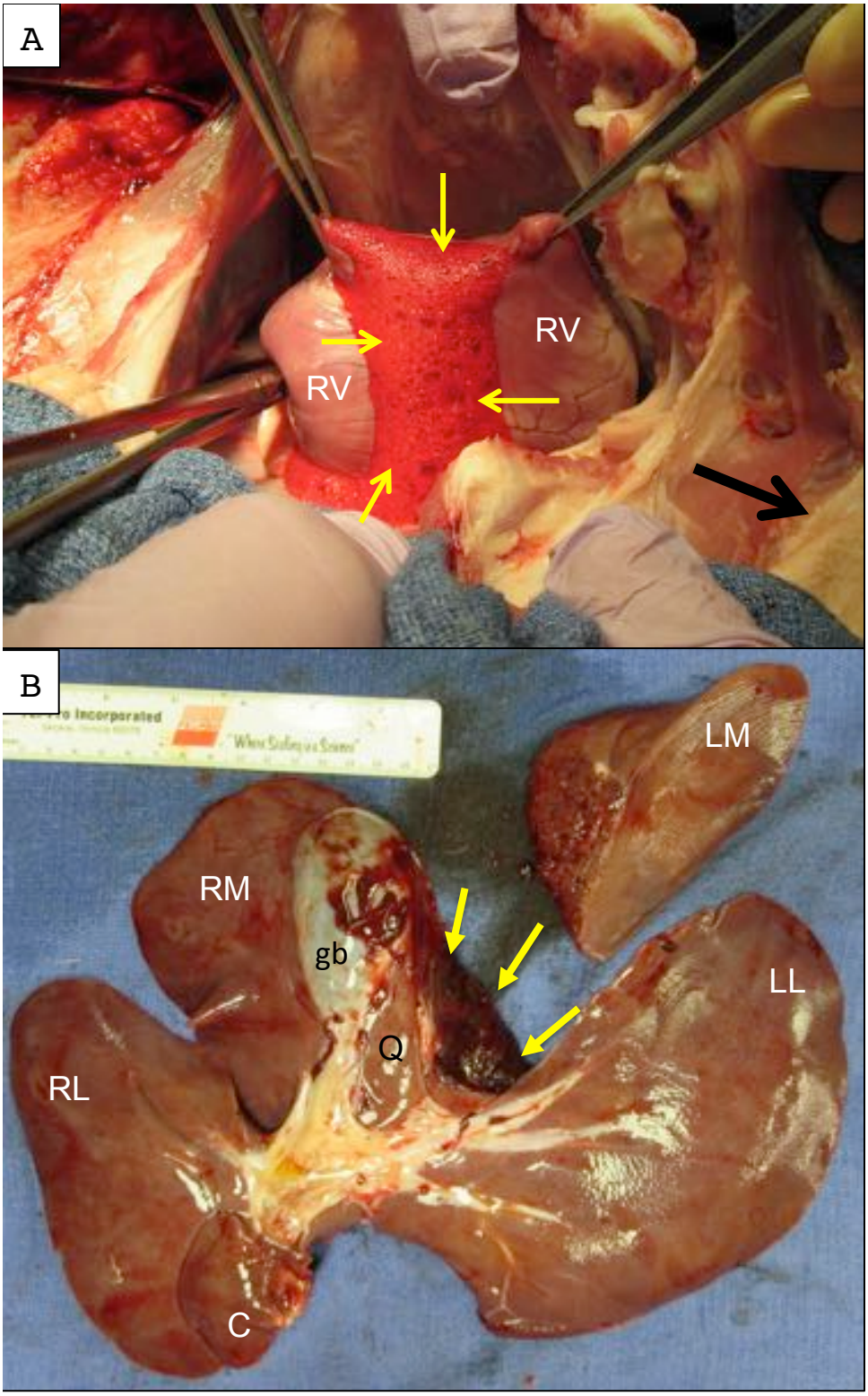


Fig. 2, Swine 308, necropsy.
(A) Thoracotomy and right ventriculotomy of heart, demonstrating gas/foam embolism (yellow arrows) pouring out of the right ventricle (RV). Black arrow = cephalad.
(B) Liver *ex vivo*, inferior surface. Liver lobes labeled: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Arrows = resection/injury site at base of LM lobe, covered with very thin layer of fibrin, but no foam. Anterior is at top of image.

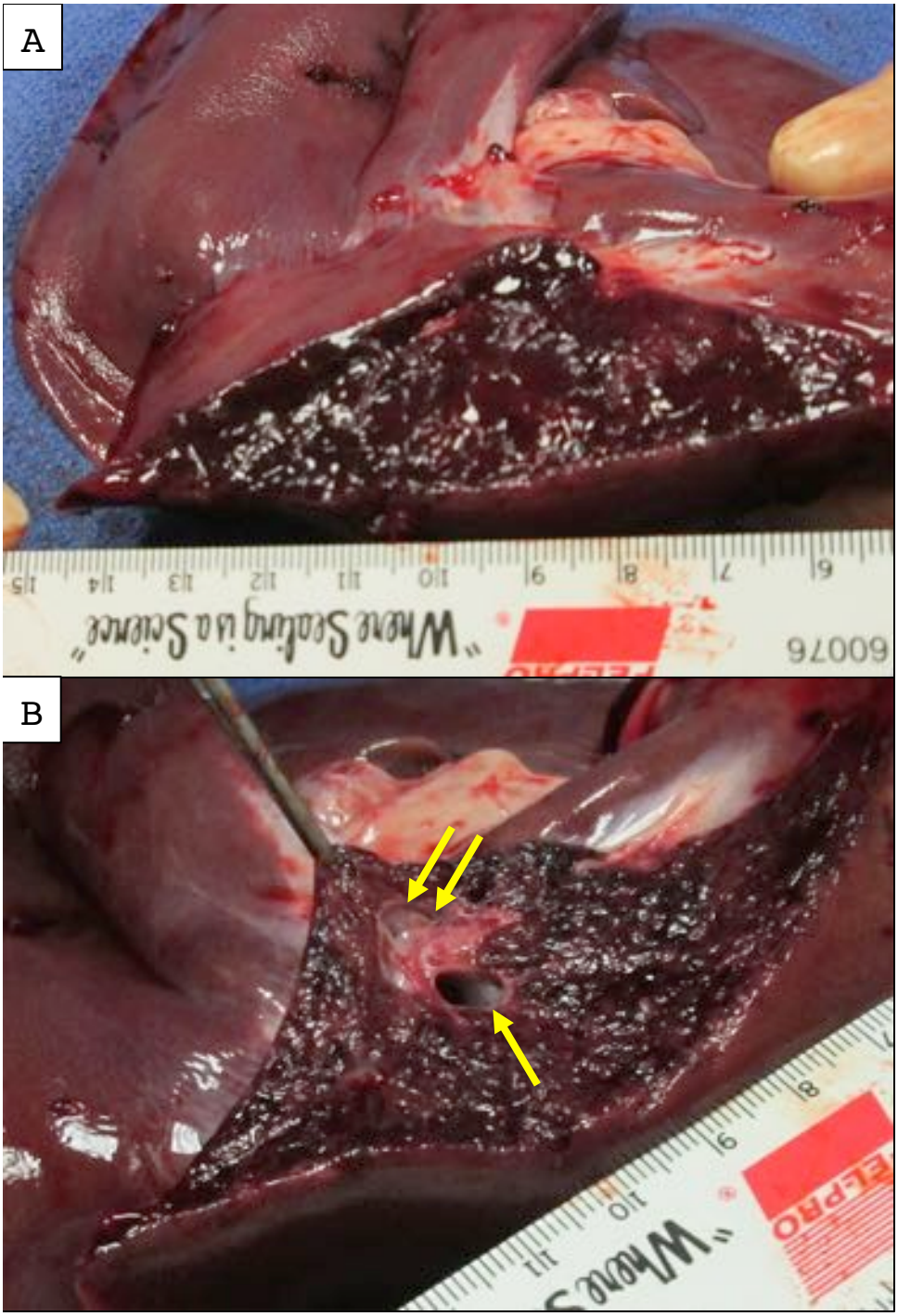


Fig. 3, swine 308. Liver *ex vivo*. (A) Close-up of injury site with very thin clot layer, without adherent foam. (B) Close-up of injury site after removal of thin clot layer, with demonstration of transected hepatic vein (arrow) to LM lobe and transected PV branch (double arrow).

I. OVERVIEW
Date: August 28, 2015
Swine no: 309
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Fibrin Sealant
Personnel: Carlson, Hansen, Siford, Fabian, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:45 AM
Swine sex: male
Date swine received from UNL Mead: 08/18/2015
Pre-procedure wt: 35 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 107
- MAP: 91
- Temp: 37.1
- EtCO2: 35

Blood draw no. 1 (initial): 8:20 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:30 AM
Spleen wt: 224.2gm
LR (22°C) infused after splenectomy: 700 mL at 150 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 224.2+ 30 + 2 = 256.2 mL
- LR (22°C) infused (spleen replacement + incidental): 700 + 10= 710 mL

Pre-resection VS
- HR: 101
- MAP: 115
- Temp: 36.2

- EtCO2 : 39

III. RESECTION & TREATMENT PHASE

Time resection began: 08:43AM, closed 8:58AM (t = 0; 8:48 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized through the midline incision, and then resected using the short curved single scissors (4 cm blade). The level of resection was at the junction of the LM lobe with the RM lobe. During resection and while applying the bandage + FS, the left hand pinched the base of the LM lobe to stanch blood loss. Immediately after resection, the PCL bandage (pre-wet with FS while the lobe was being cut) was applied onto the bleeding resection site, and fibrin sealant was sprayed on top of the PCL until all the FS was consumed. I held pressure for 5 min (max hold time I will use in this protocol) after completion of the FS spray. This was done with a bimanual technique (see Figs), with the left hand over the liver dome, and the right hand against the bandage on the injury site. After this 5 min hold, it was noted that the medial corner of the injury site was not covered with bandage, and was bleeding freely. I attempted to re-position the bandage so that all the injury site was covered, but I could not get the bandage to get secondary adherence. The subject continued to bleed profusely from the injury site during this secondary application, and I could never obtain hemostasis. The subject essentially exsanguinated during the treatment phase. We did manage to get the abdomen closed with towel clips and observe for a short period of time, but the MAP was 20 by this time, and the subject did not survive much longer.
Bandage treatment: PCL, triple ply soft open-pore mesh, 16 x 16 cm starting size, 8 x 16 cm size after one fold (as used on the injury)
Clotting factors: pdFI, rFII, rFXIII (252 mg FI, 10.08 mg FXIII, 2956.8 U thrombin, 0.34 mL CaCl2)
Abdominal closure: “fully-closed” technique, as described above
Resuscitation target MAP: 90
Resuscitation fluid: warm LR given at constant rate of 9.4 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 248.9+ 526.5 (suction + clots + sponges) = 775.4 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (12 min post-resection): 09:00 AM

12 min post-resection VS
- HR: 80
- MAP: 7
- Temp: 35.9
- EtCO2: 9

Survival at 60 min? No
Target MAP attained? Very briefly
Time of death: 9:05 AM
Cause of death: intra-operative blood loss
Interval from completion of resection to death: 12 min

Post-treatment fluid data:
- Blood loss: 269.7 mL (suction) + 548.9mL (clot + lap pads) + 60 mL (phlebotomies) = 878.6 mL
- IV fluid given: LR (37°C): 150 mL + 100 mL (ear) + 0 (incidental) = 250 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, blood and clot surrounding injury site (see Figs). Bandage not adherent to injury site (see Figs).
Heart: not examined.
Number of hepatic veins transected: 2, to LM lobe.
Portal vein(s) transected: 2, to LM lobe
Other: none
Ex vivo liver wt: 147.2 (resected LM lobe) + 682.1 (remaining liver) = 829.3 g
Tissue harvested: none

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage + FS. The sealant set up fine, but secondary to technical error, the bandage did not cover the entire injury site. A secondary application of the bandage failed, because the bandage did not have any ability to re-adhere after the initial application. The bandage interstices became filled with fibrin and clot, and the bandage surface subsequently was very slick/slippery, sliding all over the place and not sticking to anything (see Figs). So this subject died from blood loss during the resection, a first for this type of experiment.

My current iteration of model may allow us to collect reproducible data because it is too difficult to get good reproducible bandage placement on the relatively large injury area that has ongoing high-flow bleeding. So I will decrease the injury size in hopes of getting a compressible solid organ planar injury model that will have a greater technical reproducibility.

One issue that has become apparent with the past three subjects is that if bandage adherence is not obtained perfectly during the first several seconds of application, then there really is no 2nd opportunity for bandage application, because the bandage becomes unusable. After the bandage interstices have been filled with fibrin, the bandage becomes so slippery and non-adhering that it is pretty much useless. Perhaps a bandage re-design which incorporates 3D surface appendages ("hooks" or "cleats") that aid in adherence and prevent slippage (analogous to the self-gripping hernia meshes that have appeared on the market in past decade) would improve the performance of the hemostatic PCL mesh in this respect. For the next several subjects we will try using plain PCL without exogenous factors to see if the mesh "slipperiness" is affected. But I suspect if I make the injury smaller, with less hemorrhage flow, then I should have a better chance at getting bandage-wound adherence on the first try.

VII. PLAN

Perform the next subject in this protocol, using plain PCL without clotting factors, on Wed Sep 2nd.

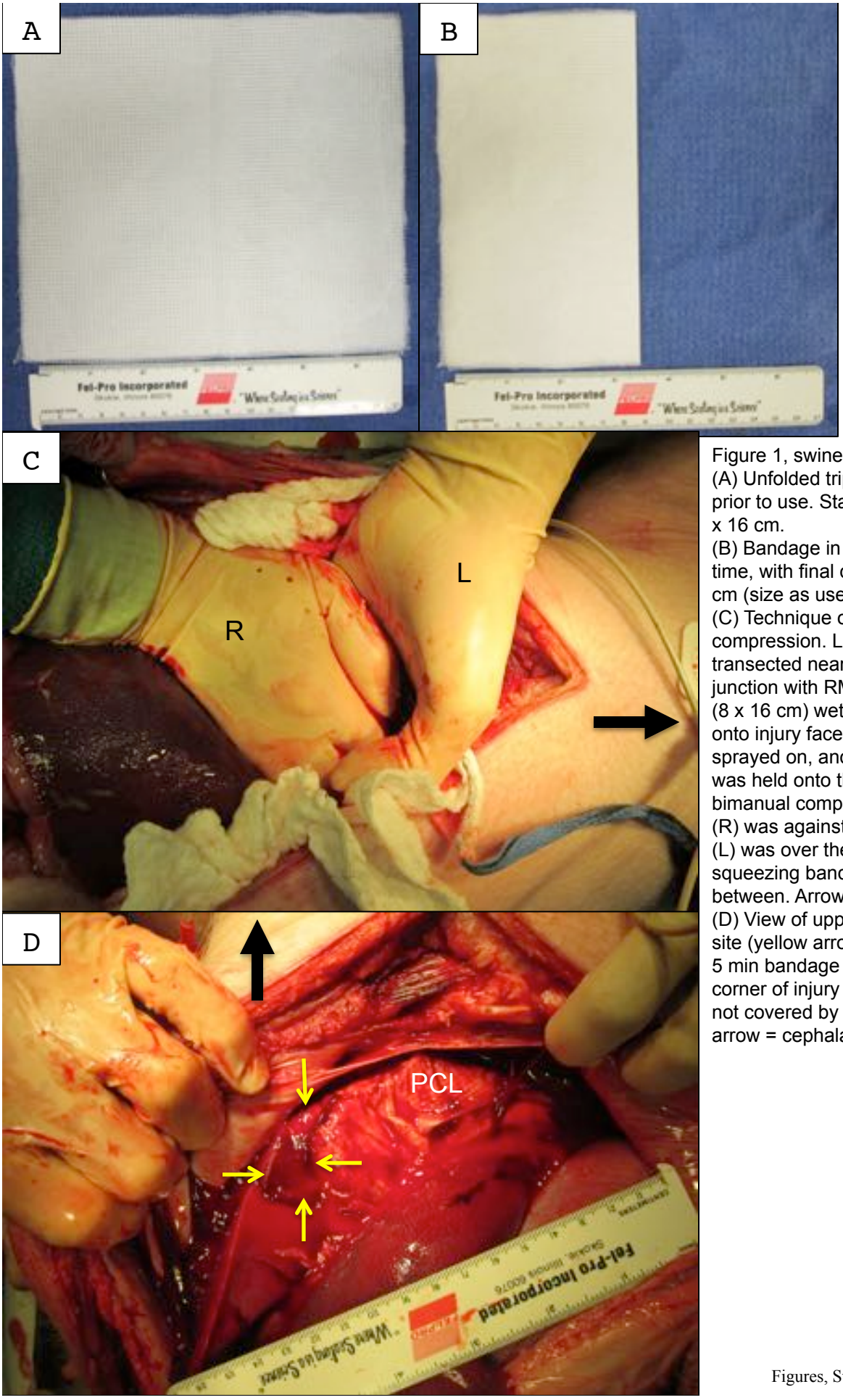
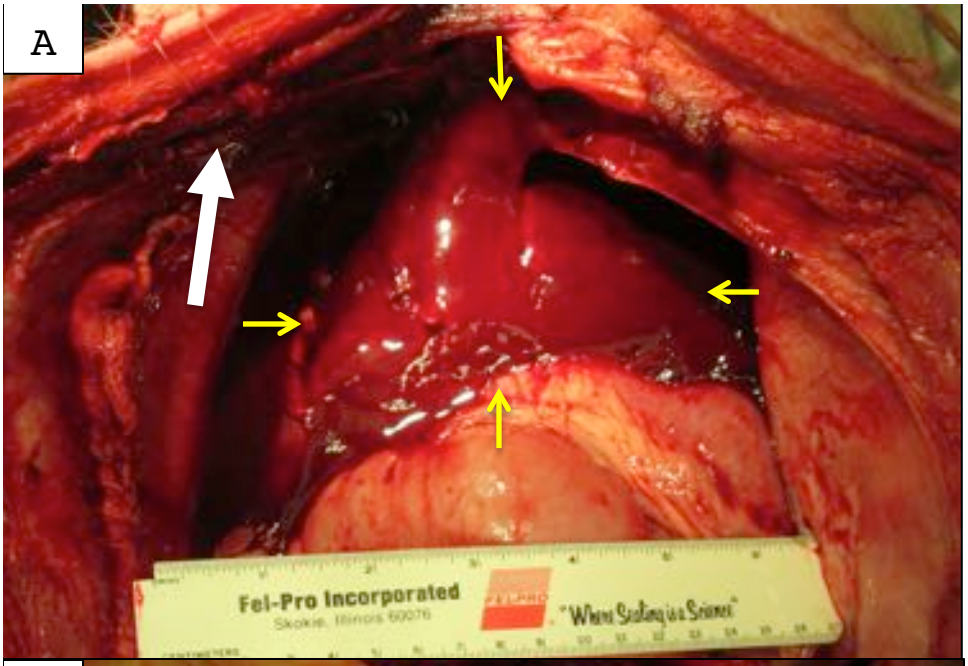
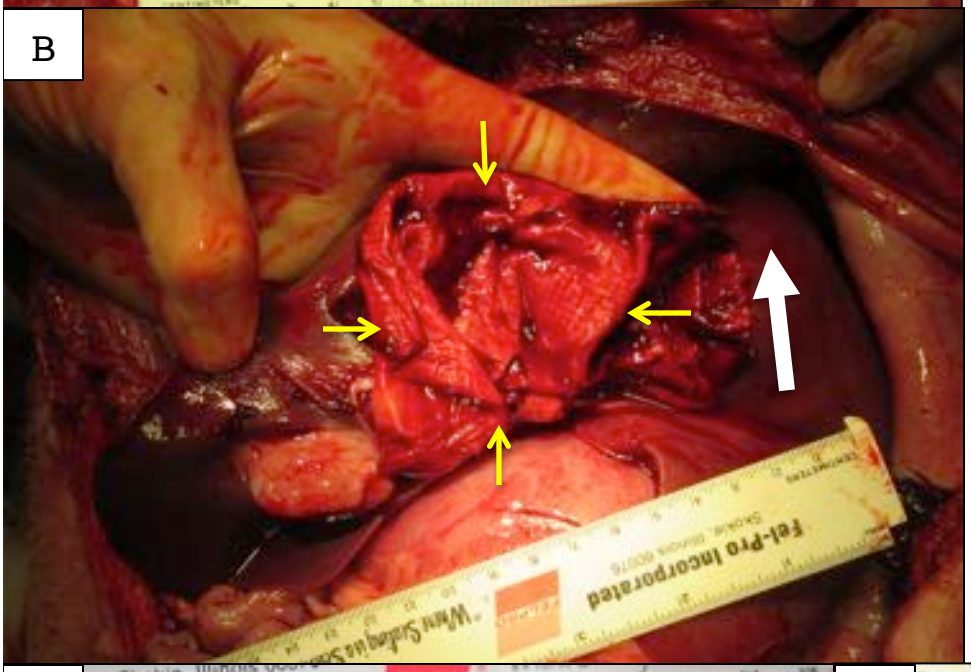
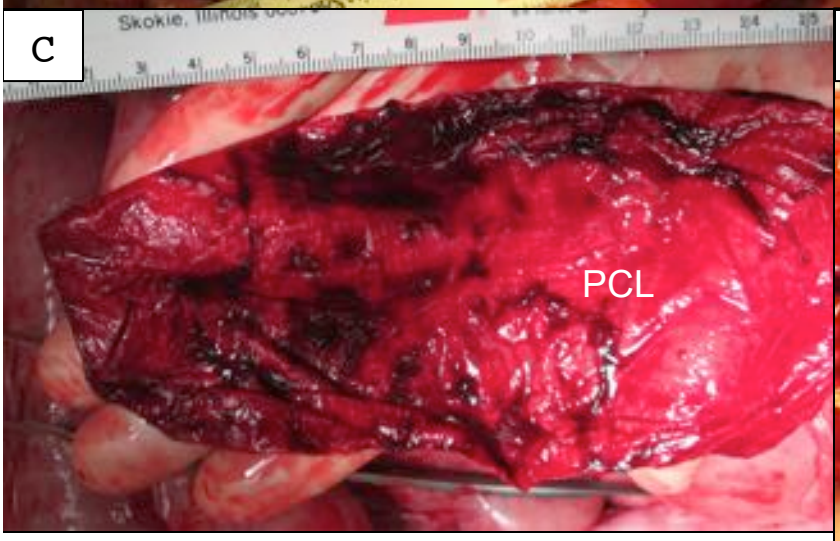


Figure 1, swine 309. Injury & Rx.
(A) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 16 x 16 cm.
(B) Bandage in panel A folded one time, with final dimensions of 8 x 16 cm (size as used *in vivo*).
(C) Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) wet with FS was applied onto injury face, further FS was sprayed on, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was over the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad.
(D) View of upper abdomen & injury site (yellow arrows) immediately after 5 min bandage compression. Medial corner of injury (yellow arrows) was not covered by PCL bandage. Black arrow = cephalad.







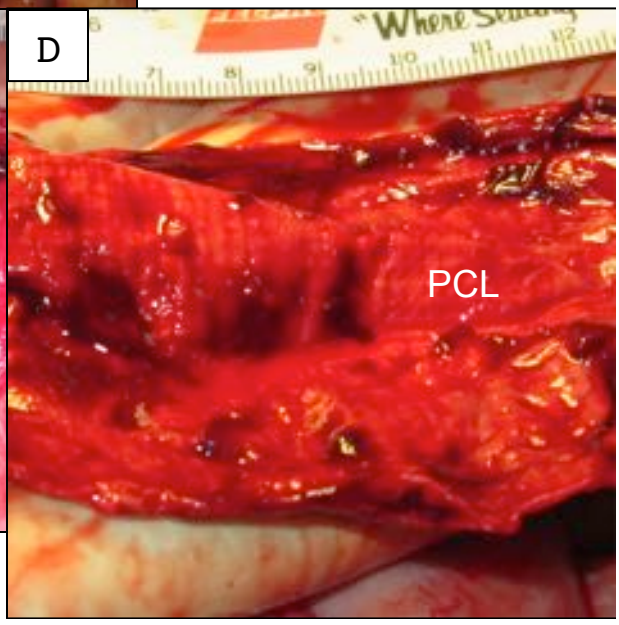


Figure 2, Swine 309. Necropsy
(A) View of upper abdomen immediately after expiration. Injury site (small yellow arrows) is inundated with fresh blood. Large white arrow = cephalad.
(B) Similar view as in panel A, except that blood and clot have been removed to reveal injury site. PCL bandage (asterisks) was not able to adhere during secondary application, and has appearance of crumple up piece of paper, sitting ontop of injury site with no adherence.
(C) Close-up views of PCL bandage after removal and uncrumpling, side visible is that which was against the injury site. The interstices of the bandage were filled with fibrin clot, and the surface was very slick and mucousy, not likely to stick to anything.

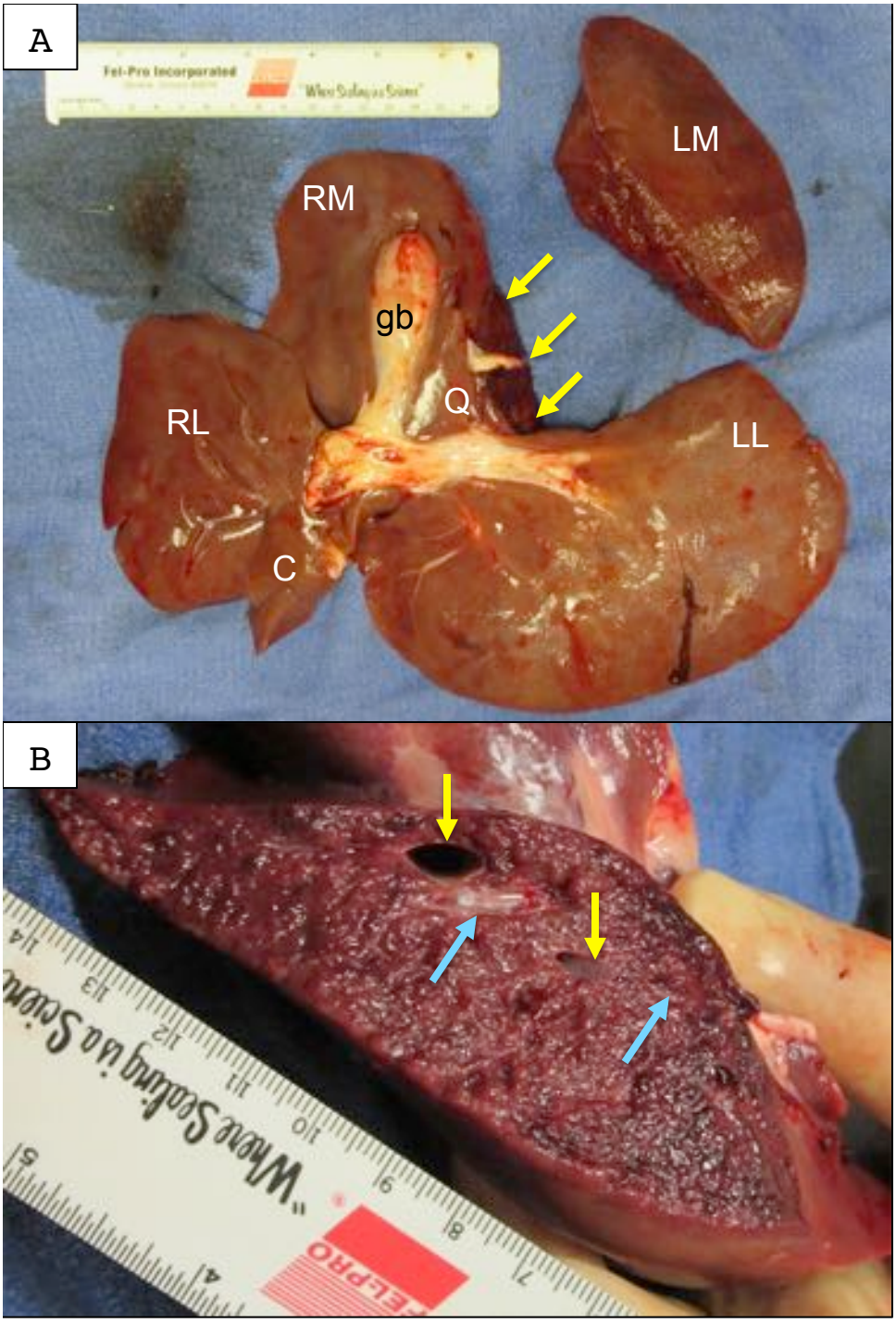


Figure 3, Swine 309. Liver *ex vivo*. (A) Liver inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Injury site only covered with thin film of fibrin clot.
(B) Close-up of injury site, with fibrin clot wiped away. There were two hepatic vein branches (yellow arrows) and two PV branches (blue arrows) that were transected. Further images on file.

I. OVERVIEW
Date: September 2, 2015
Swine no: 310
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, distal LM liver lobe resection
Treatment: PCL Bandage (16 x 16 cm) without biologics
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:15 AM
Swine sex: male
Date swine received from UNL Mead: 08/26/2015
Pre-procedure wt: 34.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 81
- MAP: 93
- Temp: 38.4
- EtCO2: 54

Blood draw no. 1 (initial): 7:25 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 07:32 AM
Spleen wt: 270.1gm
LR (22°C) infused after splenectomy: 800 mL at 50 mL/min (A drop in the MAP prompted us to run the fluid at a slower rate)

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 270.1+ 30 + 3.4 = 303.5 mL
- LR (22°C) infused (spleen replacement + incidental): 800 + 460= 1,260 mL

Pre-resection VS
- HR: 110
- MAP: 72

- Temp: 36.8
- EtCO2 : 56

III. RESECTION & TREATMENT PHASE

Time resection began: 08:21AM, closed : 9:30AM (t = 0 began at 8:21 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. Prior to the resection, the lower half of the ventral midline incision was closed with towel clips. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and line of resection on the LM lobe 3 cm from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the left hand, in order to minimize procedural blood loss. The resected liver lobe was removed from the abdomen. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was applied onto the bleeding resection site without any fibrin sealant. I held pressure for 5 min, compressing the bandage to the injury with bimanual compression (see Figs). After compression, the shed blood was carefully collected with a lap pad, and the incision was closed with towel clips.
Bandage treatment: PCL, open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 60
Resuscitation fluid: warm LR given at constant rate of 9.6 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 0 + 0 + 99.5 (suction + clots + sponges) = 99.5 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:45 AM

15 min post-resection VS
- HR: 81
- MAP: 83
- Temp: 38.4
- EtCO2: 51
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 9:30AM
Cause of death: Exsangunation from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 mins

Post-treatment fluid data:
- Blood loss: 67 mL (suction) + 0 mL (clot + lap pads) + 60 mL (phlebotomies) = 127 mL
- IV fluid given: LR (37°C): 350 mL + 0mL (ear) + 0 (incidental) = 350 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, low amounts of blood and few clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 66.4 (resected LM lobe) + 885.4(remaining liver) = 951.8 g
Tissue harvested: No

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage only. The extent of the LM lobe resection (3 cm distal from junction with RM lobe; see Figs) was decreased from previous runs, and the bleeding was easily controlled during the bandage application. There were no issues with bandage adhesion, even though this run was done without FS. Blood loss was 100 mL during the resection (the amount lost while we obtained hemostasis) + 67 mL shed during the 60 minute observation period = 167 mL total. Bandage was adherent to the wound at necropsy. So this less severe resection technique was more tractable in this first attempt, using plain PCL without biologics.

VII. PLAN

Repeat this injury/treatment in next subject on Sep 3rd.

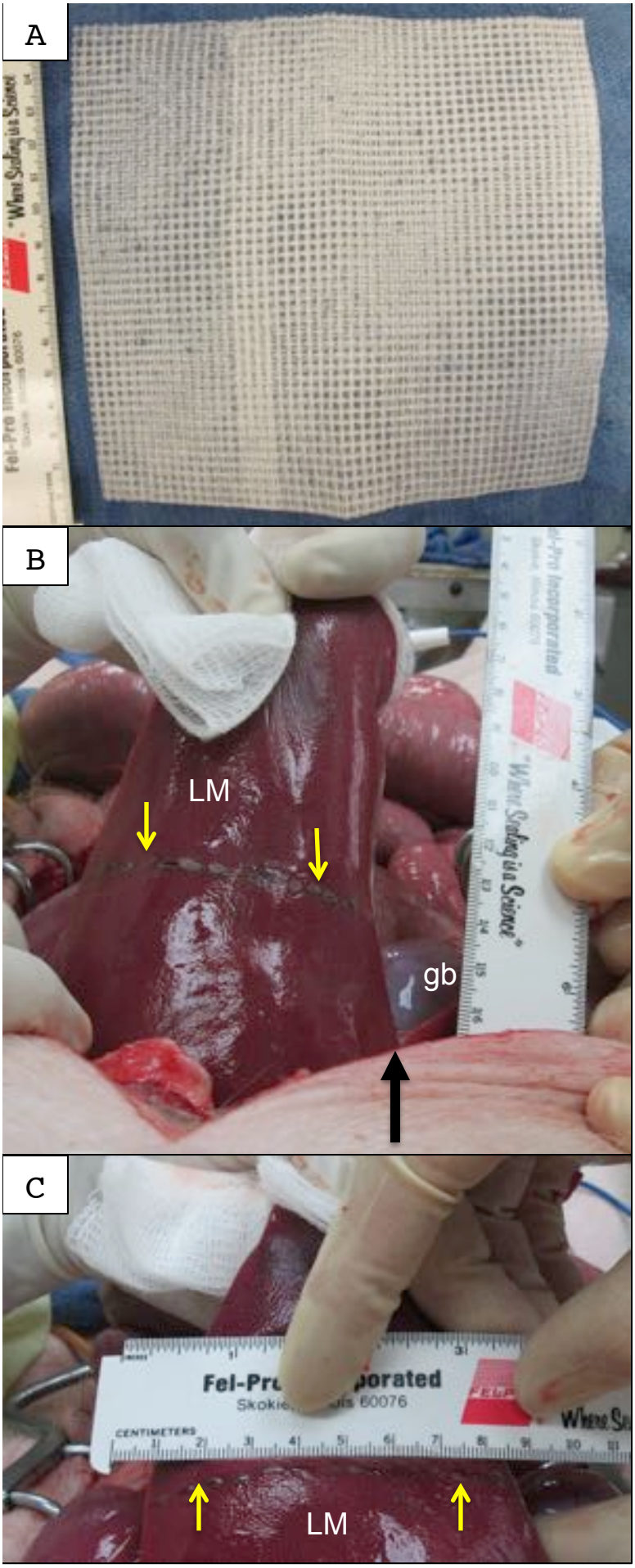


Figure 1, swine 310. Injury set-up.
(A) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 15 x 15 cm.
(B) Scored line (yellow arrows) on capsule of LM lobe indicated where lobe will be cut for partial lobectomy. Level of score was 3 cm distal to intersection (black arrow) of LM and RM lobes. gb = gallbladder. LM lobe is being suspended out of midline incision. View is looking down from head toward hindlimbs.
(C) Similar view as in panel B, measuring width of resection (~8 cm).

Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) wet with FS was applied onto injury face, further FS was sprayed on, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was over the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad.
(D) View of upper abdomen & injury site (yellow arrows) immediately after 5 min bandage compression. Medial corner of injury (yellow arrows) was not covered by PCL bandage. Black arrow = cephalad.

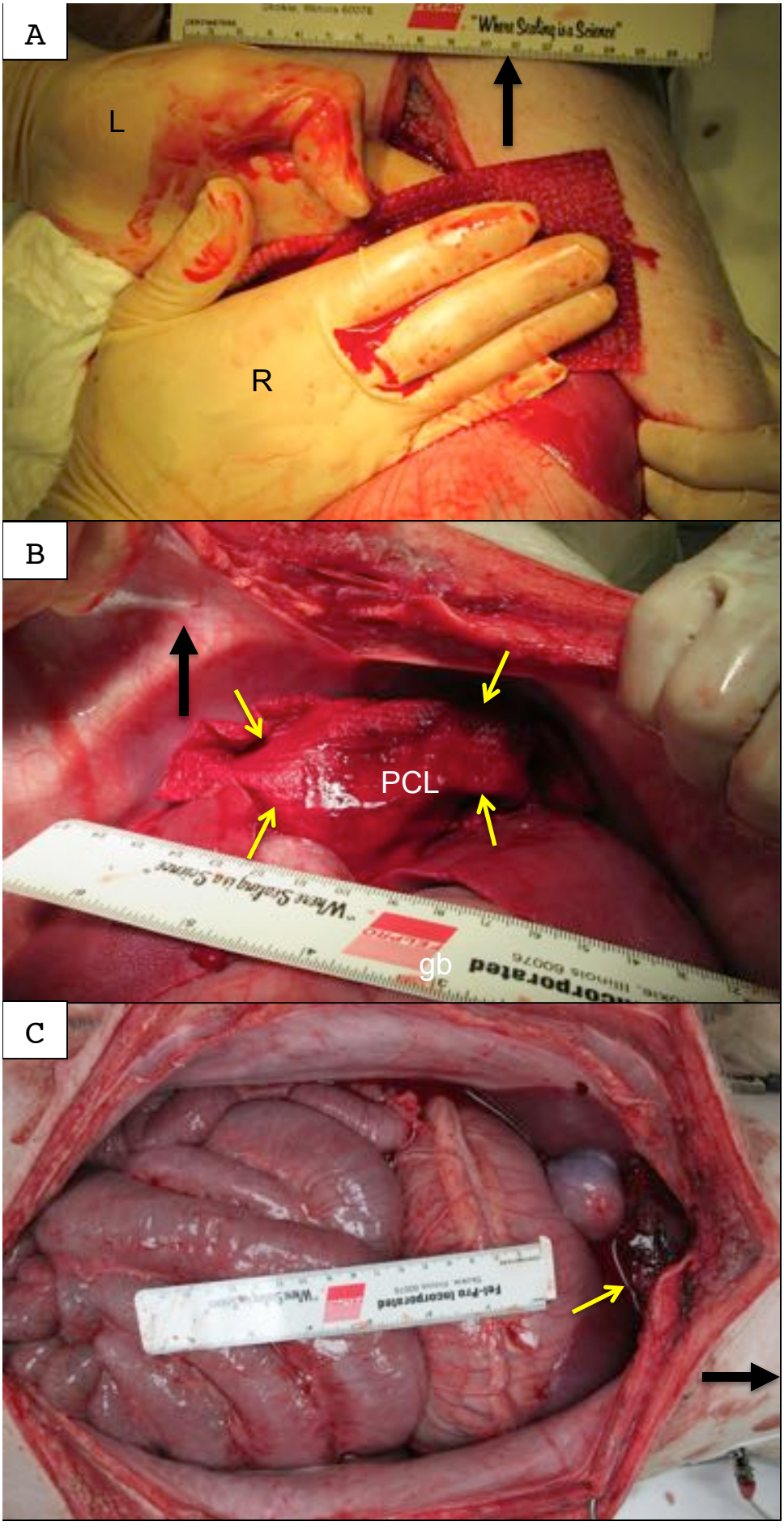
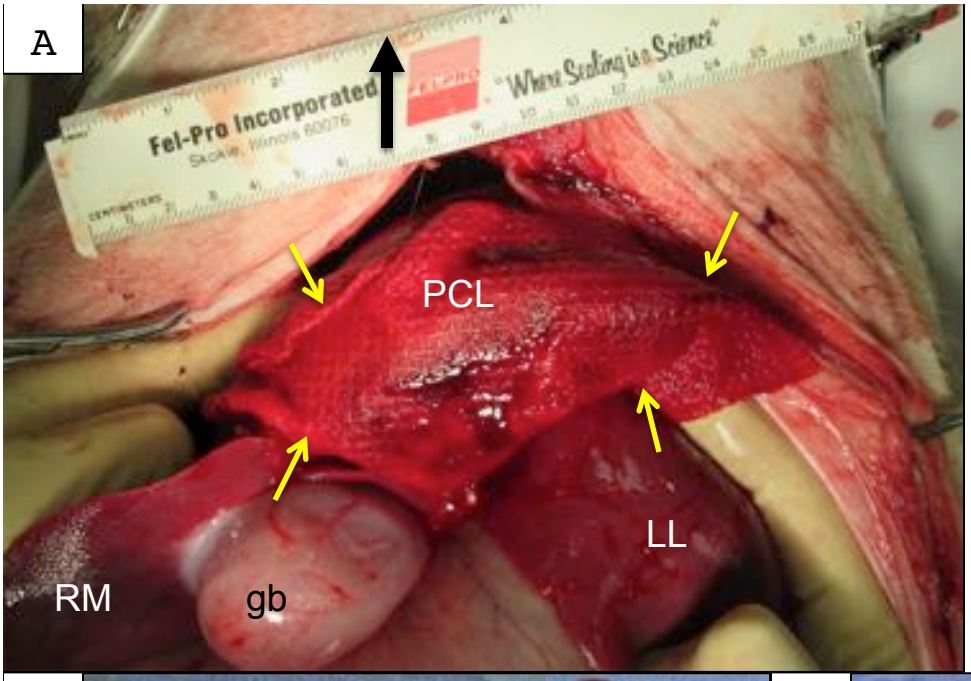
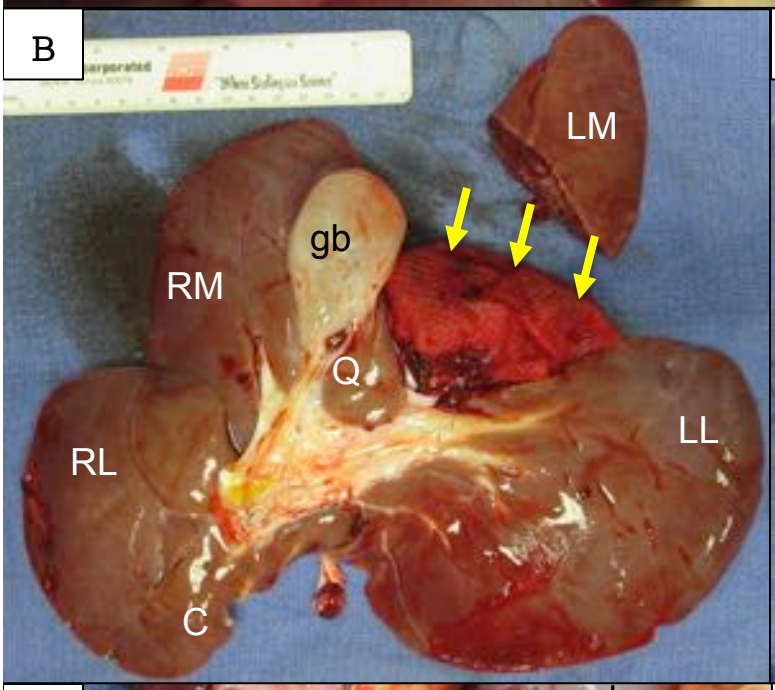
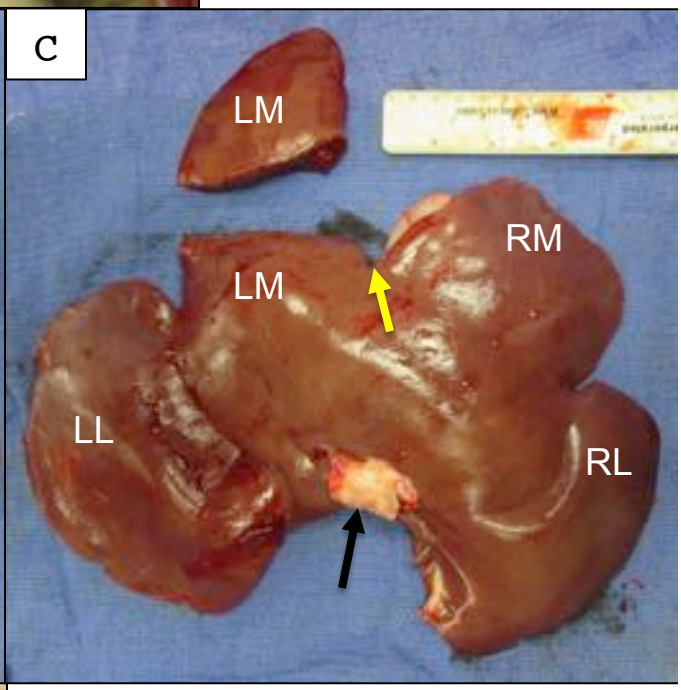


Fig. 2, swine 310. Injury treatment. (A) Technique of manual compression. LM lobe has been transected near its base, 3 cm above level of junction with RM lobe. Dry PCL bandage (~8 x 15 cm) was applied onto injury face, and the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was over the dome of the liver, squeezing bandage and liver in between. Black arrow = cephalad. (B) View of upper abdomen & injury site (yellow arrows) immediately after 5 min bandage compression. Injury site completey covered and hemostatic. (C) Overhead view of abdomen re-opened 1 h after panel B. Yellow arrow = injury site, still covered and hemostatic.







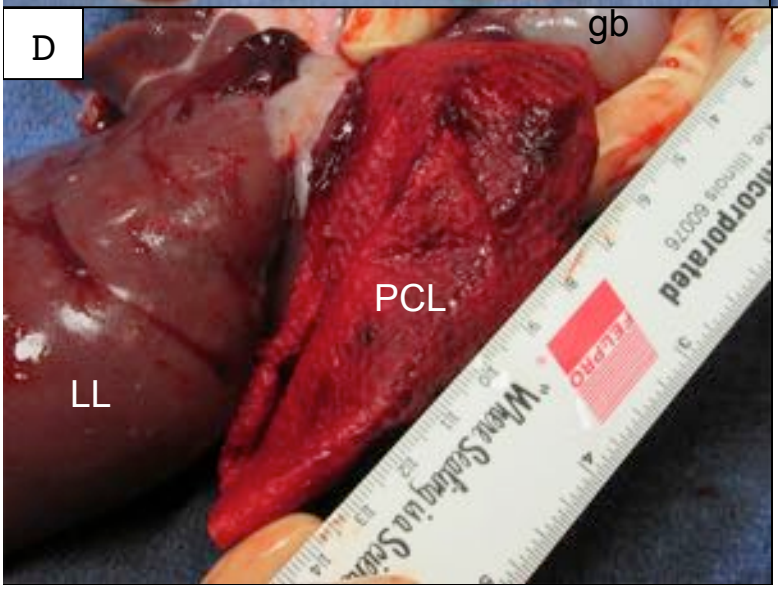


Fig. 3, swine 310. Necropsy.
(A). View of upper abdomen immediately after 1 h observation. Subject still alive. Liver and injury site (yellow arrows; still covered and hemostatic with PCL) being elevated out of incision with hands.
(B) Liver *ex vivo* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Injury site only covered with thin film of fibrin clot.

(C) Liver, superior surface. Yellow arrow = junction of RM & LM lobes; level of transection = 3 cm above this junction. Black arrow = suprahepatic IVC.
(D) Close-up of injury site, with intact PCL bandage.

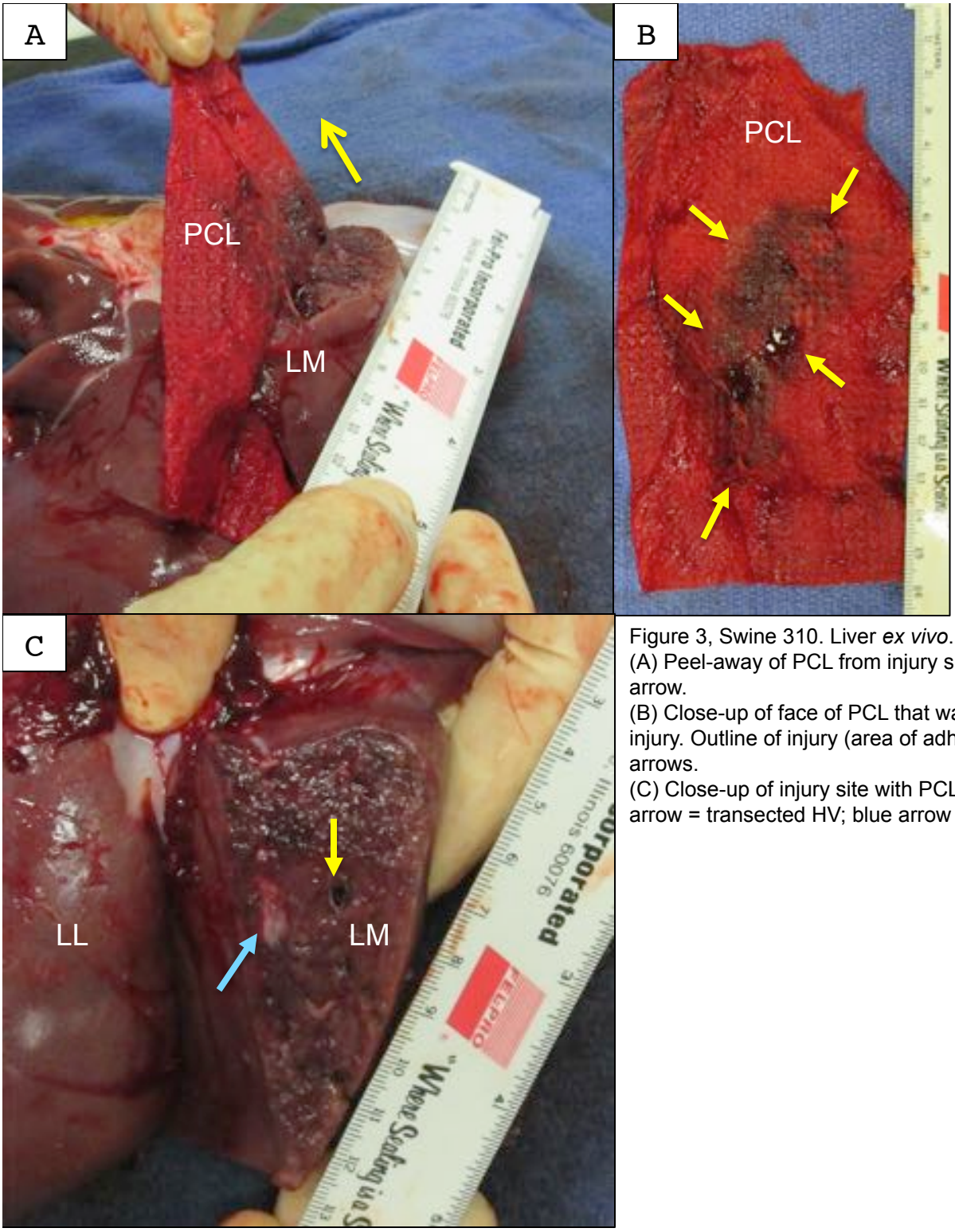


Figure 3, Swine 310. Liver *ex vivo*.
(A) Peel-away of PCL from injury site, in direction of arrow.
(B) Close-up of face of PCL that was against the injury. Outline of injury (area of adherence) noted with arrows.
(C) Close-up of injury site with PCL removed. Yellow arrow = transected HV; blue arrow = transected PV.

I. OVERVIEW
Date: September 3, 2015
Swine no: 311
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, distal LM liver lobe resection
Treatment: PCL Bandage (16 x 16 cm) without biologics
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:40 AM
Swine sex: male
Date swine received from UNL Mead: 08/26/2015
Pre-procedure wt: 35.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 104
- MAP: 85
- Temp: 37.8
- EtCO2: 43

Blood draw no. 1 (initial): 8:00 AM (ABG, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:18 AM
Spleen wt: 212.8gm
LR (22°C) infused after splenectomy: 650 mL at 150 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 212.8 + 30 + 78.4 = 321.2 mL
- LR (22°C) infused (spleen replacement + incidental): 650 + 20= 670 mL

Pre-resection VS
- HR: 84
- MAP: 108
- Temp: 37.3

- EtCO2 : 48

III. RESECTION & TREATMENT PHASE

Time resection began: 08:27 AM, closed : 8:35 AM (t = 0 began at 8:35 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. The resected liver lobe was removed from the abdomen. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was applied onto the bleeding resection site without any clotting factors. I held pressure for 5 min, compressing the bandage to the injury with bimanual compression (see Figs). After compression, the shed blood was carefully collected with a lap pad, and the incision was closed with towel clips.
Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm; NO biologics
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 80
Resuscitation fluid: warm LR given at constant rate of 9.8 mL/min (wt in kg x 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 0 + 0 + 42.7 (suction + clots + sponges) = 42.7 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:50 AM

15 min post-resection VS
- HR: 105
- MAP: 88
- Temp: 38
- EtCO2: 47
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 9:35AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 0 mL (suction) + 64.9 mL (clot + lap pads) + 60 mL (phlebotomies) = 124.9 mL
- IV fluid given: LR (37°C): 150 mL + 10 mL (ear) + 350 (incidental) = 510 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, low amounts of blood and few clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 87.2 (resected LM lobe) + 956.2 (remaining liver) = 1,043.4 g
Tissue harvested: yes, injury site specimen with PCL bandage in formalin (see Figs)

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage only, no biologics. Similar to subject #310, the extent of the LM lobe resection (3 cm distal from junction with RM lobe; see Figs) was decreased from previous runs, and the bleeding was easily controlled during the bandage application. There were no issues with bandage adhesion (even though no biologics used). Blood loss was 43 mL during the resection (the amount lost while we obtained hemostasis) + 65 mL shed during the 60 minute observation period = 108 mL total. Bandage was adherent to the wound at necropsy. So this less severe resection technique was again tractable in this 2nd attempt, using plain PCL without biologics.

So using a less severe extent of LM hepatic lobe resection produces a model which is technically easier and more reproducible, but it also appears to be easily treatable by simply applying the PCL without biologics (as long as the bandage can be applied perfectly the first time and held there for 5 min). So this model may not be able to discriminate hemostatic efficacy of the PCL bandage ± biologics. One option may be to partially heparinize these subjects so that PCL on its own will be ineffective.

VII. PLAN

Repeat this injury/treatment in next subject on Tue Sep 8th.

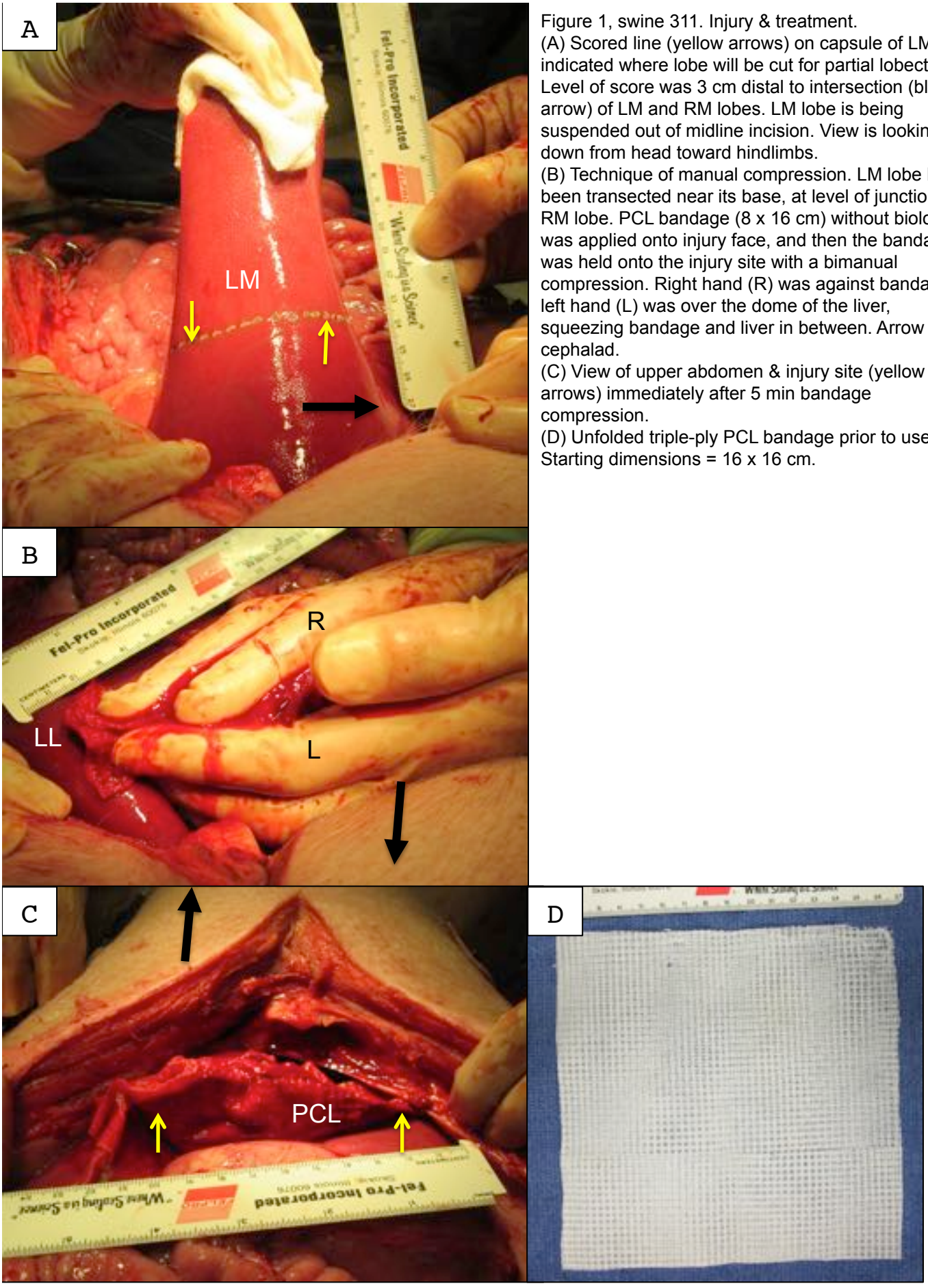


Figure 1, swine 311. Injury & treatment.
(A) Scored line (yellow arrows) on capsule of LM lobe indicated where lobe will be cut for partial lobectomy. Level of score was 3 cm distal to intersection (black arrow) of LM and RM lobes. LM lobe is being suspended out of midline incision. View is looking down from head toward hindlimbs.
(B) Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) without biologics was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was over the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad.
(C) View of upper abdomen & injury site (yellow arrows) immediately after 5 min bandage compression.
(D) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 16 x 16 cm.

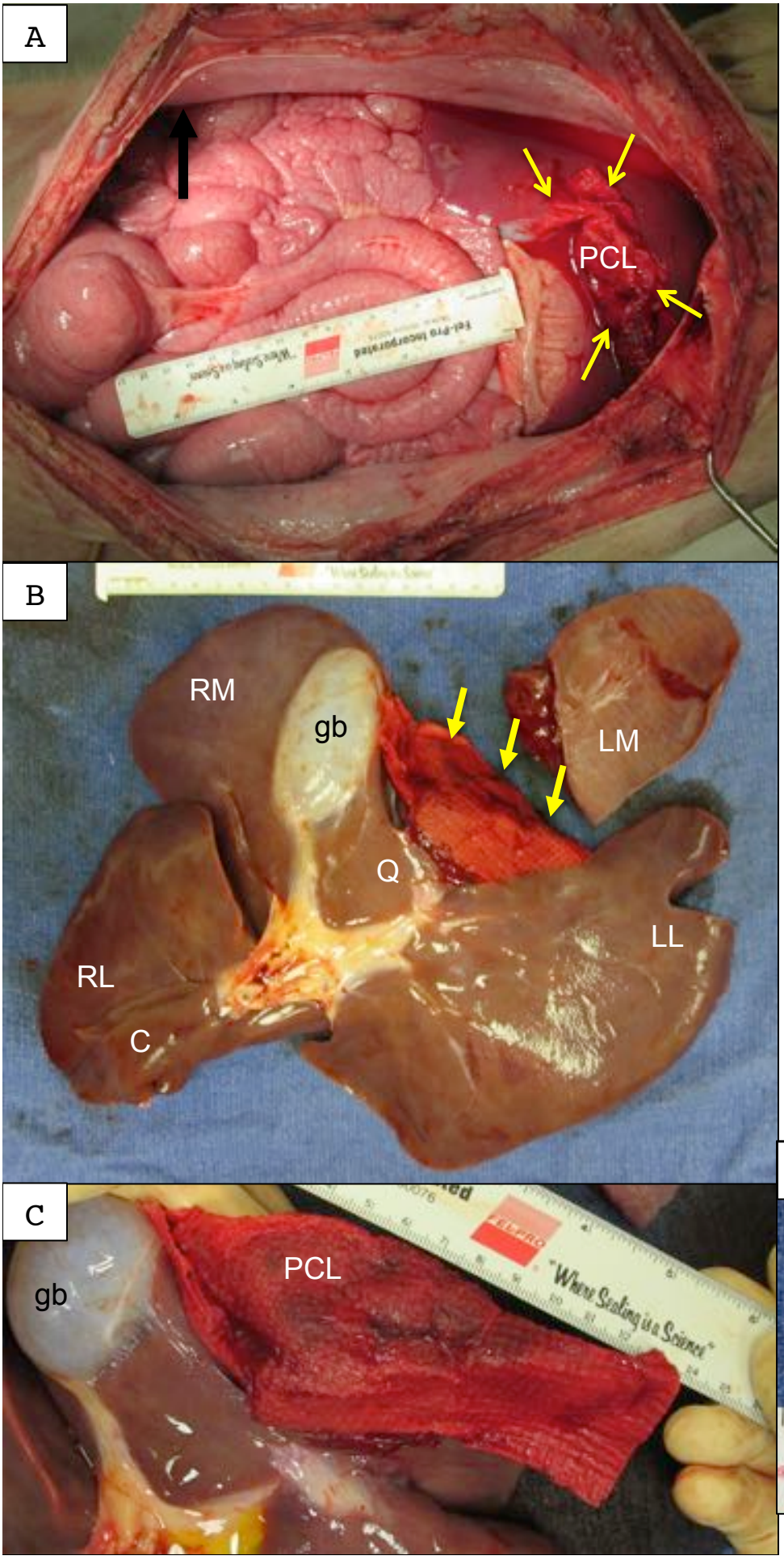


Fig. 2, swine 311. Necropsy.
(A). View of upper abdomen immediately after 1 h observation. Subject still alive. Injury site (yellow arrows) still covered and hemostatic with PCL).
(B) Liver *ex vivo* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; Q = quadrate; gb = gallbladder. Injury site with PCL bandage well adherent.
(C) Close-up of PCL covering injury site on LM lobe.
(D) Section of injury site with adherent PCL (arrows) taken for histology.

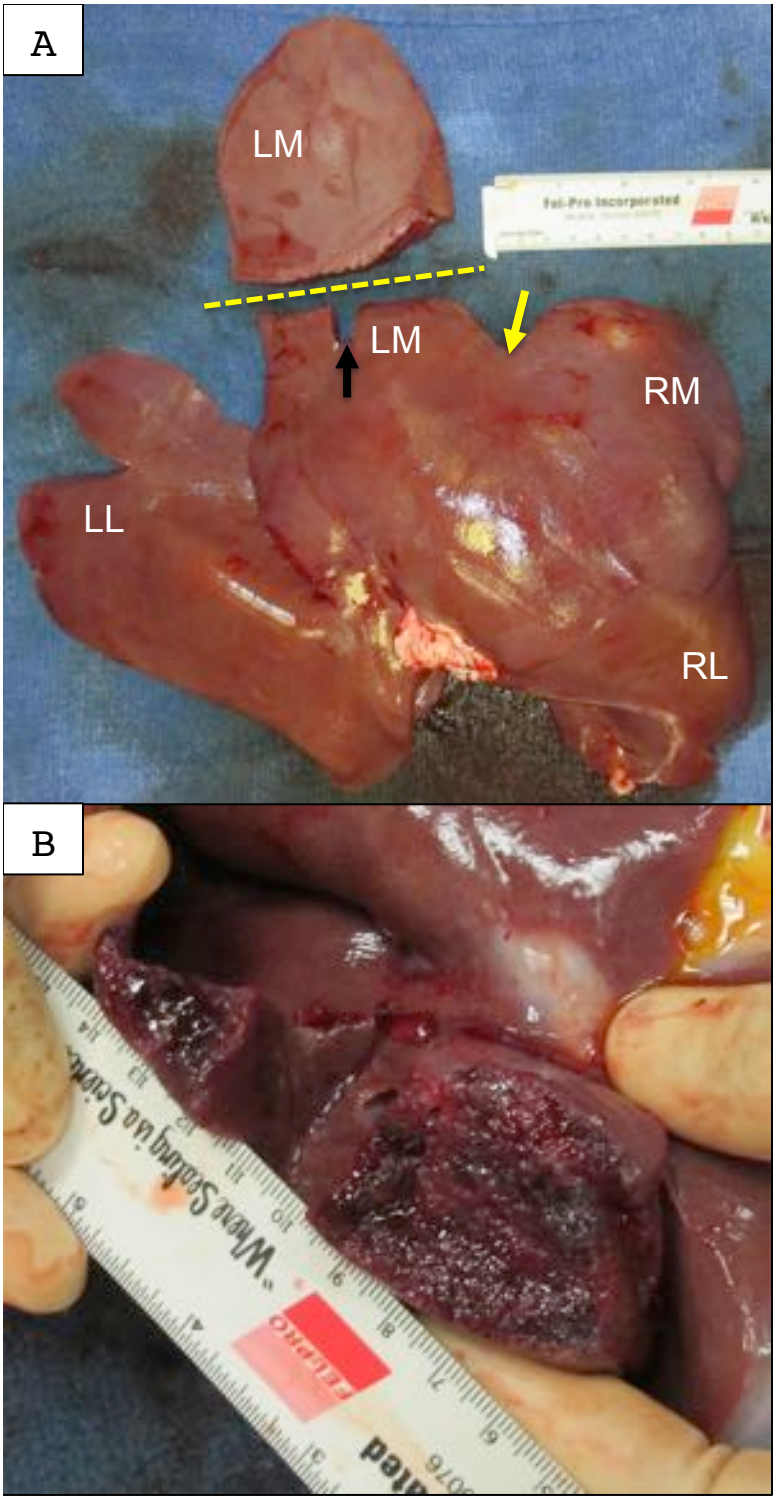


Fig. 3, swine 311. Necropsy.
(A). Liver, superior surface. PCL bandage has been removed. Yellow arrow = junction of RM & LM lobes; level of transection (dashed yellow line) = 3 cm above this junction. Black arrow = area that was removed for histology (Fig. 3D).
(B) Close-up of injury site, with PCL and fibrin removed. Transected PV and HV are smaller at this level than when transection made at junction of LM & RM lobes.

I. OVERVIEW
Date: September 8, 2015
Swine no: 312
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:30 AM
Swine sex: Male
Date swine received from UNL Mead: 08/26/2015
Pre-procedure wt: 36.6 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support

1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS

- HR: 98
- MAP: 106
- Temp: 36.8
- EtCO2: 37

Blood draw no. 1 (initial): 7:55 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:03 AM
Spleen wt: 319.1gm
LR (22°C) infused after splenectomy: 950 mL at 100 mL/min (rate of replacement slowed from 150 mL/min to minimize risk of random/unexplainable/counterintuitive hypotension we occasionally see with rapid infusion of warm LR)

Pre-resection fluid data:

- Blood loss (spleen weight + phlebotomies + incidental): 319.1+ 30 + 76.7 = 425.8 mL
- LR (22°C) infused (spleen replacement + incidental): 950 + 0 = 950 mL

Pre-resection VS

- HR: 108
- MAP: 143
- Temp: 36.2
- EtCO2 : 37

III. RESECTION & TREATMENT PHASE

Time resection began: 08:17AM, closed : 8:25AM (t=0; 8:35 AM)

Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was applied onto the bleeding resection site without any clotting factors. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with bimanual compression (see Figs). After compression, the shed blood was carefully collected with a lap pad, and the incision was closed with towel clips.

Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm; NO biologics

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 105

Resuscitation fluid: warm LR given at constant rate of 10.2 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.

Blood loss during resection: 147.3 + 0 + 14.8 (suction + clots + sponges) = 162.1 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:30 AM

15 min post-resection VS

- HR: 94
- MAP: 136
- Temp: 36.7
- EtCO2: 32
- IAP: 0

Survival at 60 min? Yes

Target MAP attained? Yes

Time of death: 9:25AM

Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)

Interval from completion of resection to death: 60 mins

Post-treatment fluid data:

- Blood loss: 124 mL (suction) + 0 mL (clot + lap pads) + 60 mL (phlebotomies) = 124 mL
- IV fluid given: LR (37°C): 1,050 mL + 10 mL (ear) + 0 (incidental) = 1,050 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, low amounts of blood and few clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 54.6 (resected LM lobe) + 746.9 (remaining liver) = 801.5 g
Tissue harvested: yes, injury site specimen with PCL bandage in formalin.

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage only, no biologics. Similar to subject #310 & 311, the extent of the LM lobe resection (3 cm distal from junction with RM lobe; see Figs) was decreased from previous runs, though we did have more blood loss during the application (~160 mL) compared to previous subjects. There also was more blood loss during the 1 h observation (~124 mL), for a total of ~280 mL. Bandage was adherent to the wound at necropsy. So this less severe resection technique still was tractable in this 3rd attempt, using plain PCL without biologics, though with somewhat more blood loss with the current subject.

VII. PLAN

Perform next subject in this series on 09/09/2015.

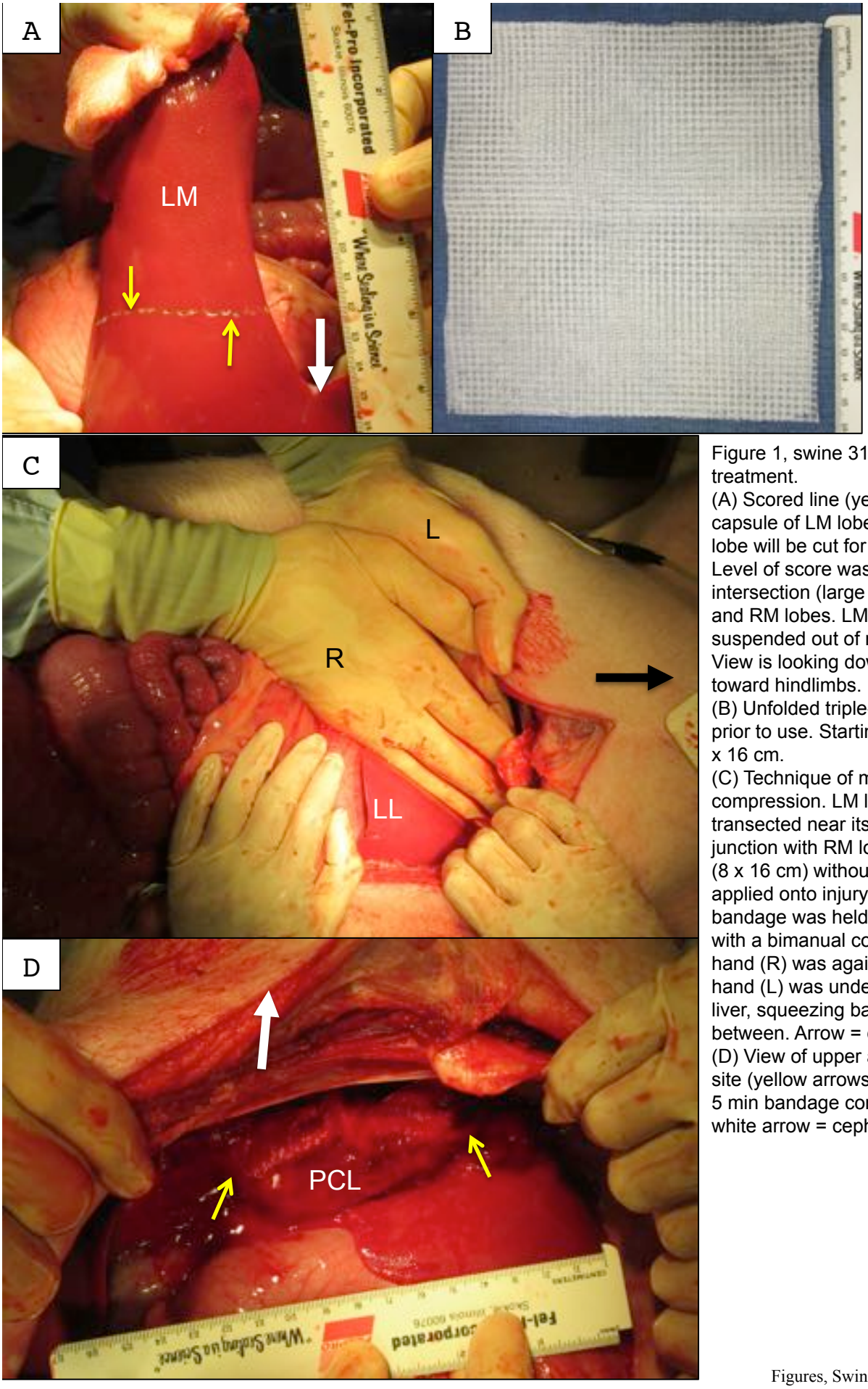


Figure 1, swine 312. Injury & treatment.
(A) Scored line (yellow arrows) on capsule of LM lobe indicated where lobe will be cut for partial lobectomy. Level of score was 3 cm distal to intersection (large white arrow) of LM and RM lobes. LM lobe is being suspended out of midline incision. View is looking down from head toward hindlimbs.
(B) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 16 x 16 cm.
(C) Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) without biologics was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was under the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad.
(D) View of upper abdomen & injury site (yellow arrows) immediately after 5 min bandage compression. Large white arrow = cephalad.

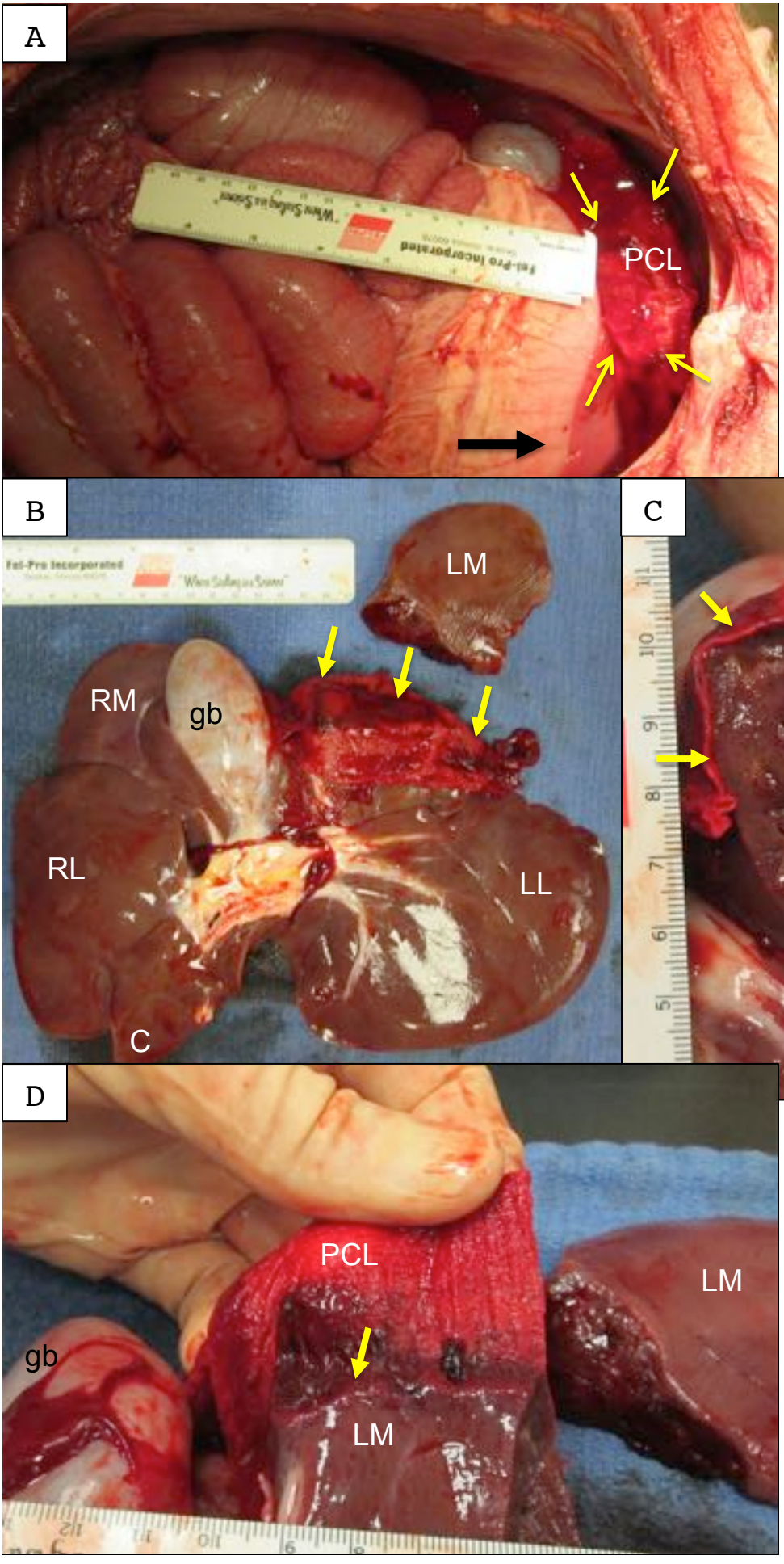
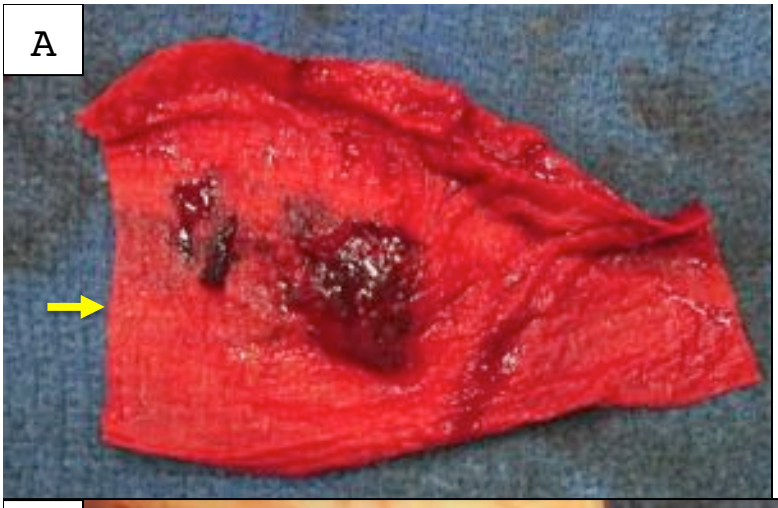
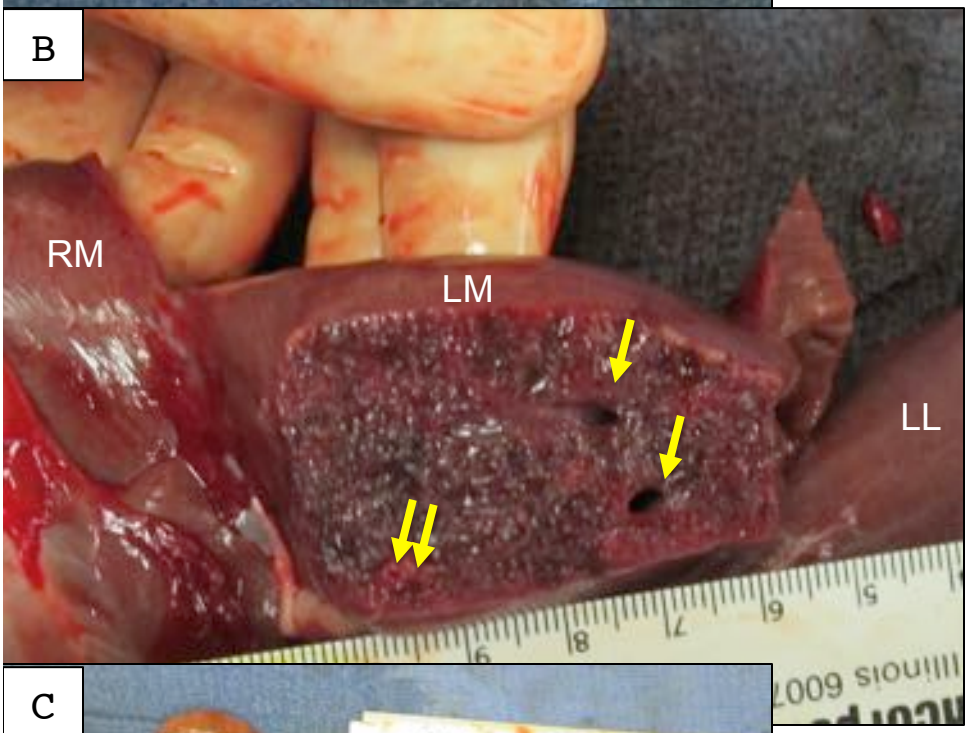


Fig. 2, swine 312. Necropsy.
(A). View of upper abdomen immediately after 1 h observation. Subject alive & well. Injury site (yellow arrows) still covered and hemostatic with PCL.
(B) Liver *ex vivo* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows).
(C) Close-up (cross-section) of PCL covering injury site on LM lobe (arrows).

(D) Peel-off of PCL from injury site (arrow) on LM lobe.





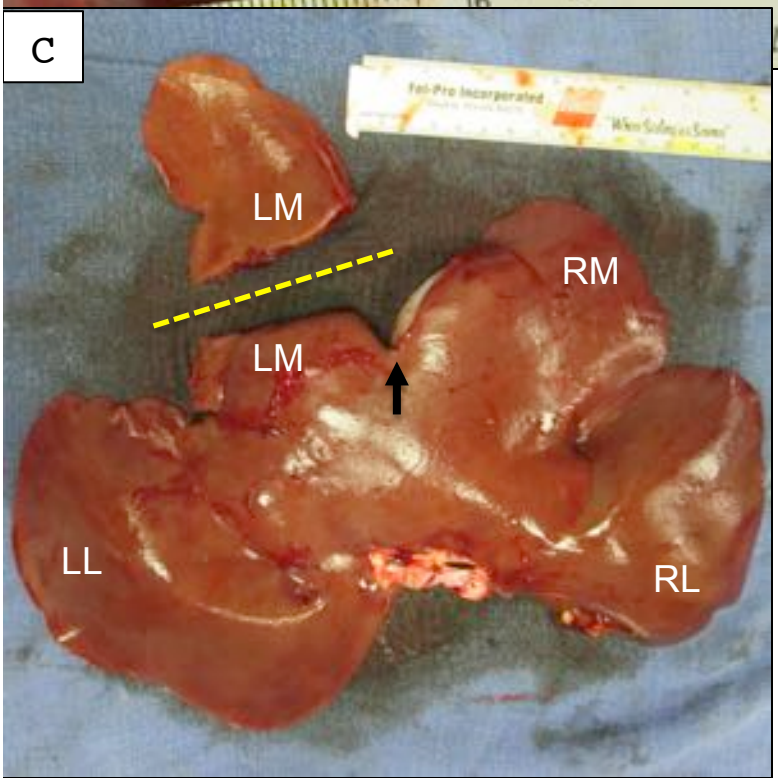


Fig. 3, swine 312. Necropsy.
(A). PCL, removed from injury. Side facing the wound. Edge with arrow was transected during harvest of section for histology. Note clot in central area of bandage, where PCL had been apposed to the liver parenchyma.
(B) Close-up of injury site, with PCL and fibrin removed. Transected PV branch (double arrow) and HV branches (single arrows) are smaller at this level than when transection made at junction of LM & RM lobes.
(C) Liver, superior surface. PCL bandage has been removed. Arrow = junction of RM & LM lobes; level of transection (dashed yellow line) = 3 cm above this junction. LM resection specimen in approximate anatomic position.

I. OVERVIEW
Date: September 9, 2015
Swine no: 313
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, LM liver lobe resection (3 cm from RM lobe)
Treatment: PCL Bandage, no biologics
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:45 AM
Swine sex: Male
Date swine received from UNL Mead: 09/04/2015
Pre-procedure wt: 45.2 kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 70
- MAP: 96
- Temp: 37.5
- EtCO2: 43

Blood draw no. 1 (initial): 8:20 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:30 AM
Spleen wt: 318.8gm
LR (22°C) infused after splenectomy: 980 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 318.8+ 30 + 42.2= 391 mL
- LR (22°C) infused (spleen replacement + incidental): 980+ 0= 980 mL

Pre-resection VS
- HR: 87
- MAP: 107

- Temp: 36.9
- EtCO2 : 49

III. RESECTION & TREATMENT PHASE

Time resection began: 08:42AM, closed : 8:50AM (t=0; 8:50 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was applied onto the bleeding resection site without any clotting factors. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). The MAP dropped during this compression, probably because I kinked the IVC and reduced venous cardiac return during this compression. After compression, the shed blood was carefully collected with a lap pad and sucker, and the incision was closed with towel clips. The injury site appeared hemostatic.
Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm; NO biologics
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 85
Resuscitation fluid: warm LR given at constant rate of 12.6 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 185.2 + 0+ 48.7 (suction + clots + sponges) = 233.9 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:05 AM

15 min post-resection VS
- HR: 104
- MAP: 78
- Temp: 36.4
- EtCO2: 43
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? No
Time of death: 9:55AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:

- Blood loss: 262.9 mL (suction) + 233.6 mL (clot + lap pads) + 90 mL (phlebotomies) = 586.5 mL
- IV fluid given: LR (37°C): 930 mL + 10 mL (ear) + 0 (incidental) = 940 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, moderate amounts of blood and clot were seen around the injury site (see Figs). There may have been some oozing from the resection site (see Figs). Bandage was completely covering injury site without any gaps.
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 115.1 (resected LM lobe) +1220 (remaining liver) = 1335.1 g
Tissue harvested: No

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage only, no biologics. Similar to last three subjects, the extent of the LM lobe resection (3 cm distal from junction with RM lobe; see Figs) was decreased from previous runs. Blood loss was 234 mL during the resection, and then ~500 mL after the 1 h observation… a bit more than during the first two attempts of PCL without biologics in this modified resection model. Grossly, the PCL appeared adherent at necropsy. Difficult to say where the leakage is occurring. I assume it is around the bandage, in small breaks of adhesion with the liver, and not through the bandage, because the bandage appears to be impenetrable after it gets filled with clot.

VII. PLAN

Perform next subject in this series on 09/11/2015.

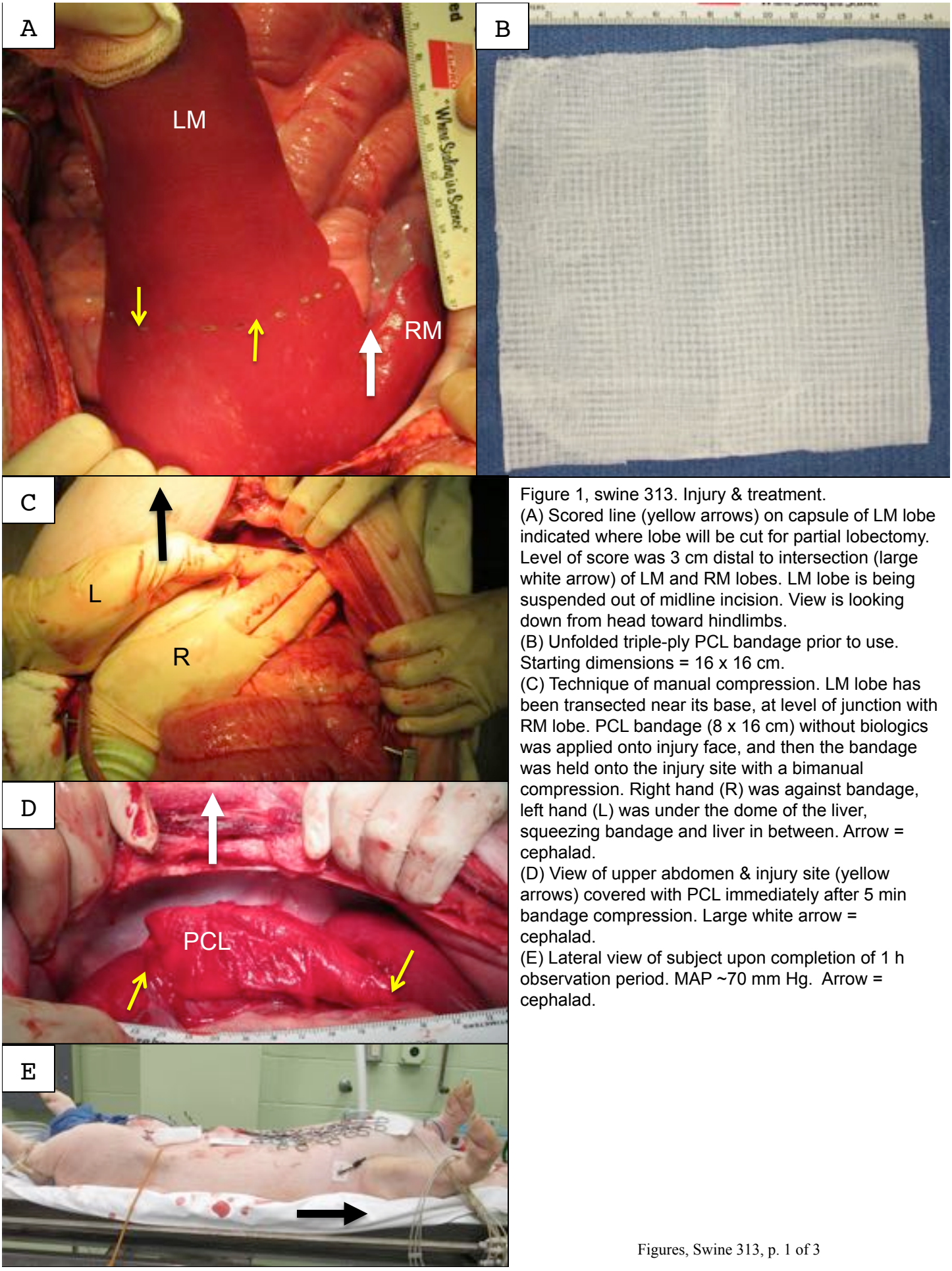
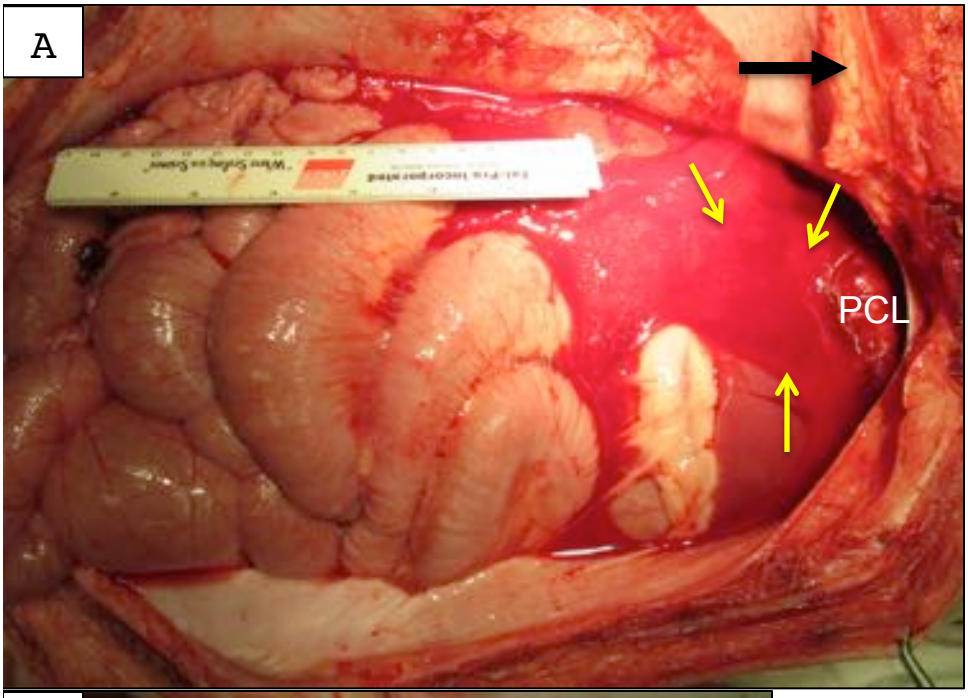
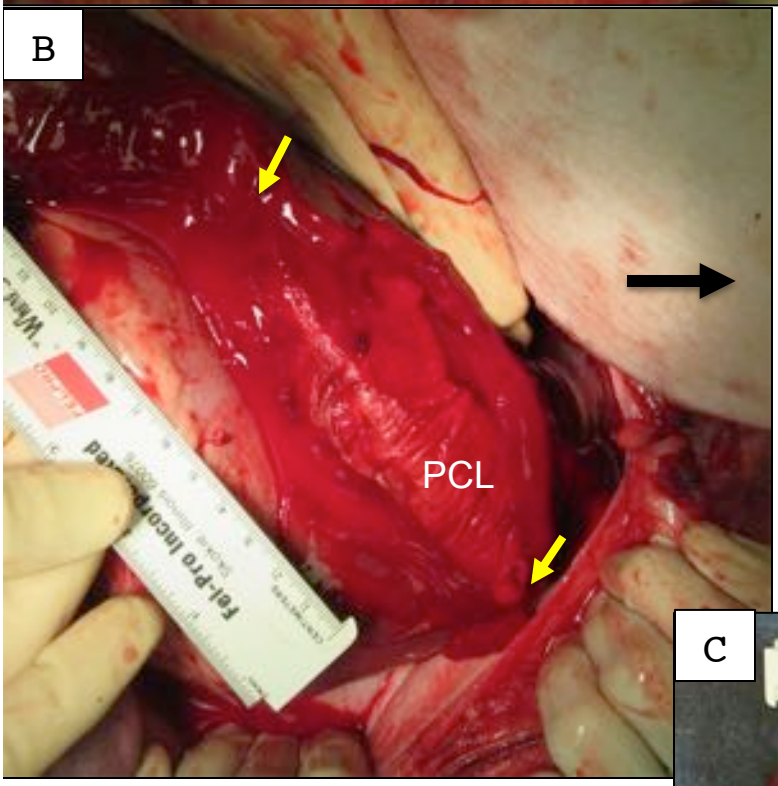


Figure 1, swine 313. Injury & treatment.
(A) Scored line (yellow arrows) on capsule of LM lobe indicated where lobe will be cut for partial lobectomy. Level of score was 3 cm distal to intersection (large white arrow) of LM and RM lobes. LM lobe is being suspended out of midline incision. View is looking down from head toward hindlimbs.
(B) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 16 x 16 cm.
(C) Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) without biologics was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was under the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad.
(D) View of upper abdomen & injury site (yellow arrows) covered with PCL immediately after 5 min bandage compression. Large white arrow = cephalad.
(E) Lateral view of subject upon completion of 1 h observation period. MAP ~70 mm Hg. Arrow = cephalad.





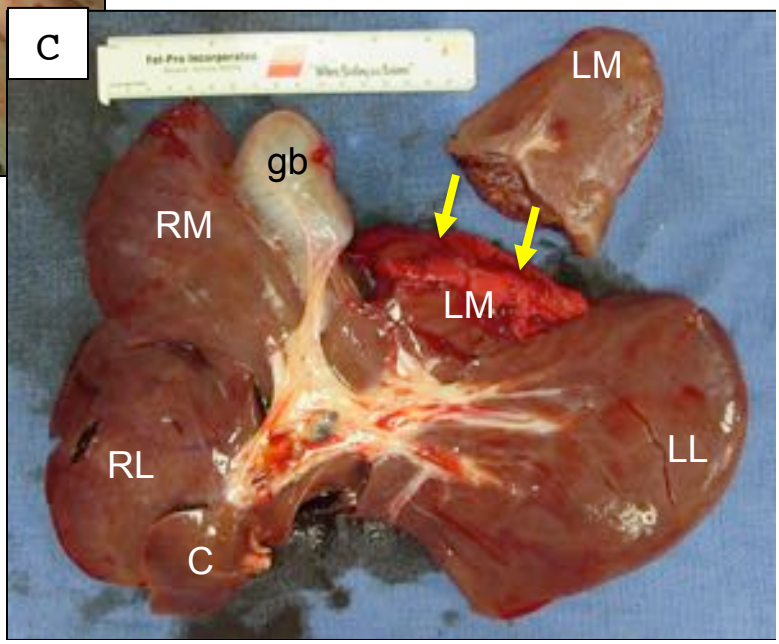
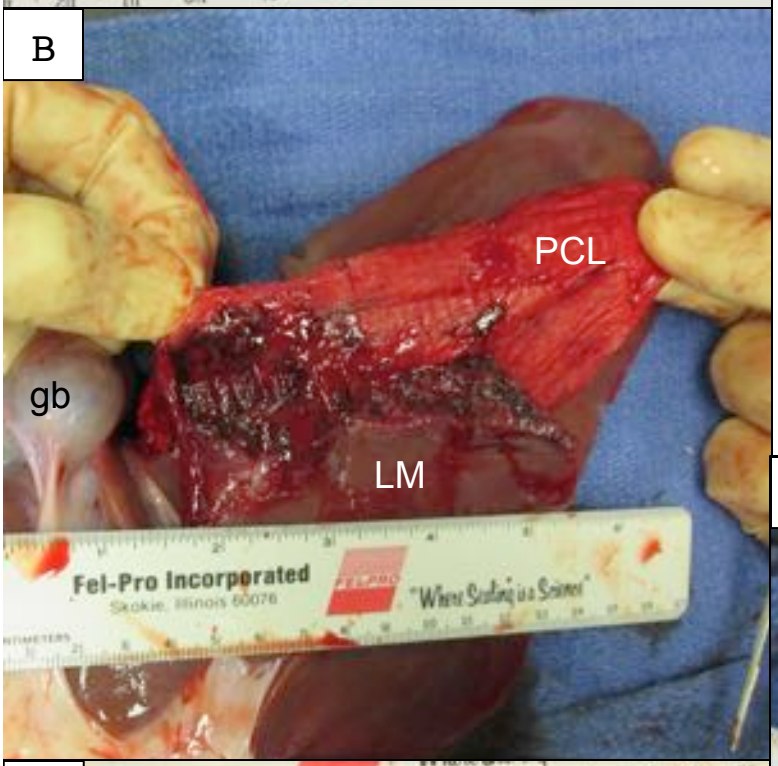
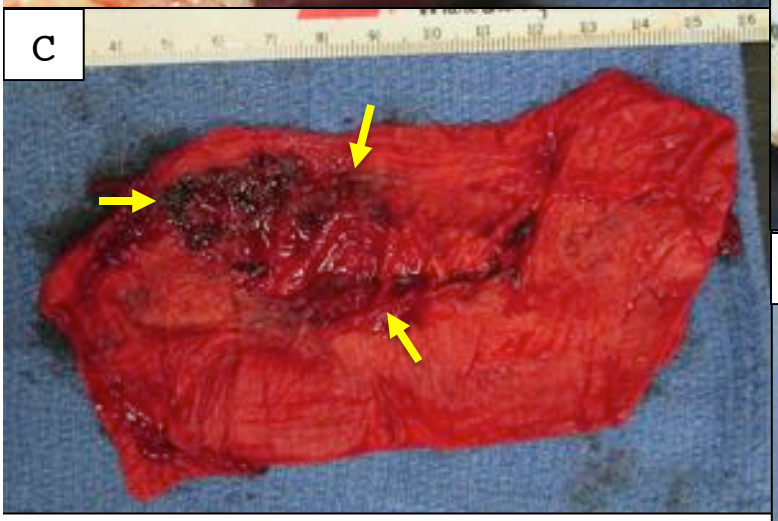
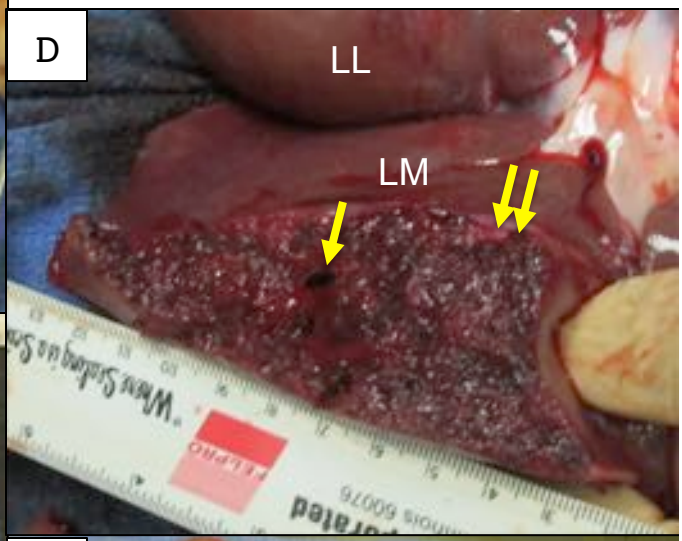


Fig. 2, swine 313. Necropsy.
(A). View of upper abdomen immediately after 1 h observation. Subject alive. Injury site still covered with PCL, but partially obscured by pool of blood (yellow arrows). Black arrow = cephalad.
(B) Close-up of injury site (yellow arrows) in vivo, still covered with PCL, with surrounding clot.
(C) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows).







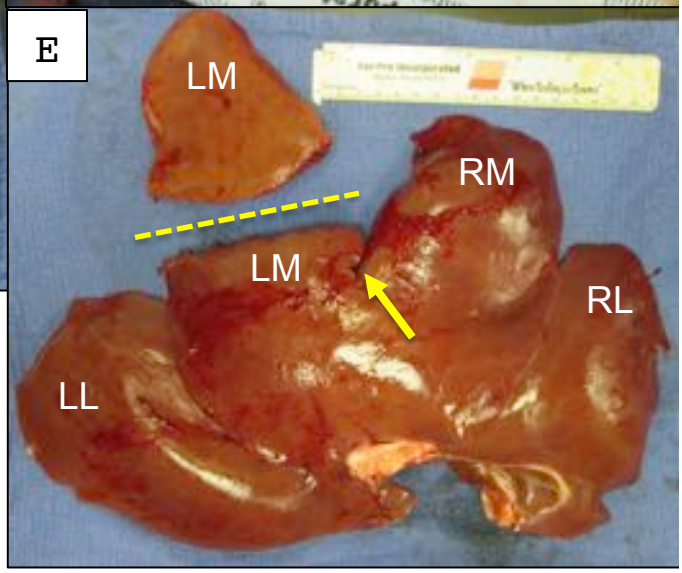


Fig. 3, swine 313. Necropsy.
(A) Close-up of injury site, with PCL intact/adherent.
(B) Peel-off of PCL from injury site.
(C) PCL, removed from injury. Side facing the wound. Note clot in central area of bandage (arrows), where PCL had been apposed to the liver parenchyma.
(D) Close-up of injury site, with PCL and fibrin removed. Transected PV branch (double arrow) and HV branches (single arrows) are smaller at this level than when transection made at junction of LM & RM lobes.
(E) Liver, superior surface. PCL bandage has been removed. Arrow = junction of RM & LM lobes; level of transection (dashed yellow line) = 3 cm above this junction. LM resection specimen in approximate anatomic position.

I. OVERVIEW
Date: September 11, 2015
Swine no: 314
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, LM liver lobe resection (3 cm from RM lobe)
Treatment: PCL Bandage, no biologics
Personnel: Carlson, Hansen, Siford, Spretz, Aravind

II. PRE-RESECTION PHASE

Start time: 07:35 AM
Swine sex: Male
Date swine received from UNL Mead: 09/04/2015
Pre-procedure wt: 37.8kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 85
- MAP: 89
- Temp: 36.2
- EtCO2: 36

Blood draw no. 1 (initial): 8:00 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:07 AM
Spleen wt: 292.2gm
LR (22°C) infused after splenectomy: 900 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 292.2+ 30 + 6.4= 328.6 mL
- LR (22°C) infused (spleen replacement + incidental): 900+ 0= 900 mL

Pre-resection VS
- HR: 114
- MAP: 110

- Temp: 36.1
- EtCO2 : 40

III. RESECTION & TREATMENT PHASE

Time resection began: 08:20AM, closed: 8:30AM (t = 0 defined as 8:30 AM)
Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was applied onto the bleeding resection site without any clotting factors. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). The MAP dropped during this compression, probably because I kinked the IVC and reduced venous cardiac return during this compression. After compression, the shed blood was carefully collected with the sucker, and the incision was closed with towel clips. The injury site appeared hemostatic.
Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm; NO biologics
Abdominal closure: "fully-closed" technique, as described above
Resuscitation target MAP: 90
Resuscitation fluid: warm LR given at constant rate of 10.5 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.
Blood loss during resection: 100.9 + 0 + 0 (suction + clots + sponges) = 100.9 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:45 AM

15 min post-resection VS
- HR: 117
- MAP: 83
- Temp: 35.3
- EtCO2: 38
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 9:30AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 mins

Post-treatment fluid data:

- Blood loss: 58.2 mL (suction) + 38.7 mL (clot + lap pads) + 90 mL (phlebotomies) +20ml (blood loss from arterial line)= 206.9 mL
- IV fluid given: LR (37°C): 800 mL + 0 mL (ear) + 50 (incidental) = 850 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, small amount of blood around injury site (see Figs). No active hemorrhage from resection site (see Figs). There was a heavy clot on the lateral aspect of the bandage (see Figs). The bandage appeared to be completely adherent to the injury site.
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 52.5 (resected LM lobe) +786.2 (remaining liver) =838.7 g
Tissue harvested: No

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage only, no biologics. Similar to last four subjects, the extent of the LM lobe resection was 3 cm distal from junction with RM lobe. Blood loss was 100 mL during the resection, and another ~100 mL after the 1 h observation, for a total of ~200 mL attributable to the resection (not bad). But there was a big clot on the lateral aspect of the bandage, suggesting some leakage at least early on during the observation period, which the animal was able to stop.

VII. PLAN

Perform next subject in this protocol on 09/15/2015, this time treatment will be PCL + Factor II + Factor XIII.

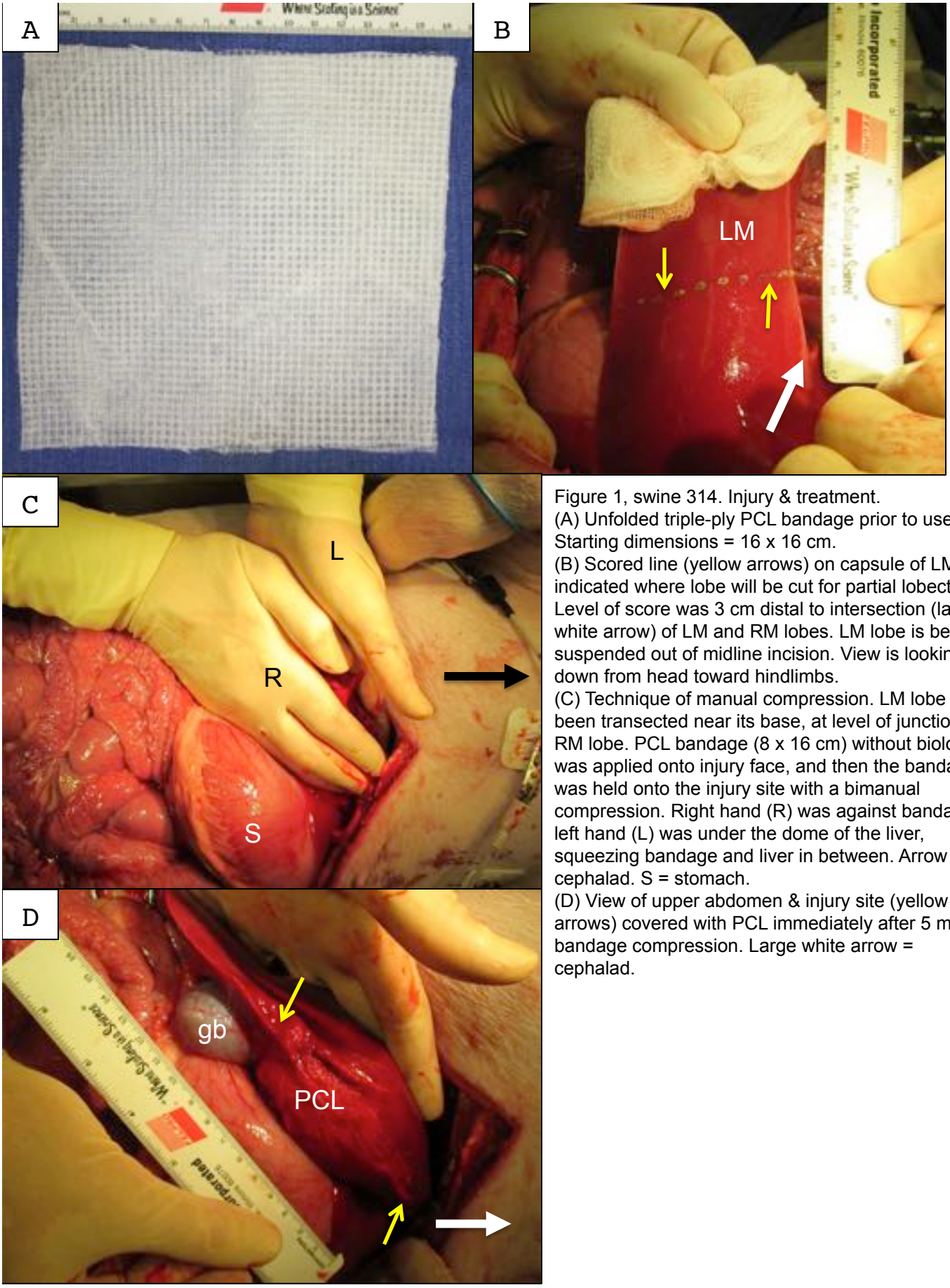


Figure 1, swine 314. Injury & treatment.
(A) Unfolded triple-ply PCL bandage prior to use. Starting dimensions = 16 x 16 cm.
(B) Scored line (yellow arrows) on capsule of LM lobe indicated where lobe will be cut for partial lobectomy. Level of score was 3 cm distal to intersection (large white arrow) of LM and RM lobes. LM lobe is being suspended out of midline incision. View is looking down from head toward hindlimbs.
(C) Technique of manual compression. LM lobe has been transected near its base, at level of junction with RM lobe. PCL bandage (8 x 16 cm) without biologics was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was under the dome of the liver, squeezing bandage and liver in between. Arrow = cephalad. S = stomach.
(D) View of upper abdomen & injury site (yellow arrows) covered with PCL immediately after 5 min bandage compression. Large white arrow = cephalad.

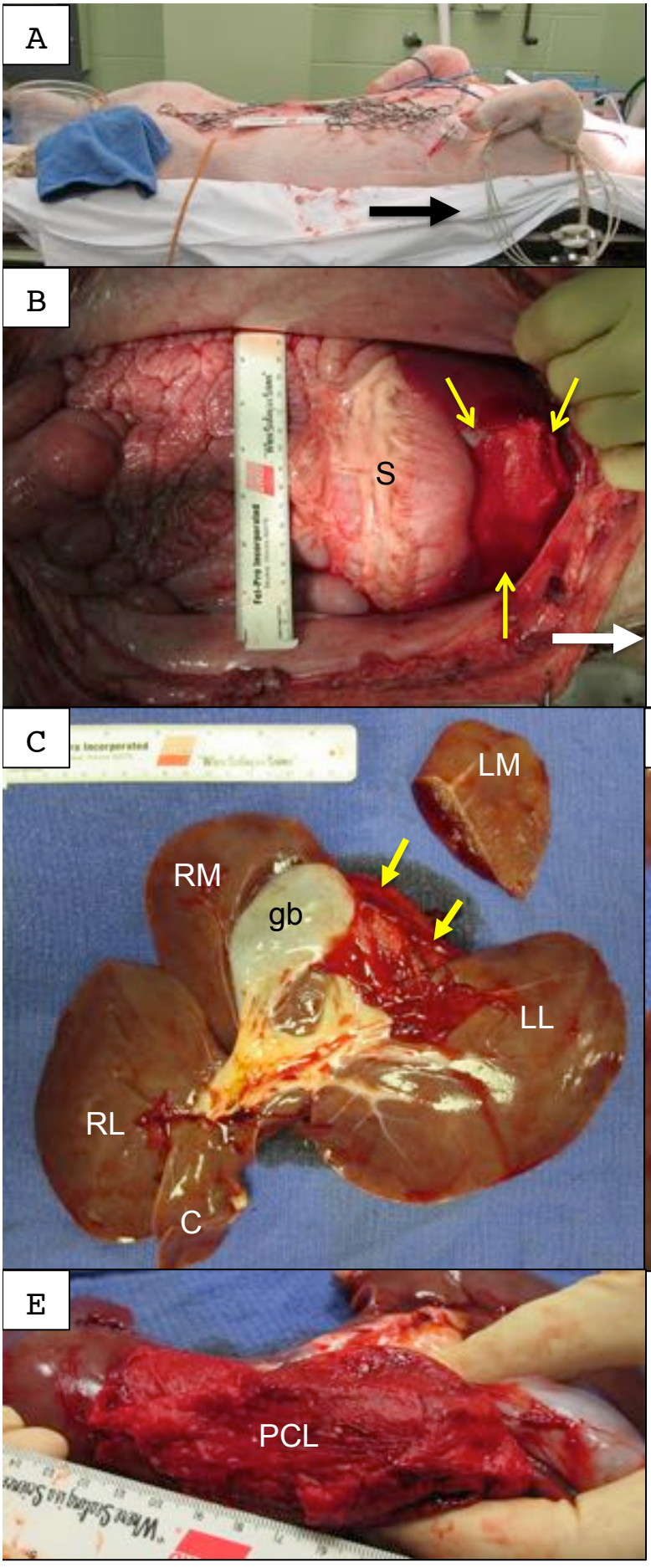
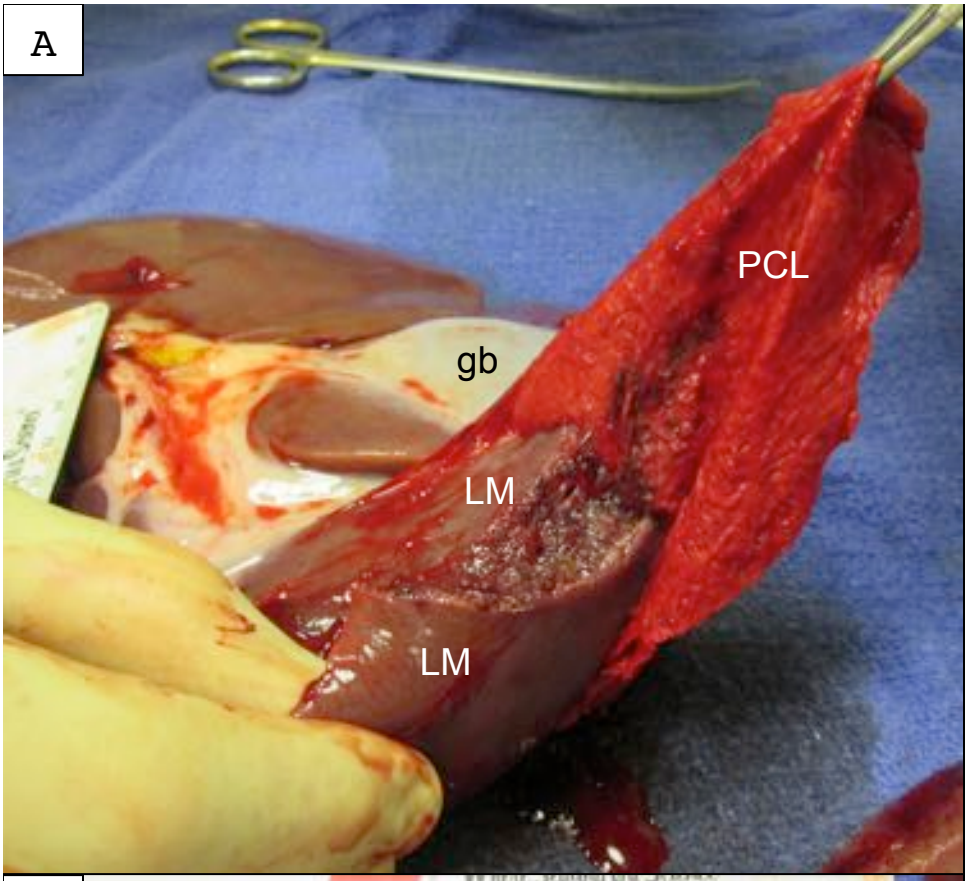
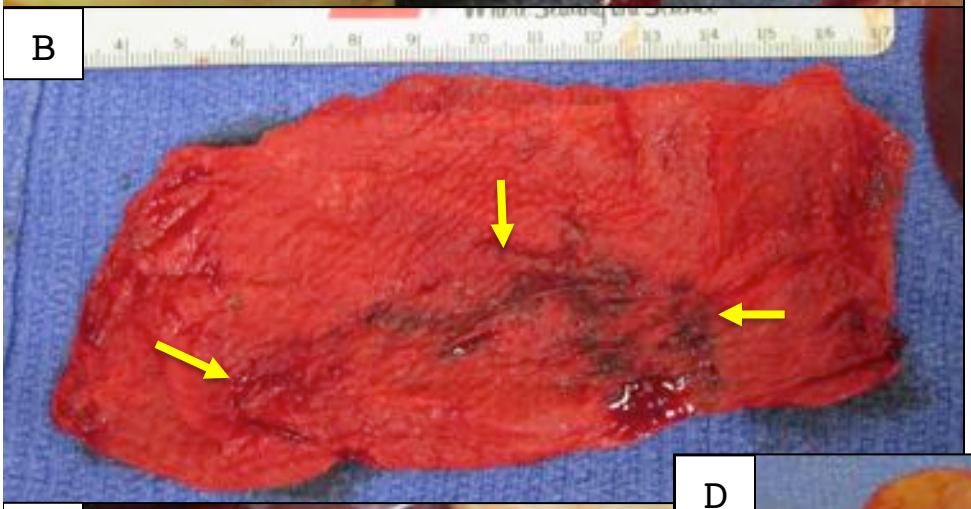
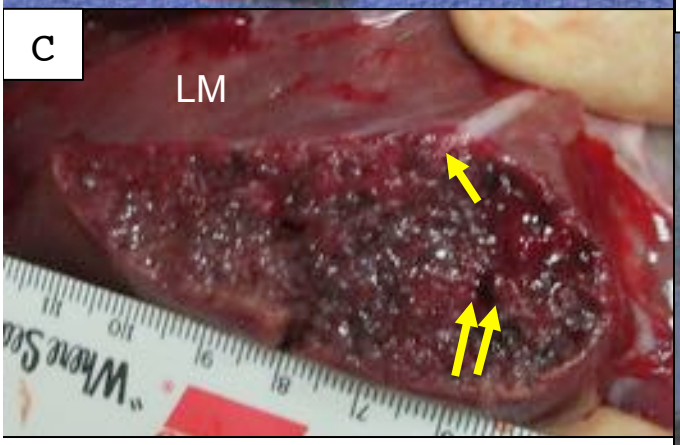


Fig. 2, swine 314. Necropsy.
(A). Lateral view of subject upon completion of 1 h observation period. MAP ~60 mm Hg. Arrow = cephalad.
(B) View of upper abdomen immediately after 1 h observation. Subject alive. Injury site still covered with PCL, but partially obscured by pool of blood (yellow arrows). White arrow = cephalad.
(C) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows).
(D) Close-up of injury site ex vivo, with large adherent clot (arrows) adjacent to injury, indicative of some hemorrhage during the the observation period.
(E) Same view as panel D, with clot removed. Bandage was completely covering and adherent to the injury site.







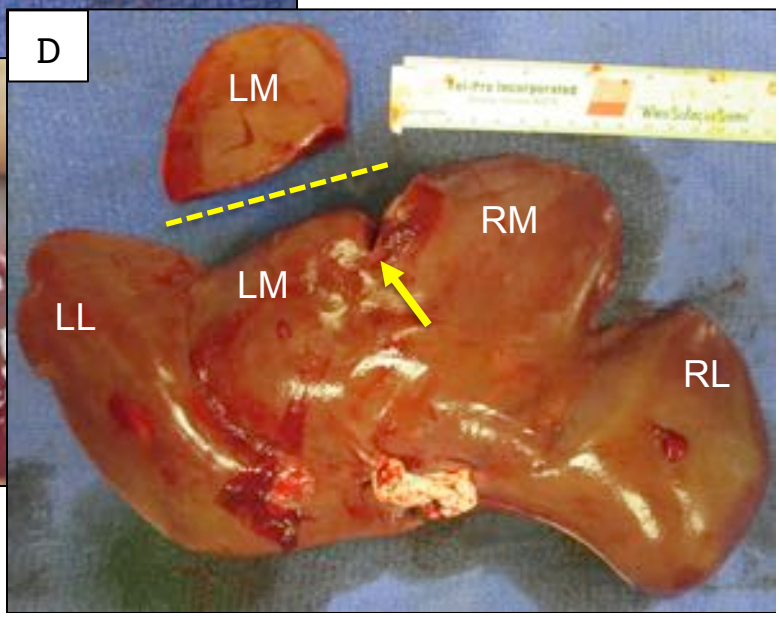


Fig. 3, swine 314. Necropsy.
(A) Peel-off of PCL from injury site. PCL was fully adherent, no gaps.
(B) PCL, removed from injury. Side facing the wound. Note outline on bandage (arrows), where PCL had been apposed to liver parenchyma.
(C) Close-up of injury site, with PCL and fibrin removed. Transected PV branch (single arrow) and HV branch (single arrow) are smaller at this level than when transection made at junction of LM & RM lobes.
(D) Liver, superior surface. PCL bandage has been removed. Arrow = junction of RM & LM lobes; level of transection (dashed yellow line) = 3 cm above this junction. LM resection specimen in approximate anatomic position.

I. OVERVIEW
Date: September 15, 2015
Swine no: 315
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Factor II + Factor XIII
Personnel: Carlson, Hansen, Siford, Spretz, Aravind, Fabian, Ragusa

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II. PRE-RESECTION PHASE

Start time: 07:30 AM
Swine sex: Male
Date swine received from UNL Mead: 09/04/2015
Pre-procedure wt: 40kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 102
- MAP: 106
- Temp: 37.3
- EtCO2: 42

Blood draw no. 1 (initial): 7:55 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:08 AM
Spleen wt: 290.6gm
LR (22°C) infused after splenectomy: 900 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 290.6 + 30 + 7.4= 328 mL
- LR (22°C) infused (spleen replacement + incidental): 900 + 10 = 910 mL

Pre-resection VS
- HR: 99
- MAP: 109

- Temp: 36.7
- EtCO2 : 44

III. RESECTION & TREATMENT PHASE

Time resection began: 08:20AM, closed: 8:30AM (t=0; 8:30 AM)

Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was sprayed with biologics, and applied onto the bleeding resection site. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. Additional biologics then were sprayed onto the exposed PCL surface. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). After the 5 min compression, the shed blood was carefully collected with the sucker, the site photographed, and the incision was closed with towel clips. The injury site appeared hemostatic.

Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm, with Factor II and Factor XIII

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 90

Resuscitation fluid: warm LR given at constant rate of 11.1 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.

Blood loss during resection: 80.4 + 38.6 + 0 (suction + clots + sponges) = 119 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:45 AM

15 min post-resection VS

- HR: 96
- MAP: 85
- Temp: 36.2
- EtCO2: 41
- IAP: 0

Blood draw no 3. (30 min post-resection): 9:00 AM

30 min post-resection VS

- HR: 96
- MAP: 95
- Temp: 35.9
- EtCO2: 41

- IAP: 0

Blood draw no 4. (1 hour post-resection): 9:30 AM

60 min post-resection VS
- HR: 87
- MAP: 81
- Temp: 35.6
- EtCO2: 39
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 9:30AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 58.1 mL (suction) + 0 mL (clot + lap pads) + 90 mL (phlebotomies) = 148.1 mL
- IV fluid given: LR (37°C): 700 mL + 0 mL (ear) + 0 (incidental) = 700 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, minimal amounts of blood and few clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 78.6 (resected LM lobe) +931.4 (remaining liver) =1010 g
Tissue harvested: Clots in formalin for H&E (porcine fibrinogen control for FI IHC)

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage with FII/FXIII, first subject with modified resection treated in this manner. Excellent result, only 120 mL blood loss during resection and very little (~60 mL, pretty much bloody ascites) during 1 h observation. No clots around bandage at end of 1 h, suggesting no leakage.

VII. PLAN

Perform next subject in this series on 09/16/2015.

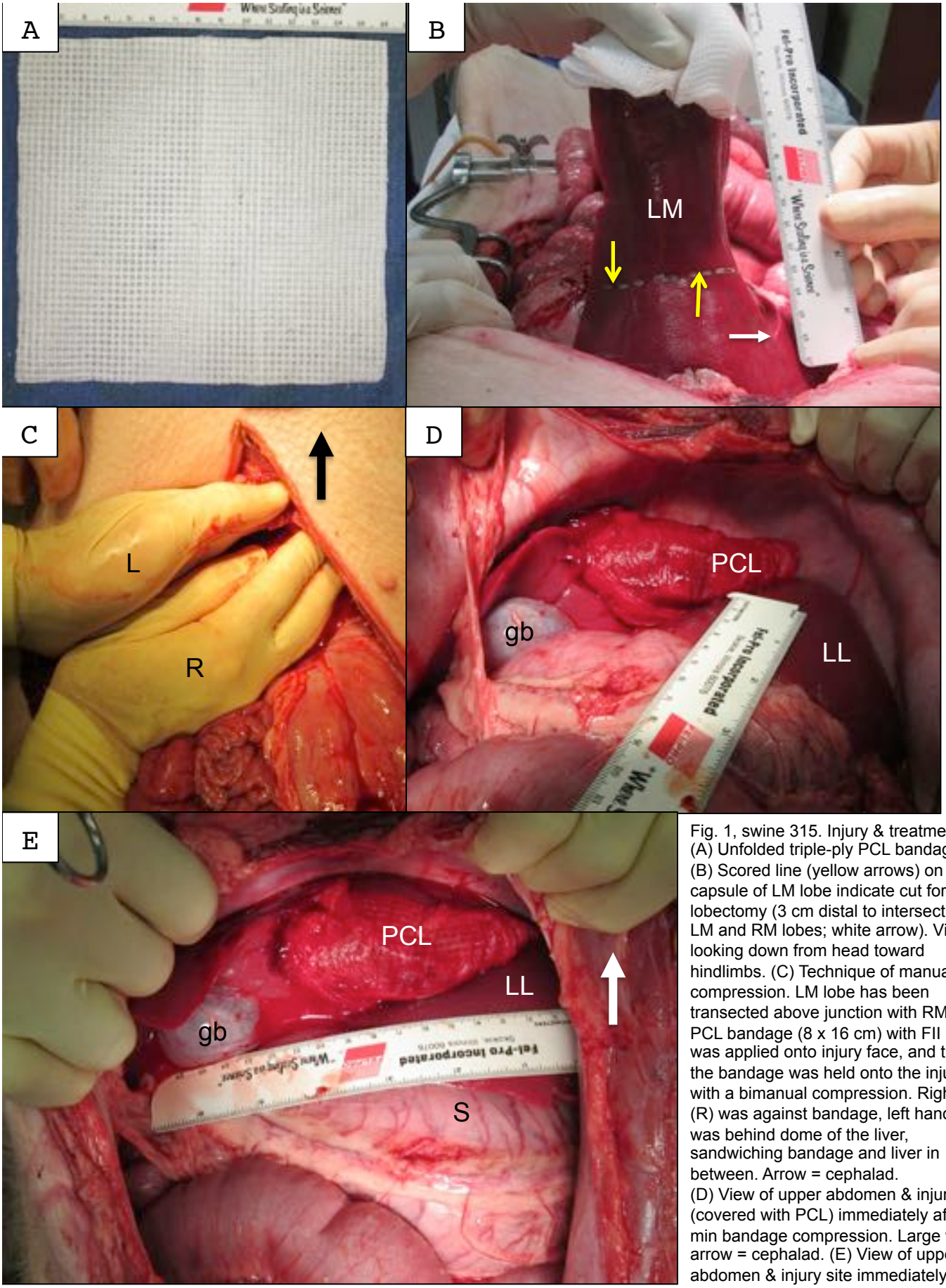

Fig. 1, swine 315. Injury & treatment. (A) Unfolded triple-ply PCL bandage. (B) Scored line (yellow arrows) on capsule of LM lobe indicate cut for partial lobectomy (3 cm distal to intersection of LM and RM lobes; white arrow). View is looking down from head toward hindlimbs. (C) Technique of manual compression. LM lobe has been transected above junction with RM lobe. PCL bandage (8 x 16 cm) with FII + FXIII was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was behind dome of the liver, sandwiching bandage and liver in between. Arrow = cephalad. (D) View of upper abdomen & injury site (covered with PCL) immediately after 5 min bandage compression. Large white arrow = cephalad. (E) View of upper abdomen & injury site immediately after 1 h observation period. Very little blood evident. S = stomach.

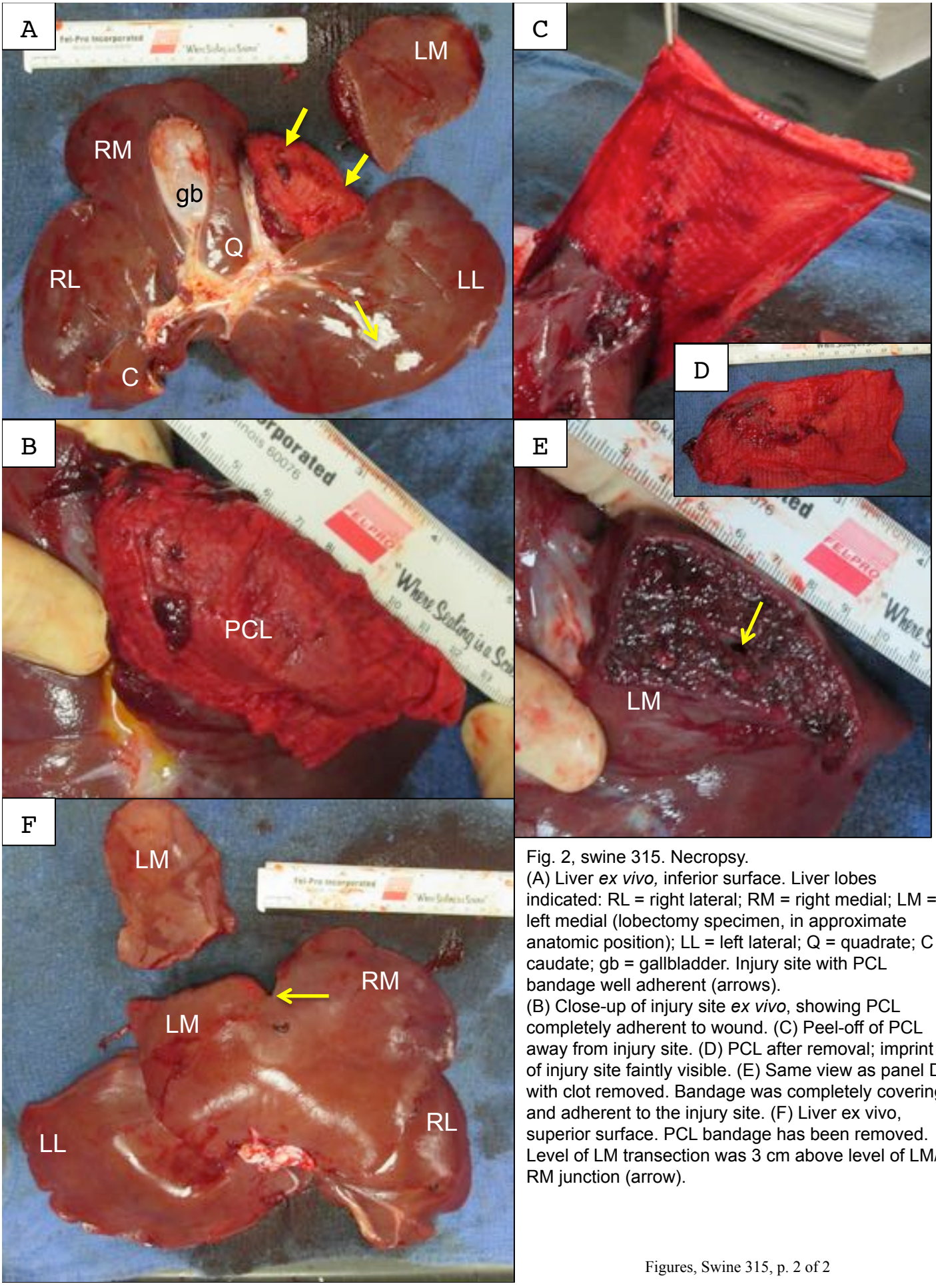

Fig. 2, swine 315. Necropsy.
(A) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, in approximate anatomic position); LL = left lateral; Q = quadrate; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows).
(B) Close-up of injury site *ex vivo*, showing PCL completely adherent to wound. (C) Peel-off of PCL away from injury site. (D) PCL after removal; imprint of injury site faintly visible. (E) Same view as panel D, with clot removed. Bandage was completely covering and adherent to the injury site. (F) Liver ex vivo, superior surface. PCL bandage has been removed. Level of LM transection was 3 cm above level of LM/ RM junction (arrow).

I. OVERVIEW
Date: September 16, 2015
Swine no: 316
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Factor II + Factor XIII
Personnel: Carlson, Hansen, Siford, Spretz, Aravind, Fabian, Ragusa

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II. PRE-RESECTION PHASE

Start time: 08:05 AM
Swine sex: Male
Date swine received from UNL Mead: 09/04/2015
Pre-procedure wt: 40.8kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 106
- MAP: 116
- Temp: 38.6
- EtCO2: 47

Blood draw no. 1 (initial): 8:25 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:35 AM
Spleen wt: 245.3gm
LR (22°C) infused after splenectomy: 750 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 245.3 + 30 + 5.5 = 280.8 mL
- LR (22°C) infused (spleen replacement + incidental): 750 + 0= 750 mL

Pre-resection VS
- HR: 85
- MAP: 110

- Temp: 38
- EtCO2 : 48

III. RESECTION & TREATMENT PHASE

Time resection began: 08:45AM, closed: 8:52AM (t=0; 8:52 AM)

Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was sprayed with biologics, and applied onto the bleeding resection site. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. Additional biologics then were sprayed onto the exposed PCL surface. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). After the 5 min compression, the shed blood was carefully collected with the sucker, the site photographed, and the incision was closed with towel clips. The injury site appeared hemostatic.

Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm, with Factor II and Factor XIII

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 95

Resuscitation fluid: warm LR given at constant rate of 11.3 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.

Blood loss during resection: 74.3 + 129.2 (suction + clots + sponges) = 203.5 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 09:08 AM

15 min post-resection VS

- HR: 96
- MAP: 85
- Temp: 36.2
- EtCO2: 41
- IAP: 0

Blood draw no 3. (30 min post-resection): 9:23 AM

30 min post-resection VS

- HR: 88
- MAP: 76
- Temp: 37.2
- EtCO2: 40

- IAP: 0

Blood draw no 4. (1 hour post-resection): 9:52 AM

60 min post-resection VS- Final VS
- HR: 87
- MAP: 81
- Temp: 35.6
- EtCO2: 39
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Yes
Time of death: 9:52AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 46.1 mL (suction) + 0 mL (clot + lap pads) + 90 mL (phlebotomies) = 136 mL
- IV fluid given: LR (37°C): 550 mL + 0 mL (ear) + 0 (incidental) = 550 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, minimal amounts of blood and no clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 27.5 (resected LM lobe) + 852.9 (remaining liver) = 880.4 g
Tissue harvested: Clots in formalin for H&E (porcine fibrinogen control for FI IHC)

VI. COMMENTS

Compressible injury (LM resection) treated with current iteration of PCL bandage with FII/FXIII, 2nd subject with modified resection treated in this manner. LM lobe was small today, resection specimen weighed only 27 g. Good result, ~200 mL blood loss during resection and very little (~45 mL, pretty much bloody ascites) during 1 h observation. No clots around bandage at end of 1 h, suggesting no leakage. PCL + FII/FXIII appears to work pretty good in this model after N = 2.

VII. PLAN

Perform next subject in this series on Wed 9/23.

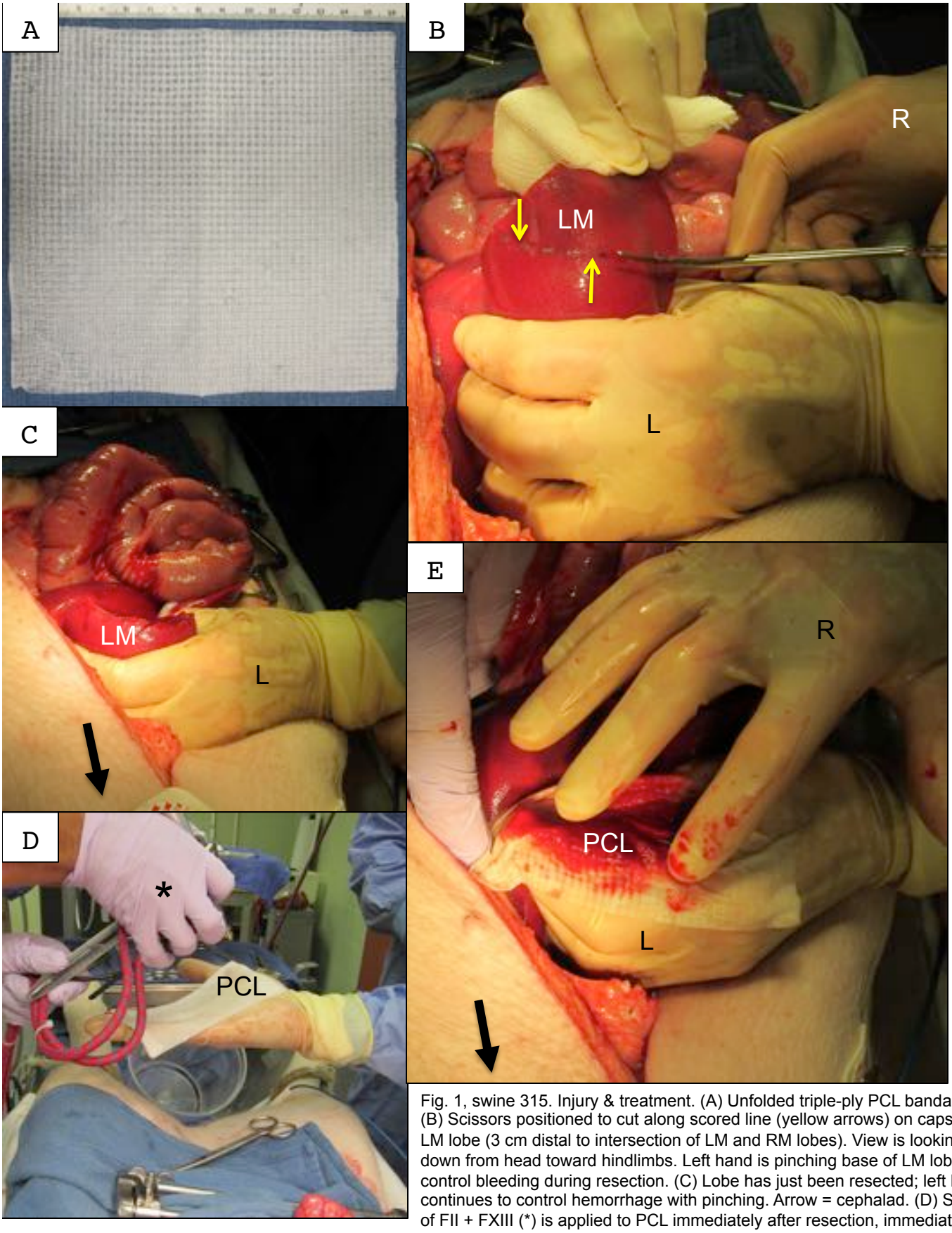


Fig. 1, swine 315. Injury & treatment. (A) Unfolded triple-ply PCL bandage. (B) Scissors positioned to cut along scored line (yellow arrows) on capsule of LM lobe (3 cm distal to intersection of LM and RM lobes). View is looking down from head toward hindlimbs. Left hand is pinching base of LM lobe to control bleeding during resection. (C) Lobe has just been resected; left hand continues to control hemorrhage with pinching. Arrow = cephalad. (D) Spray of FII + FXIII (*) is applied to PCL immediately after resection, immediately prior to PCL application onto injury. (E) PCL wet with clotting factors is applied to injury site; left hand still is pinching base of LM lobe to control hemorrhage. Additional FII + FXIII sprayed on top of PCL after bandage positioned.

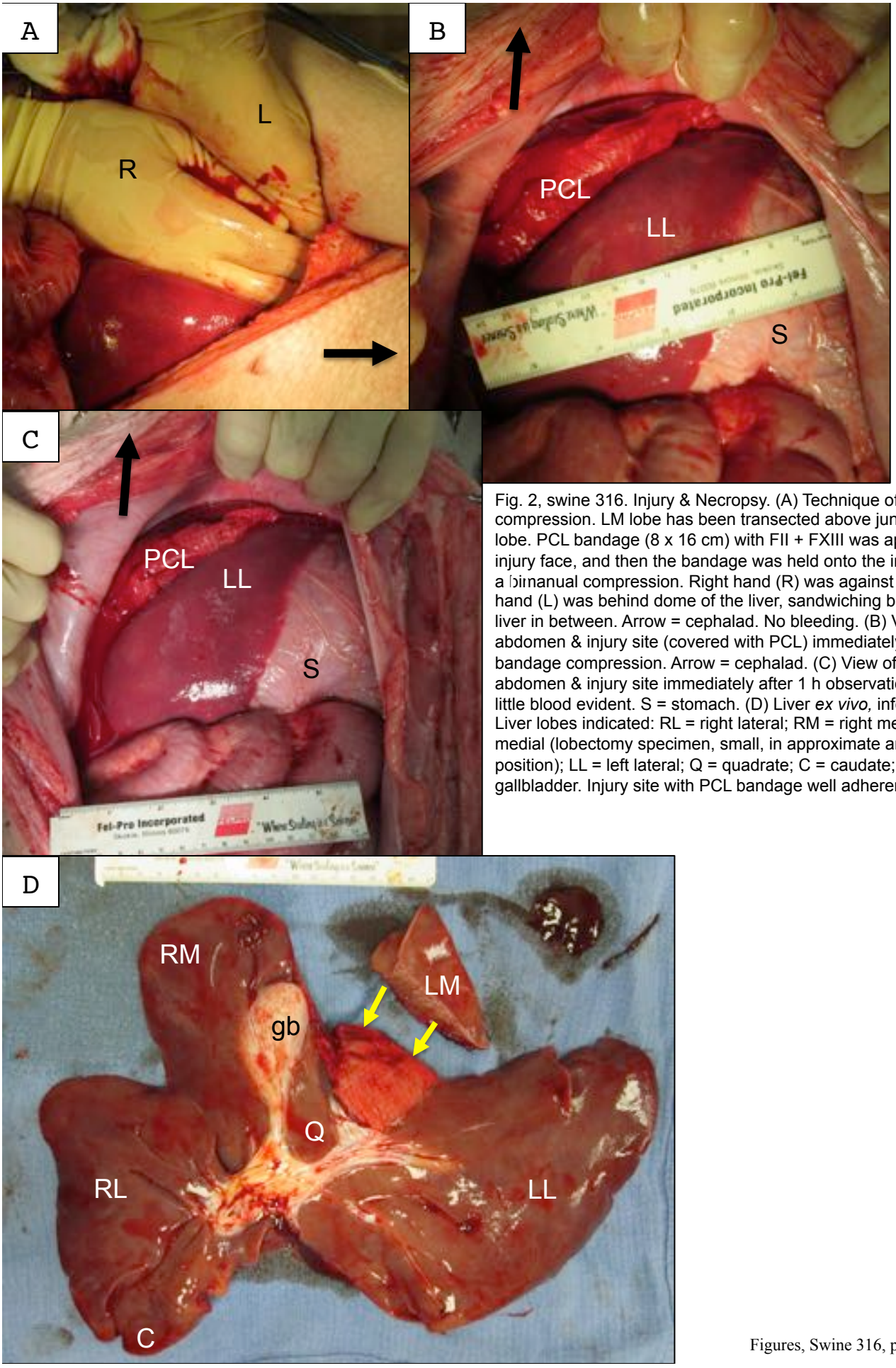


Fig. 2, swine 316. Injury & Necropsy. (A) Technique of manual compression. LM lobe has been transected above junction with RM lobe. PCL bandage (8 x 16 cm) with FII + FXIII was applied onto injury face, and then the bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was behind dome of the liver, sandwiching bandage and liver in between. Arrow = cephalad. No bleeding. (B) View of upper abdomen & injury site (covered with PCL) immediately after 5 min bandage compression. Arrow = cephalad. (C) View of upper abdomen & injury site immediately after 1 h observation period. Very little blood evident. S = stomach. (D) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, small, in approximate anatomic position); LL = left lateral; Q = quadrate; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows).

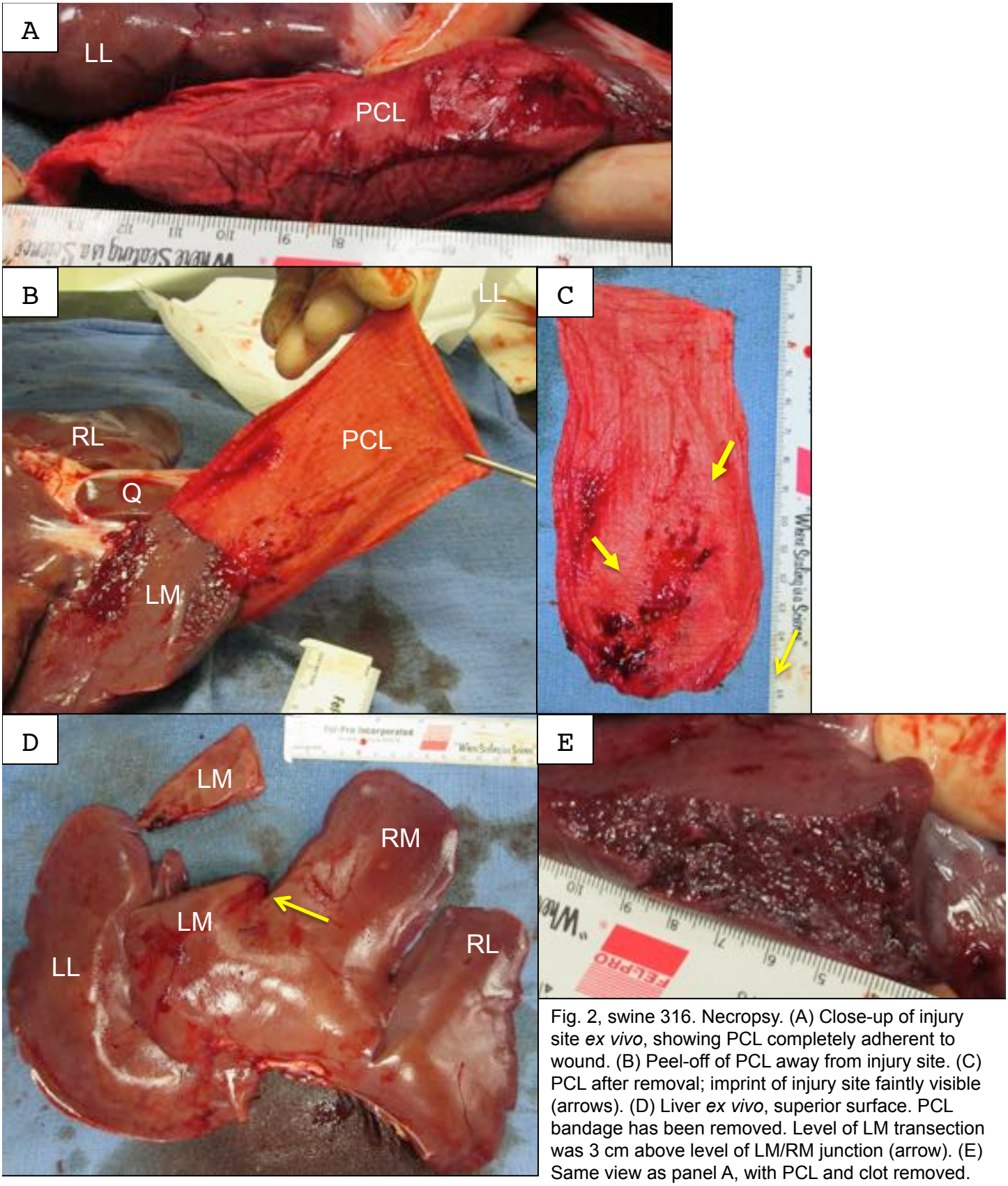


Fig. 2, swine 316. Necropsy. (A) Close-up of injury site *ex vivo*, showing PCL completely adherent to wound. (B) Peel-off of PCL away from injury site. (C) PCL after removal; imprint of injury site faintly visible (arrows). (D) Liver *ex vivo*, superior surface. PCL bandage has been removed. Level of LM transection was 3 cm above level of LM/RM junction (arrow). (E) Same view as panel A, with PCL and clot removed. Bandage was completely covering and adherent to the injury site. Transected PV and HV branches were present, but not visible in this image.

I. OVERVIEW
Date: September 23, 2015
Swine no: 317
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Factor II + Factor XIII
Personnel: Carlson, Hansen, Siford, Aravind, Fabian, Ragusa

II. PRE-RESECTION PHASE

Start time: 07:25 AM
Swine sex: Male
Date swine received from UNL Mead: 09/21/2015
Pre-procedure wt: 35.6kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 119
- MAP: 83
- Temp: 37.9
- EtCO2: 40

Blood draw no. 1 (initial): 8:15 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:05 AM
Spleen wt: 205.5 gm
LR (22°C) infused after splenectomy: 600 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 205.5 + 30 + 16.6 = 252.1 mL
- LR (22°C) infused (spleen replacement + incidental): 600 + 10= 610 mL

Pre-resection VS
- HR: 86
- MAP: 97

- Temp: 36.9
- EtCO2 : 43

III. RESECTION & TREATMENT PHASE

Time resection began: 08:24AM, closed: 8:31AM (t=0; 8:29 AM)

Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was sprayed with biologics, and applied onto the bleeding resection site. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. Additional biologics then were sprayed onto the exposed PCL surface. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). After the 5 min compression, the shed blood was carefully collected with the sucker and sponge, the site photographed, and the incision was closed with towel clips. The injury site appeared hemostatic.

Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm, with Factor II and Factor XIII

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 77

Resuscitation fluid: warm LR given at constant rate of 9.9 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.

Blood loss during resection: 25.8 + 36.1 (suction + sponges) = 61.9 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:45 AM

15 min post-resection VS

- HR: 98
- MAP: 69
- Temp: 36.2
- EtCO2: 52
- IAP: 0

Blood draw no 3. (30 min post-resection): 9:23 AM

30 min post-resection VS

- HR: 96
- MAP: 52
- Temp: 36.2
- EtCO2: 65

- IAP: 0

Blood draw no 4. (1 hour post-resection): 9:30 AM

60 min post-resection VS- Final VS
- HR: 93
- MAP: 53
- Temp: 35.7
- EtCO2: not recorded
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? No
Time of death: 9:31AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 46.1 mL (suction) + 0 mL (clot + lap pads) + 90 mL (phlebotomies) = 136.1 mL
- IV fluid given: LR (37°C): 600 mL + 0 mL (ear) + 0 (incidental) = 600 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, minimal amounts of blood and no clots were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 1, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 48.9 (resected LM lobe) +764.6 (remaining liver) =813.5 g
Tissue harvested: None

VI. COMMENTS

N = 3 with compressible injury (LM resection) treated with current iteration of PCL bandage + FII/FXIII. Blood loss during the resection (the amount lost while we obtained hemostasis) = 62 mL, plus loss during the 60 minute observation period = 46 mL, for a total loss of ~106 mL; this is pretty good. The bandage was adherent to the wound at necropsy, with no signs of leak.

VII. PLAN

Perform next subject in this series on 09/24/2015.

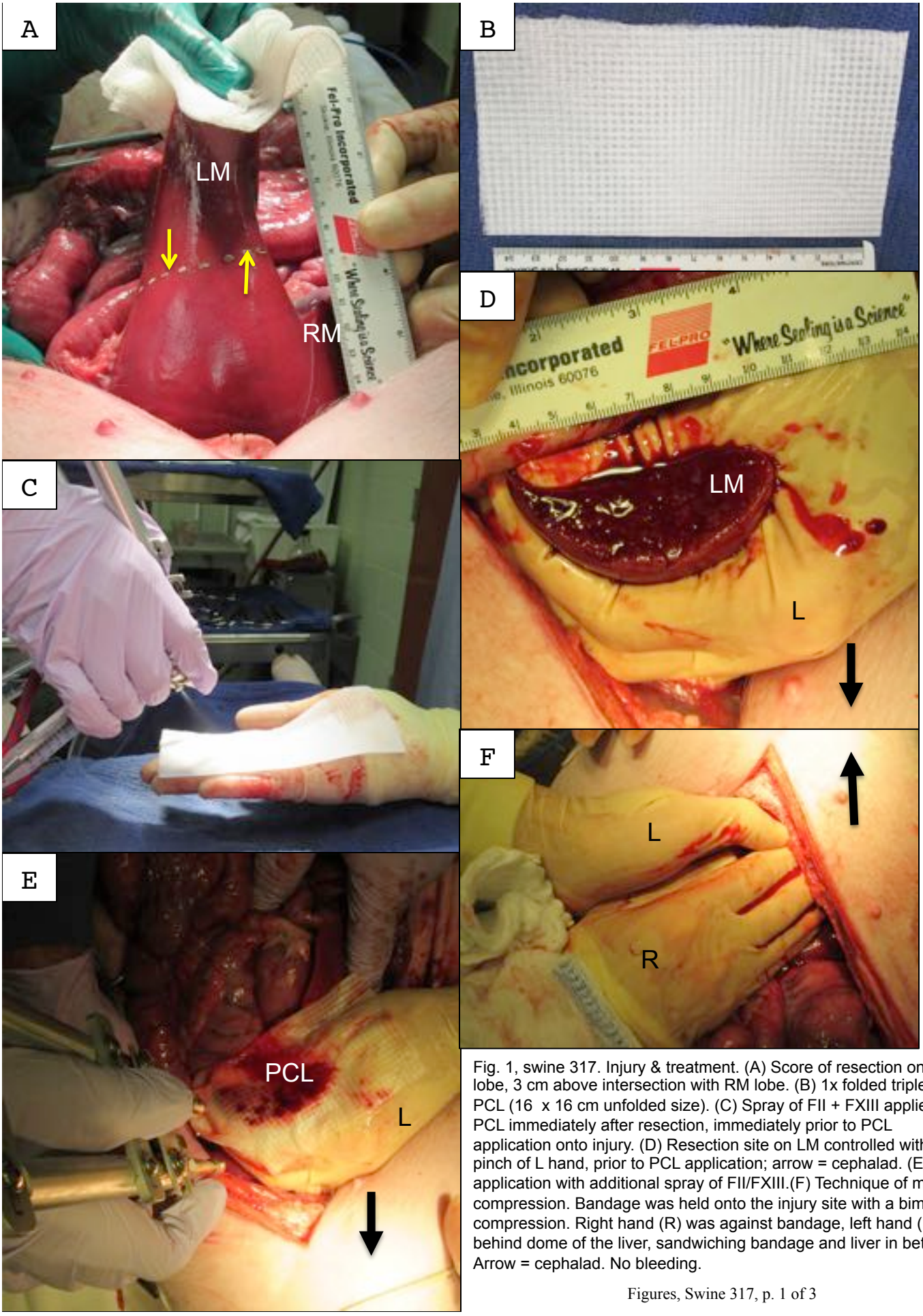


Fig. 1, swine 317. Injury & treatment. (A) Score of resection on LM lobe, 3 cm above intersection with RM lobe. (B) 1x folded triple-ply PCL (16 x 16 cm unfolded size). (C) Spray of FII + FXIII applied to PCL immediately after resection, immediately prior to PCL application onto injury. (D) Resection site on LM controlled with pinch of L hand, prior to PCL application; arrow = cephalad. (E) PCL application with additional spray of FII/FXIII.(F) Technique of manual compression. Bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was behind dome of the liver, sandwiching bandage and liver in between. Arrow = cephalad. No bleeding.

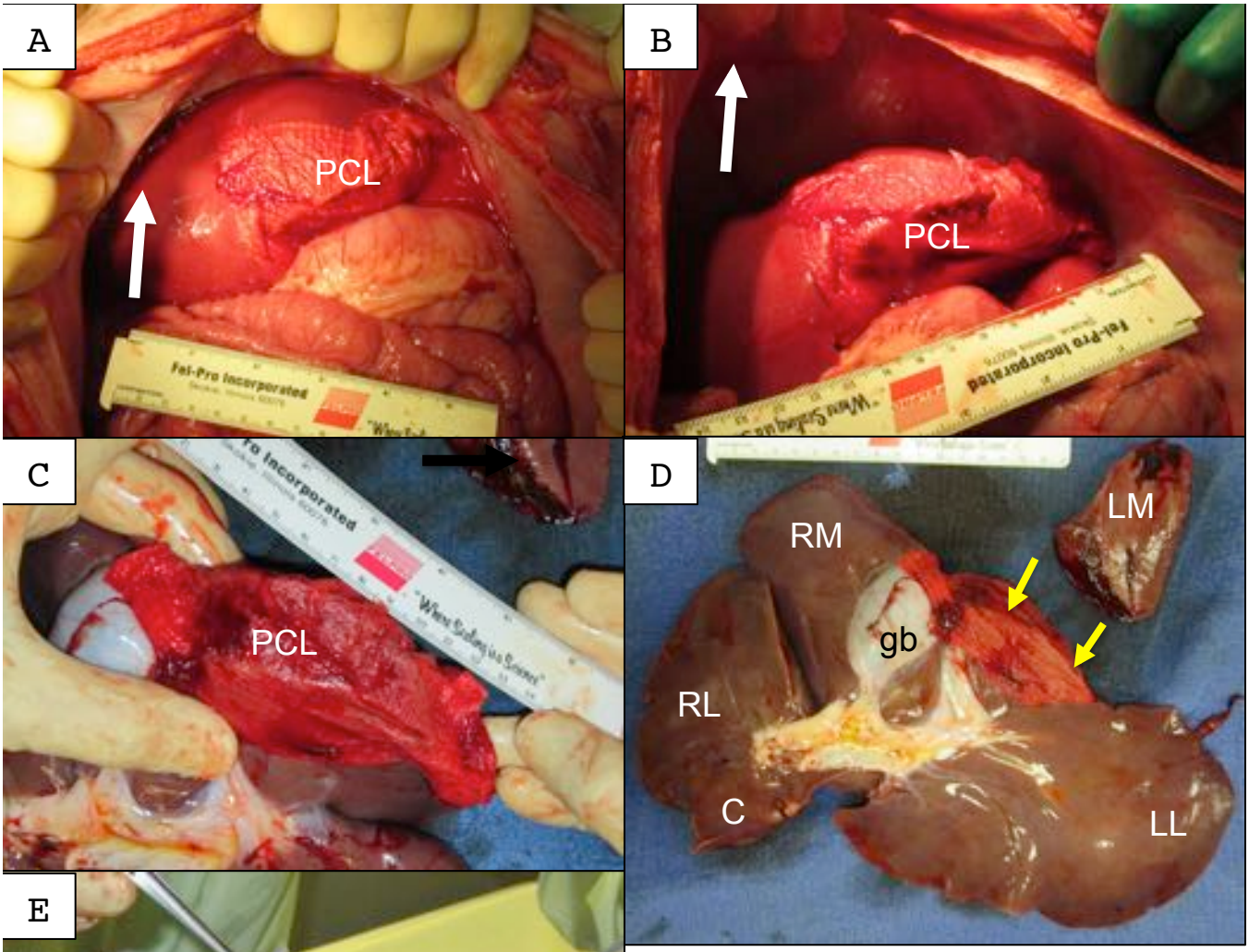


Fig. 2, swine 317. Injury & Necropsy. (A) View of upper abdomen & injury site (covered with PCL) immediately after 5 min bandage compression. Arrow = cephalad. No bleeding. (B) View of upper abdomen & injury site immediately after 1 h observation period. Very little blood evident. (C) Liver *ex vivo*, close-up of injury site with PCL intact and completely adherent. (D) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, small, in approximate anatomic position); LL = left lateral; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows). (E) Peel-off of bandage from injury site using forceps. (F) PCL bandage after peel-of; impression of injury site is visible.

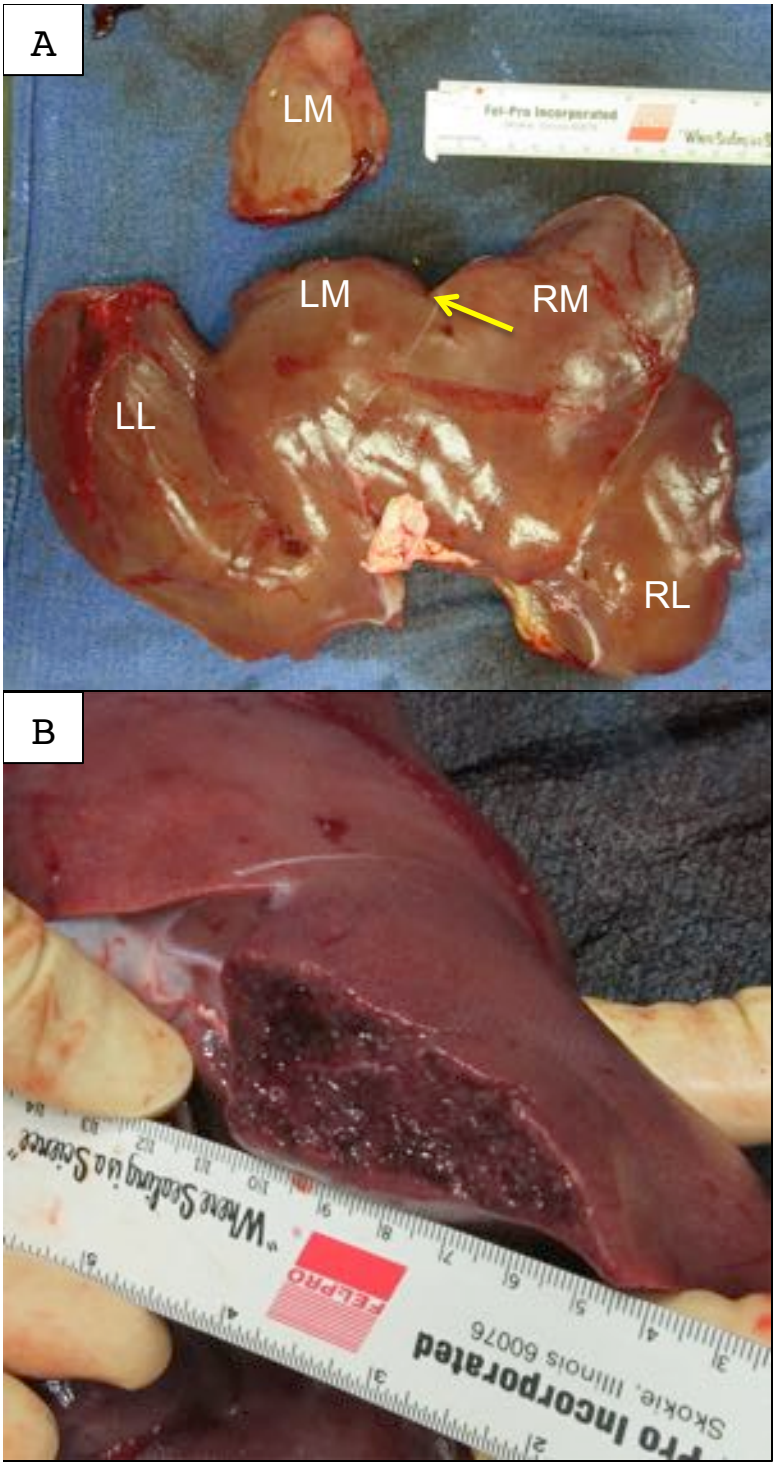


Fig. 3, swine 317. Necropsy. (A) Liver *ex vivo*, superior surface. PCL bandage has been removed. Level of LM transection was 3 cm above level of LM/RM junction (arrow). (B) Close-up of injury site. Transected PV and HV branches not obvious in this photograph.

I. OVERVIEW
Date: September 24, 2015
Swine no: 318
IACUC Protocol no. 01005
Model: Swine, normothermic, normovolemic, liver lobe resection
Treatment: PCL Bandage + Factor II + Factor XIII
Personnel: Carlson, Hansen, Siford, Aravind, Fabian, Ragusa

II. PRE-RESECTION PHASE

Start time: 07:35 AM
Swine sex: Male
Date swine received from UNL Mead: 09/21/2015
Pre-procedure wt: 30.8kg

Anesthetic Induction: Telazol (300 mg), Ketamine (90 mg), Xylazine (180 mg), given as single IM shot
Anesthetic maintenance: 0.5-1.0% inhalational isoflurane

Lines/tubes/monitors/support
1. Endotracheal tube with ETCO2 monitor
2. EKG clips
3. Left ear vein angiocath (20g) for supplemental LR
4. Right carotid artery angiocath (20g), cutdown; for BP monitor
5. Right jugular vein angiocath (16g), cutdown; connected to rapid infusion pump
6. Transabdominal cystotomy for 16 Fr Foley catheter
7. Rectal temp probe
8. Pulse oximetry
9. Heating pad below subject

Initial VS
- HR: 135
- MAP: 72
- Temp: 37.2
- EtCO2: 38

Blood draw no. 1 (initial): 8:10 AM (ABG, CMP/Lactate, hematocrit/hemoglobin, PT/PTT, qualitative fibrinogen)

Splenectomy time: 08:15 AM
Spleen wt: 178.3 g
LR (22°C) infused after splenectomy: 550 mL at 100 mL/min

Pre-resection fluid data:
- Blood loss (spleen weight + phlebotomies + incidental): 178.3 + 30 + 12.2 = 220.5 mL
- LR (22°C) infused (spleen replacement + incidental): 550 + 5= 555 mL

Pre-resection VS
- HR: 88
- MAP: 112

- Temp: 36.4
- EtCO2 : 42

III. RESECTION & TREATMENT PHASE

Time resection began: 08:27AM, closed: 8:32AM (t=0; 8:29 AM)

Resection type: hepatic left medial lobectomy, nonanatomical. The left medial lobe of the liver was exteriorized from the abdomen (see Figs), and a line of resection on the LM lobe 3 cm distal from the junction with RM lobe was scored with the cautery. The LM lobe then was transected at this score using a short curved single scissors (4 cm blade). The base of the LM lobe was controlled during the resection with a pincer grip of the index finger and thumb of the left hand, in order to minimize procedural blood loss. Immediately after resection, the PCL bandage (16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm) was sprayed with biologics, and applied onto the bleeding resection site. In detail: after the lobe was cut off along the score, the pincer grip staunched the bleeding, the PCL was applied flat onto the resection face, allowed to soak in some blood, and the PCL's final position was checked. Additional biologics then were sprayed onto the exposed PCL surface. The pincer grip then was removed, and the bimanual compression was immediately applied (see Figs). So there was a several second interval between release of the pincer grip to application of the bimanual compression during which unchecked bleeding occurred. This potentially could disrupt the bandage from the wound bed. The resected liver lobe was removed from the abdomen. I held pressure for 5 min, compressing the bandage to the injury with firm bimanual compression (see Figs). After the 5 min compression, the shed blood was carefully collected with a sponge, the site photographed, and the incision was closed with towel clips. The injury site appeared hemostatic.

Bandage treatment: PCL, soft open-pore mesh, 16 x 16 cm triple-ply, folded once to final dimensions of 8 x 16 cm, with Factor II and Factor XIII

Abdominal closure: "fully-closed" technique, as described above

Resuscitation target MAP: 95

Resuscitation fluid: warm LR given at constant rate of 8.6 mL/min (wt in kg X 50 mL/180 = mL/min), continuously during the observation period, or until animal expires. Begun at MAP less than target MAP.

Blood loss during resection: 0 + 40.3 (suction + sponges) = 40.3 mL

IV. POST-TREATMENT PHASE

Blood draw no. 2 (15 min post-resection): 08:45 AM

15 min post-resection VS

- HR: Error
- MAP: 73
- Temp: 35.5
- EtCO2: 40
- IAP: 0

Blood draw no 3. (30 min post-resection): 9:00 AM

30 min post-resection VS

- HR: Error
- MAP: 82
- Temp: 35.2
- EtCO2: 40

- IAP: 0

Blood draw no 4. (1 hour post-resection): 9:30 AM

60 min post-resection VS- Final VS
- HR: Error
- MAP: 48
- Temp: 34.7
- EtCO2: 30
- IAP: 0

Survival at 60 min? Yes
Target MAP attained? Briefly for initial 5 minutes only
Time of death: 9:32AM
Cause of death: Exsanguination from euthanasia (transection of supradiaphragmatic IVC)
Interval from completion of resection to death: 60 min

Post-treatment fluid data:
- Blood loss: 28 mL (suction) + 0 mL (clot + lap pads) + 90 mL (phlebotomies) = 118 mL
- IV fluid given: LR (37°C): 450 mL + 0 mL (ear) + 0 (incidental) = 450 mL

V. RE-EXPLORATION/POST-MORTEM PHASE

Findings upon abdominal/chest exploration: abdomen not distended, soft. Upon re-opening abdomen, minimal amounts of blood were seen (see Figs). No active hemorrhage from resection site (see Figs).
Heart: not examined.
Number of hepatic veins ligated: 2, to LM lobe.
Portal vein(s) ligated: 1 major branch, to LM lobe
Other: none
Ex vivo liver wt: 66.1 (resected LM lobe) + 756 (remaining liver) =822.1 g
Tissue harvested: None

VI. COMMENTS

N = 4 with compressible injury (LM resection) treated with current iteration of PCL bandage + FII/FXIII. Blood loss during the resection (the amount lost while we obtained hemostasis) = 40 mL, plus loss during the 60 minute observation period = 28 mL, for a total loss of ~68 mL, which was even better than previous subject (#317). The bandage was adherent to the wound at necropsy, with no signs of leak.

VII. PLAN

Perform next subject in this series in November (schedule pending).

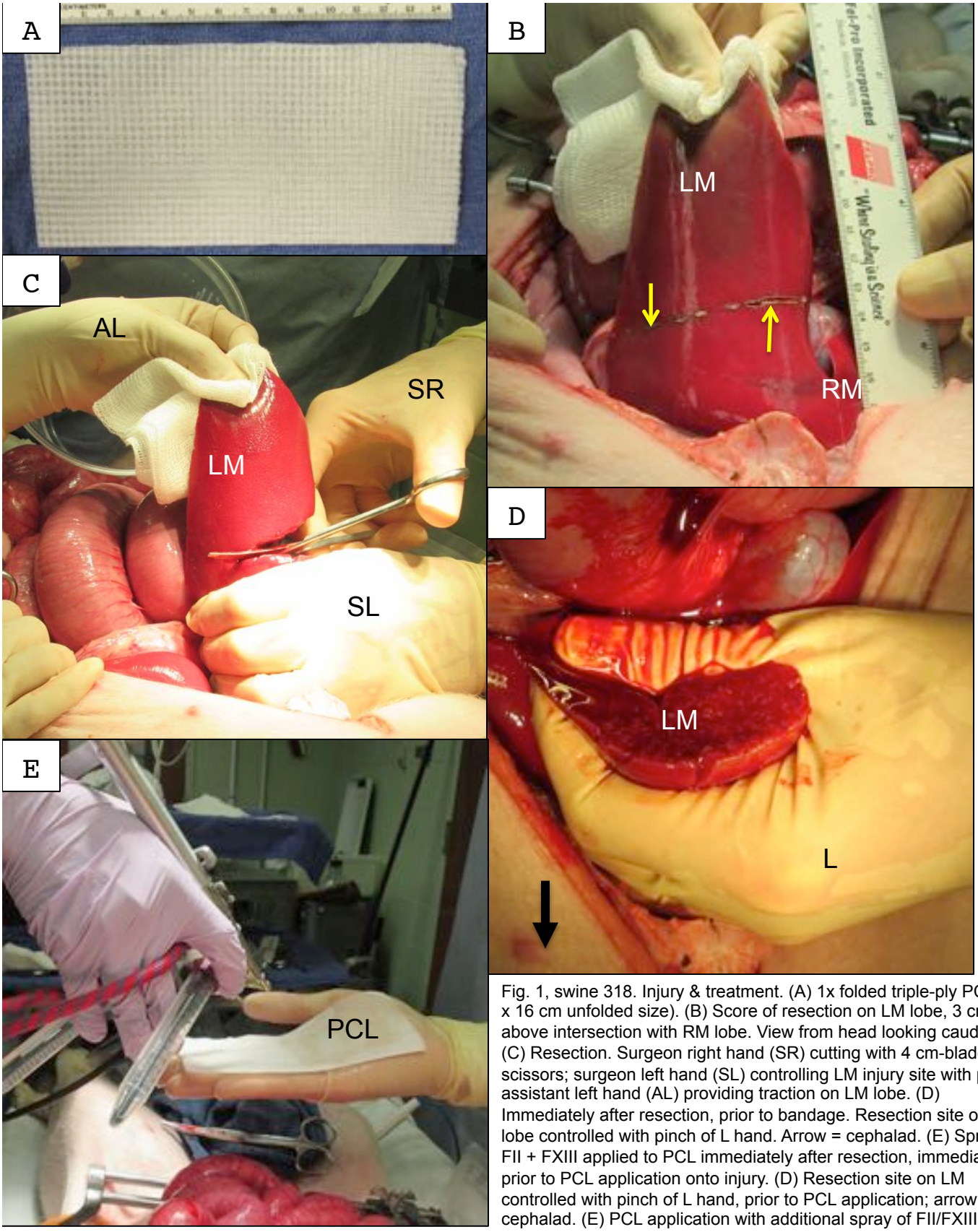
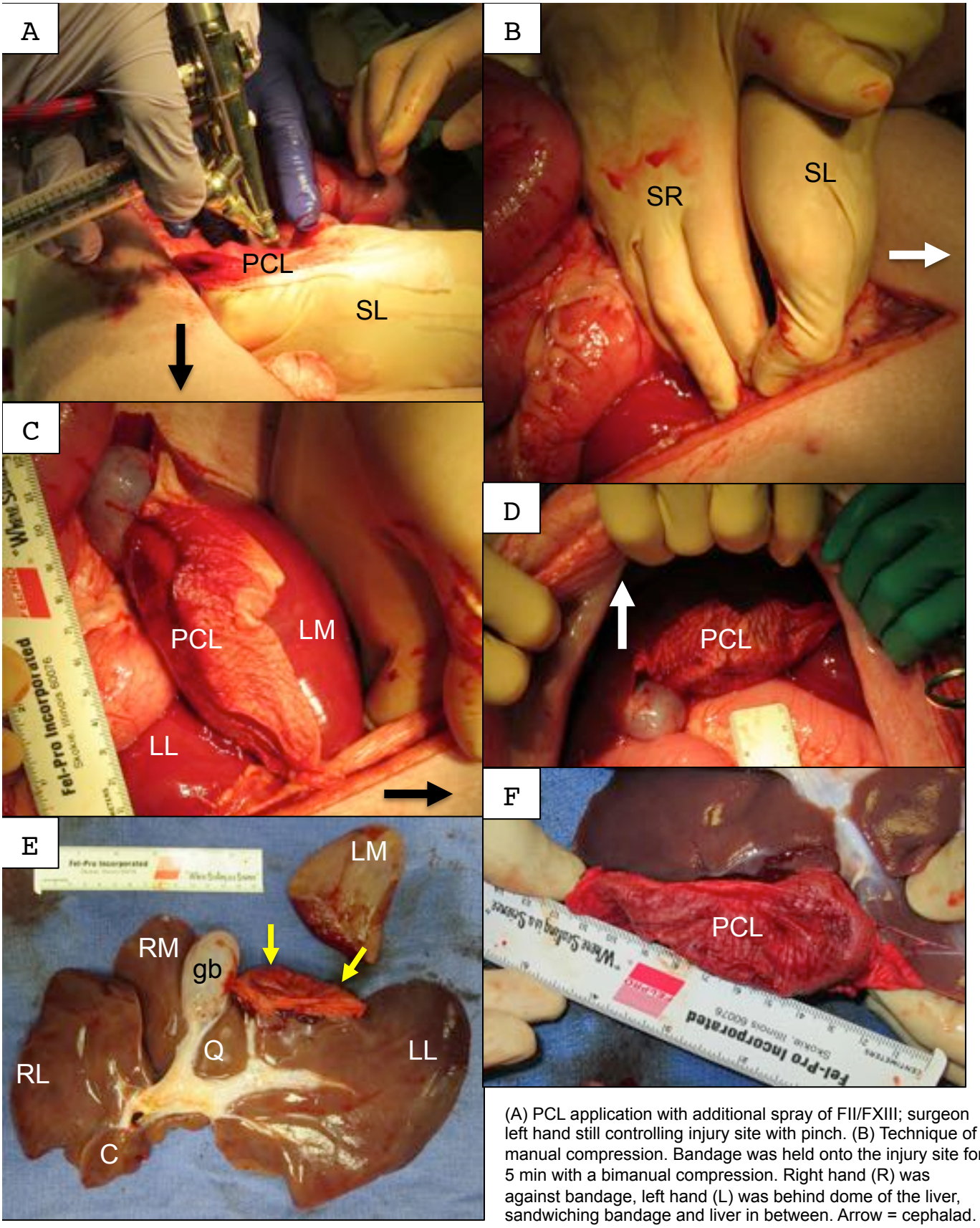


Fig. 1, swine 318. Injury & treatment. (A) 1x folded triple-ply PCL (16 x 16 cm unfolded size). (B) Score of resection on LM lobe, 3 cm above intersection with RM lobe. View from head looking caudad. (C) Resection. Surgeon right hand (SR) cutting with 4 cm-blade scissors; surgeon left hand (SL) controlling LM injury site with pinch; assistant left hand (AL) providing traction on LM lobe. (D) Immediately after resection, prior to bandage. Resection site on LM lobe controlled with pinch of L hand. Arrow = cephalad. (E) Spray of FII + FXIII applied to PCL immediately after resection, immediately prior to PCL application onto injury. (D) Resection site on LM controlled with pinch of L hand, prior to PCL application; arrow = cephalad. (E) PCL application with additional spray of FII/FXIII.(F) Technique of manual compression. Bandage was held onto the injury site with a bimanual compression. Right hand (R) was against bandage, left hand (L) was behind dome of the liver, sandwiching bandage and liver in between. Arrow = cephalad. No bleeding.



(A) PCL application with additional spray of FII/FXIII; surgeon left hand still controlling injury site with pinch. (B) Technique of manual compression. Bandage was held onto the injury site for 5 min with a bimanual compression. Right hand (R) was against bandage, left hand (L) was behind dome of the liver, sandwiching bandage and liver in between. Arrow = cephalad. No bleeding. (C) View of upper abdomen & injury site (covered with PCL) immediately after 5 min bandage compression. Arrow = cephalad. No bleeding. (D) View of upper abdomen & injury site immediately after 1 h observation period. Very little blood evident. (E) Liver *ex vivo,* inferior surface. Liver lobes indicated: RL = right lateral; RM = right medial; LM = left medial (lobectomy specimen, small, in approximate anatomic position); LL = left lateral; Q = quadrate; C = caudate; gb = gallbladder. Injury site with PCL bandage well adherent (arrows). (F)Liver *ex vivo*, close-up of injury site with PCL intact and completely adherent.

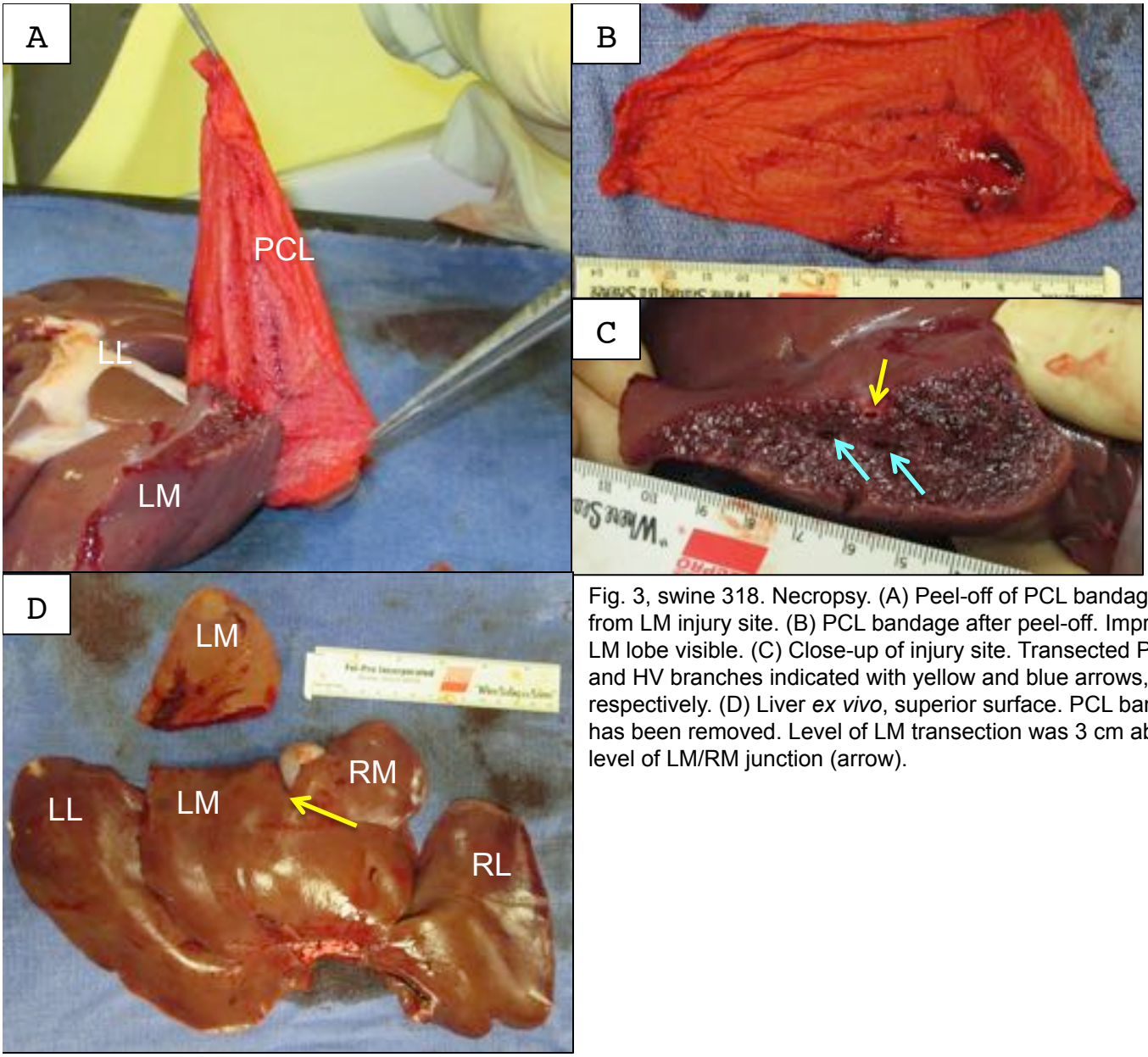


Fig. 3, swine 318. Necropsy. (A) Peel-off of PCL bandage from LM injury site. (B) PCL bandage after peel-off. Imprint of LM lobe visible. (C) Close-up of injury site. Transected PV and HV branches indicated with yellow and blue arrows, respectively. (D) Liver *ex vivo*, superior surface. PCL bandage has been removed. Level of LM transection was 3 cm above level of LM/RM junction (arrow).

DEPARTMENT OF THE ARMY
U.S. ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
820 CHANDLER STREET
FORT DETRICK MD 21702-5014

September 15, 2015

SUBJECT: BA150221 - "Locoregional Treatment Of (1) Noncompressible Truncal Hemorrhage and (2) Coagulopathic Hemorrhage"

Mark Carlson
Nebraska, University of, Medical Center
4101 Woolworth Ave
Surgery 112, VAMC
Omaha, NE 68105

Dear Dr. Carlson:

The decision to invite/not invite your submission to the Fiscal Year 2015 (FY15) Department of Defense Broad Agency Announcement (BAA) for Extramural Medical Research is being deferred at this time. There are several reasons that a decision may be deferred, and a deferment is not an indication that your submission is more or less likely to be invited. Your submission will remain under review by the U.S. Army Medical Research and Materiel Command (USAMRMC) scientific staff and you will be notified of the final decision within 60 days.

We regret any inconvenience caused by this delay in completing our process. While you may withdraw your submission from further consideration, we hope that you will not do so.

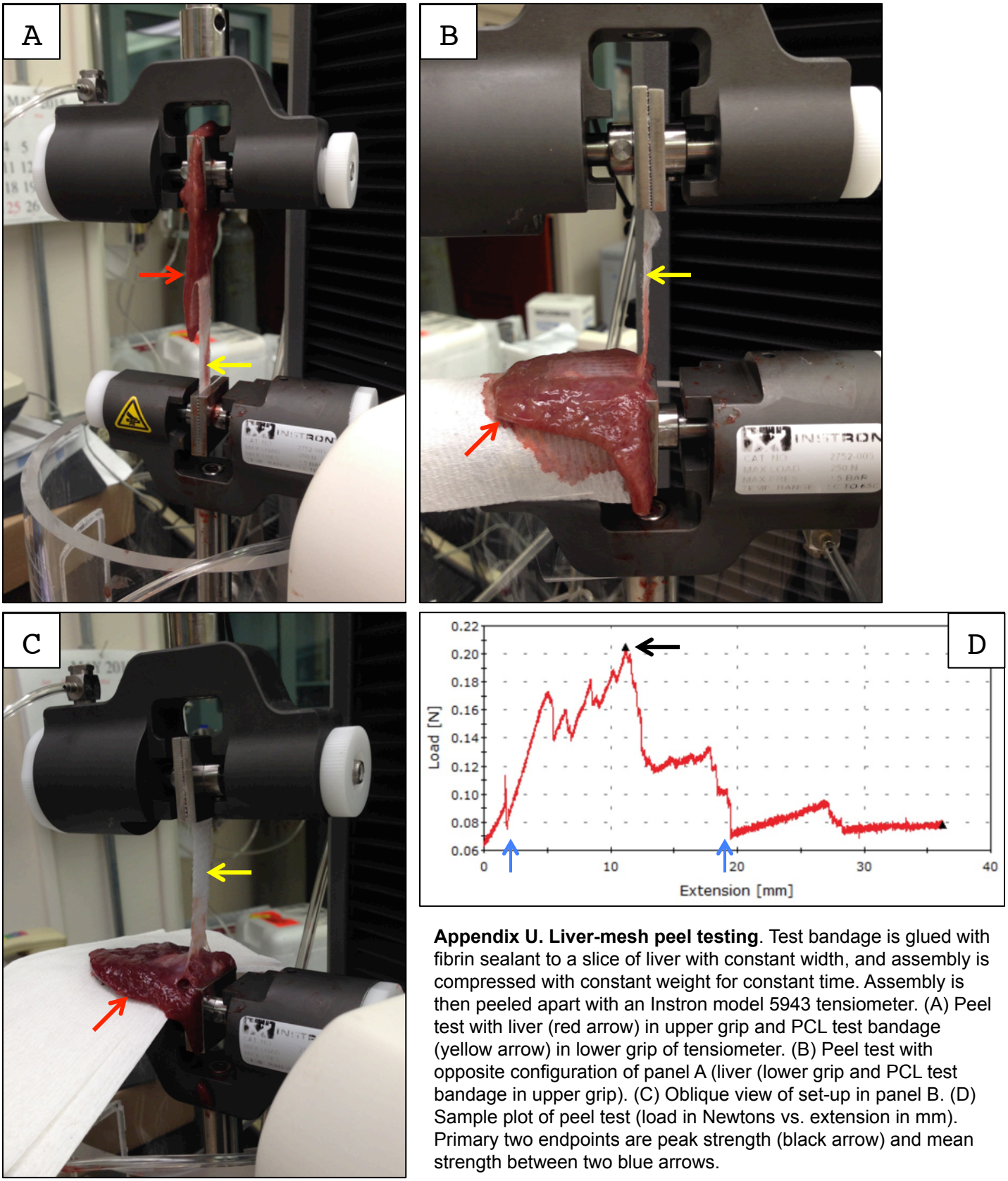
The USAMRMC appreciates your interest in our research program and the effort you have made in preparing your submission. Should you decide to withdraw your submission, please contact our help desk at help@eBRAP.org or 301-682-5507.

Sincerely,

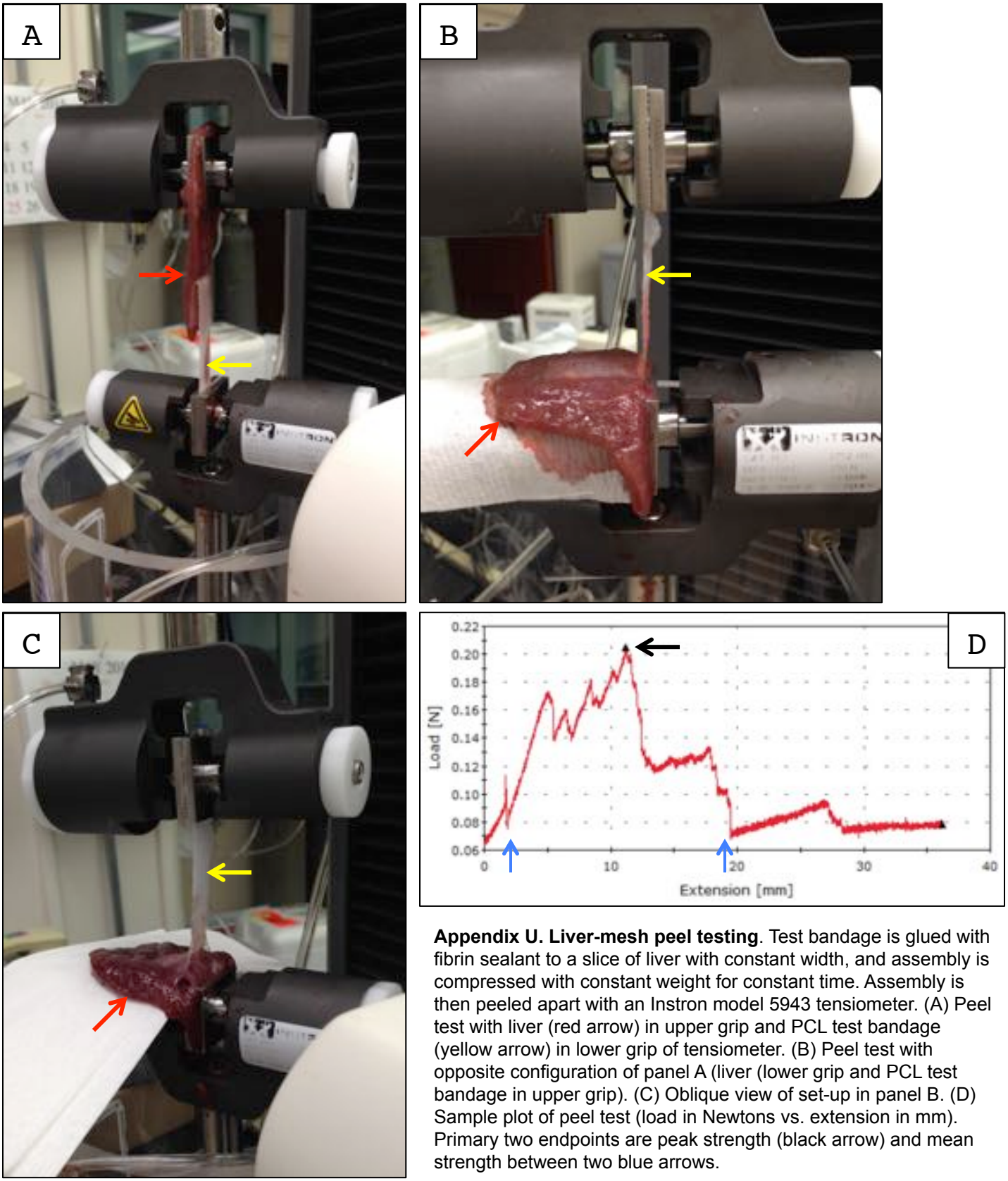
Laurie E. Hovermale

Laurie E. Hovermale
Chief, Business Operations Division

Copy furnished:
MCMR-RTC



Appendix U. Liver-mesh peel testing. Test bandage is glued with fibrin sealant to a slice of liver with constant width, and assembly is compressed with constant weight for constant time. Assembly is then peeled apart with an Instron model 5943 tensiometer. (A) Peel test with liver (red arrow) in upper grip and PCL test bandage (yellow arrow) in lower grip of tensiometer. (B) Peel test with opposite configuration of panel A (liver (lower grip and PCL test bandage in upper grip). (C) Oblique view of set-up in panel B. (D) Sample plot of peel test (load in Newtons vs. extension in mm). Primary two endpoints are peak strength (black arrow) and mean strength between two blue arrows.



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Abstract Detail

Abstract ID ASC20150945

Title Synthetic Resorbable vs. Cellulose Bandage for Minor Hemorrhage in a Porcine Model

Primary Author - **Ujwal. R. Yanala**, , MBBS [1,2]
Additional Author - **Sandra. Noriega**, [3]
Additional Author - **Ruben. Spretz**, [3]
Additional Author - **Jorge. Ragusa**, [3]
Additional Author - **Luis. Nunez**, [3]
Additional Author - **Gustavo. Larsen**, [3,4]
Senior Author - **Mark. A. Carlson**, MD , FACS [1,2]

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   Lincoln, NE USA
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   Lincoln, NE USA

Classifications

Type	Basic/Translation
Scientific Area	Wound Healing/Fibrosis
Clinical Area	General Surgery

Conflict of Interest Declarations Off Label Use: No

Introduction: Commercially-available topical hemostats for minor hemorrhage incurred during elective surgical procedures are relatively expensive. We believe that more economical synthetic hemostats could be produced. Our objective here was to compare the efficacy and toxicity of a synthetic resorbable hemostatic bandage vs. an analogous commercial product in a porcine model of minor hemorrhage.

Methods: For the nonsurvival efficacy study, anesthetized domestic swine (boars,

Abstract

3 months, 29-40 kg) underwent arterial/venous line placement and splenectomy. A 1 x 8 cm section of liver was resected from the edge of the left lateral lobe, and test bandage (macroporous polycaprolactone mesh, PCL; N = 10) or oxidized regenerated cellulose (ORC; Surgicel®, Ethicon®; N = 10) was applied with manual pressure for 5 minutes. Resuscitation then was performed with warm LR (target MAP = 80% of preinjury), and blood loss was measured 60 min after injury. For the survival toxicity study, a similar resection technique was employed (N = 6 for each material), and necropsy was performed at 30 days to evaluate for bandage toxicity (subject growth, serum chemistry, histology).

Results: Pre-injury weight, VS, and laboratory testing did not differ among groups. Resection mortality was zero. In the efficacy study, there were no differences between the PCL vs. ORC groups in blood loss or other post-injury variables (Table), except that the resuscitation fluid volume in the ORC group was greater. Other results from the efficacy study not shown in the Table include platelet counts and coagulation testing (no significant differences). Other than minor granuloma formation at the implantation site with both PCL and ORC, the survival study did not reveal any measurable toxicity.

Conclusion: The efficacy and toxicity of the PCL test bandage vs. the ORC comparator were not different in a porcine model of minor hepatic hemorrhage. Based on projected costs of production (not shown), the PCL bandage could represent a lower-cost alternative to ORC for the treatment of minor surgical bleeding.

table-29905BA4-EDFB-C89F-E6EB9A09349AB827.jpg - RESIZED for display only

Table. Data (mean ± sd) from efficacy study at the 60 min end-point

Variable	PCL	ORC	unpaired t-test
Resection mass (g)	7.6 ± 1.9	6.7 ± 2.0	0.32
Blood Loss (mL)	93 ± 27	111 ± 55	0.38
LR Resuscitation (mL)	594 ± 425	1952 ± 1363	0.01*
MAP (mm Hg)	89 ± 8	93 ± 11	0.30
Hb (g/dL)	12.8 ± 1.2	12.9 ± 0.9	0.85
Platelets (1,000/µL)	266 ± 56	327 ± 94	0.11

Overview Of Porcine Biomedical Research in Omaha

Mark A. Carlson, MD

University of Nebraska Medical Center
VA Nebraska Western Iowa Healthcare System

Omaha, Nebraska, USA

UNMC BREAKTHROUGHS FOR LIFE.

Nebraska Medicine SERIOUS MEDICINE. EXTRAORDINARY CARE.

VA Nebraska-Western Iowa Health Care System

Animal Genetics, UNL, October 16, 2014

Omaha Labs Currently Using Pigs

- Agrawal (Creighton Biomed Sci)
- Porter (UNMC Cardiology)
- Oleynikov (UNMC Surgery)
- Carlson (UNMC/VA Surgery)

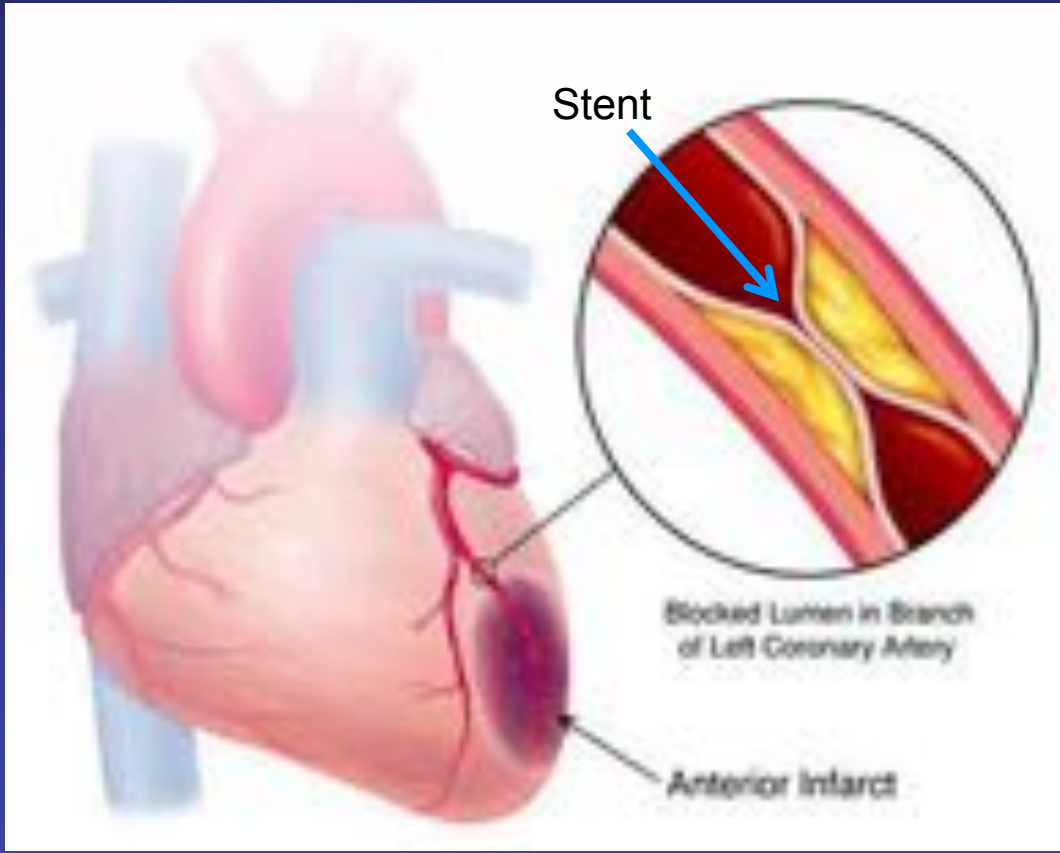
Swine Model of Atherosclerosis: Studies in Coronary Artery Disease

Laboratory of Devendra K. Agrawal

Creighton University School of Medicine

Animal Model Of Coronary Artery Disease

Coronary Artery Disease



http://medicine-science.com

Atherosclerosis

↓

Coronary Lumen

↓

Ischemia

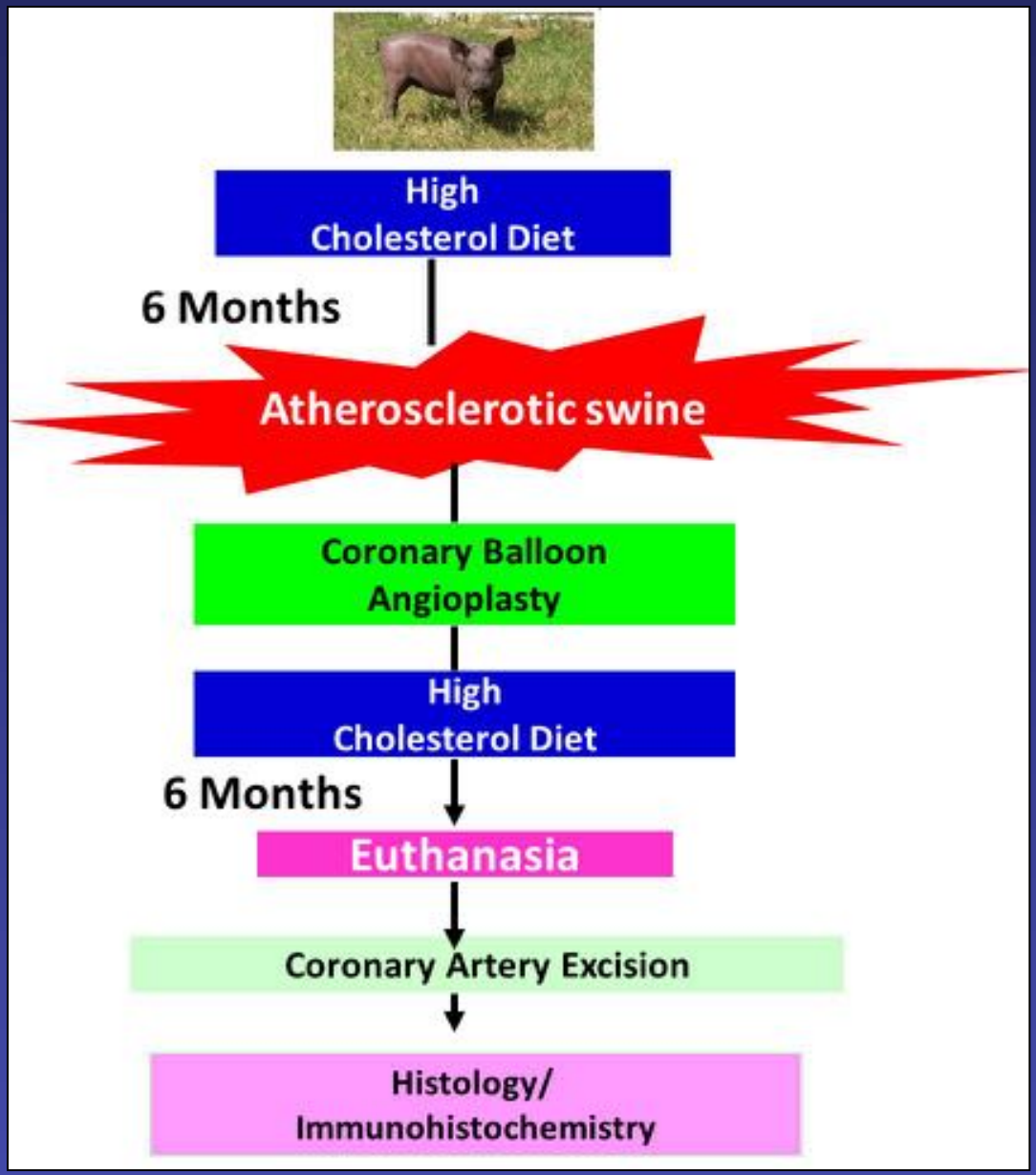
↓

Myocardial Infarction

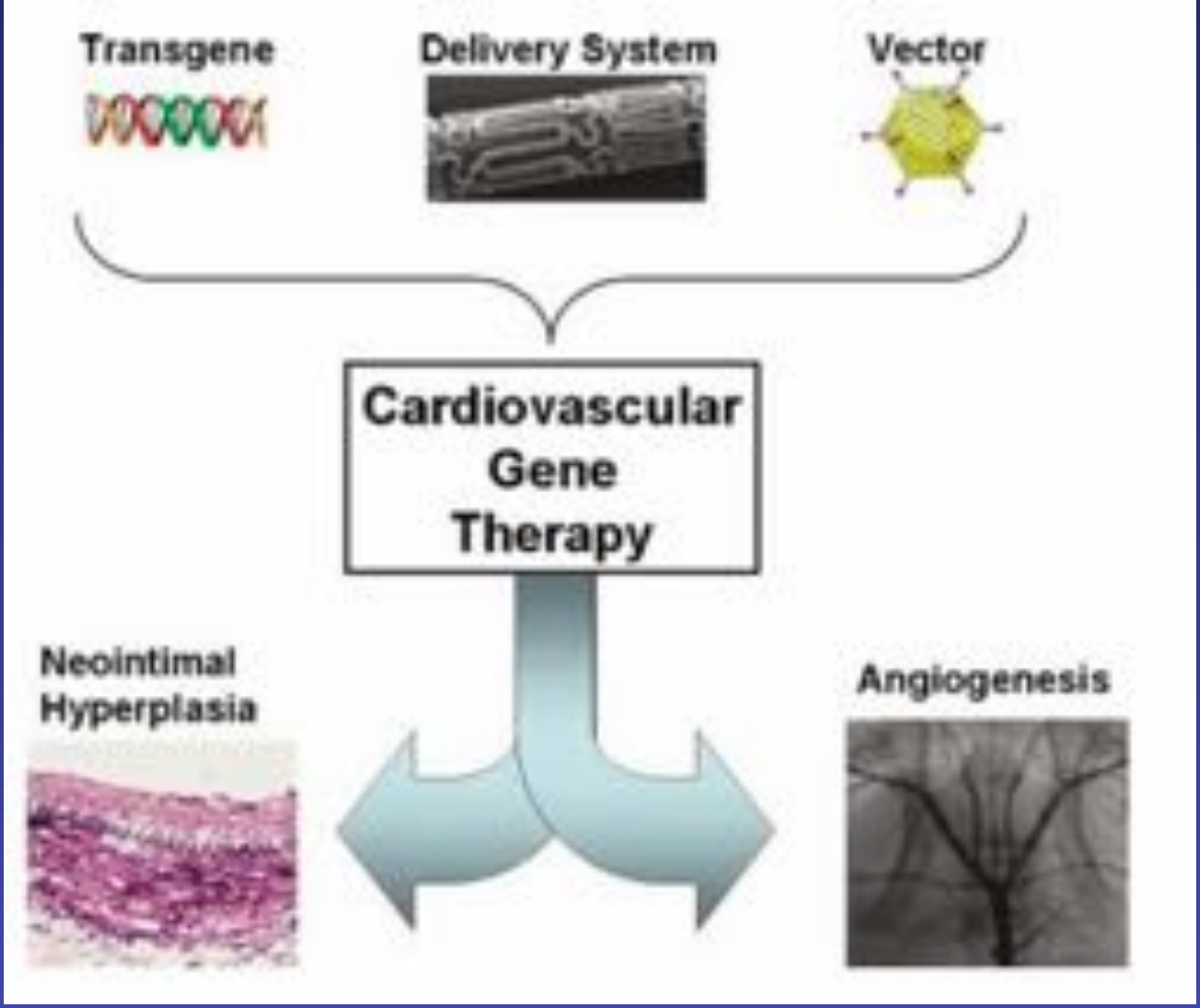
Current NIH-Funded Studies

- Gene therapy to inhibit intimal hyperplasia and in-stent restenosis in coronary arteries (R01HL104516)
- Autologous mesenchymal stem cells (MSCs) to re-endothelialize arteries following angioplasty and intravascular stenting (R01HL112597)
- Role of epicardial adipose tissue in intimal hyperplasia and in-stent restenosis in high-fat high fructose diet (R01HL120659)
- Vitamin D regulating intimal hyperplasia and in-stent restenosis (R01HL116042)

Swine Model of Coronary Restenosis



Cardiovascular Gene Therapy



MM Gaffney et al. Br J Pharmacol 2007; 152: 175–188

Diet Effect

A high-fructose (18-20%) / high-cholesterol diet induces obesity and insulin insensitivity, together with coronary artery disease — i.e., major symptoms of the metabolic syndrome

8 Weeks

High Cholesterol Diet

Atherosclerotic Microswine

High Cholesterol Diet

High Cholesterol High Fructose Diet

Coronary Balloon Angioplasty

Coronary Angioplasty With Bare-metal stent

8, 16 Weeks
Blood Draw : Vitamin D, Lipid Profile, CBC,CRP ,IL-10,TNF-α-sTNFR1

16 Weeks: Angiogram

Histology/IHC

Euthanasia Coronary Artery SMCs

Cell Culture

Goal:

To examine the effect of vitamin D deficiency and vitamin D supplementation on intimal hyperplasia and restenosis following coronary intervention in Yucatan microswine

Blood Draw : Vitamin D, Lipid Profile, CBC, CMP, CRP,IL-6,IL-10,TNF-α, IFN-γ

Vitamin D Deficient High Cholesterol Diet

High Cholesterol Diet

6 Months

Atherosclerotic Microswine

CRP, IL-6 ,CRP,IL-6,IL-10,TNF-α, IFN-γ

Blood Draw : Vitamin D Lipid Profile, CBC,CMP

Coronary Balloon Angioplasty

Coronary Angioplasty With Bare-metal stent

Vitamin D Deficient High Cholesterol diet

High Cholesterol Diet+ 1000 IU Vitamin D

High Cholesterol Diet + 3000 IU Vitamin D

6 Months

Angiogram/ OCT

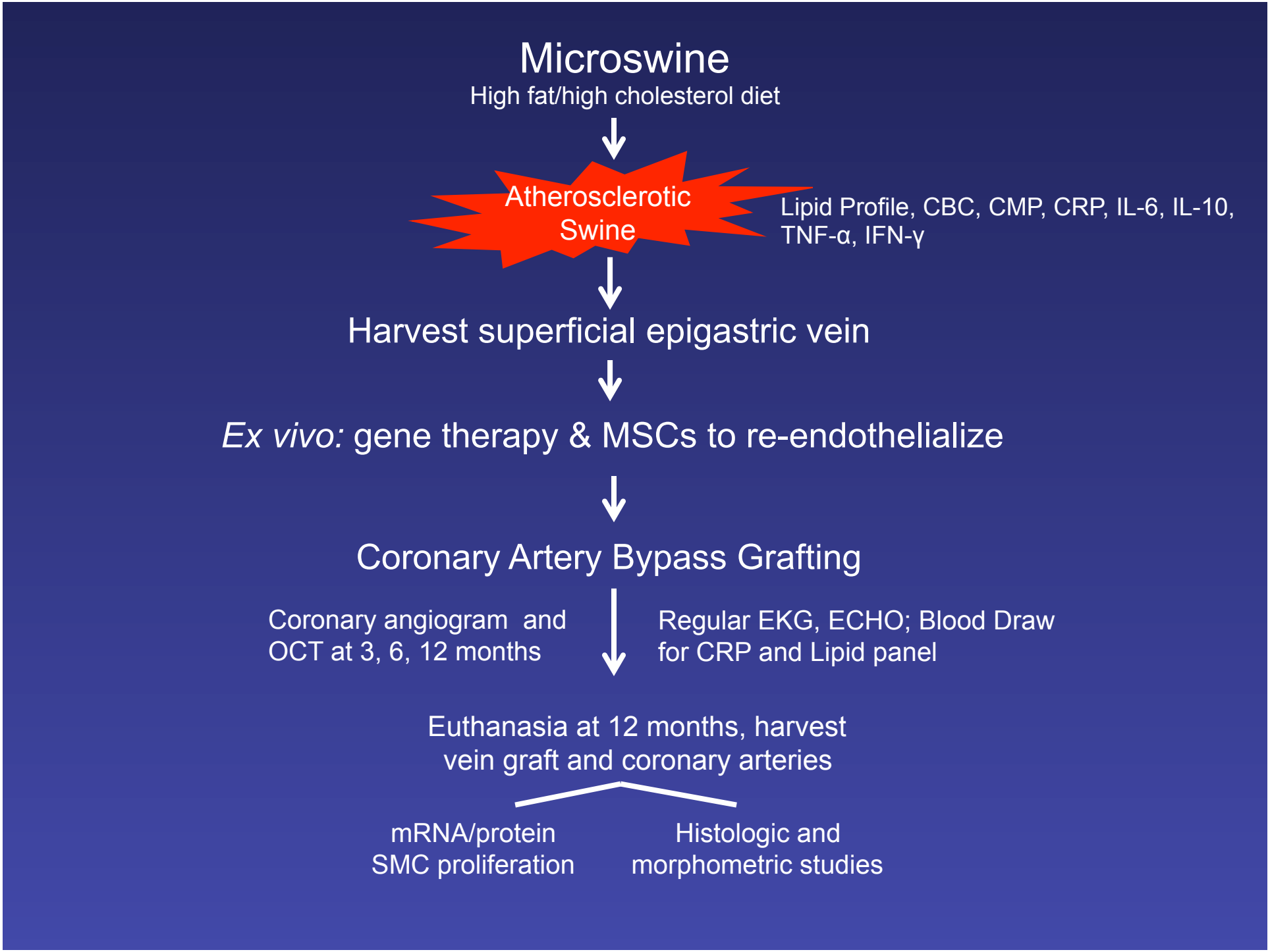
Blood Draw : Vitamin D, Lipid Profile, CBC,CRP,IL-6, IL-10,TNF-α, IFN-γ

Euthanasia

Coronary Histology/ Immunohistochemistry

Porcine Coronary Artery Bypass Graft (CABG) Model

Effect Of Gene Therapy And Stem Cell Therapy In The Prevention Of Vein Graft Disease Following CABG In Atherosclerotic Yucatan Microswine


Microswine
High fat/high cholesterol diet
Atherosclerotic Swine
Lipid Profile, CBC, CMP, CRP, IL-6, IL-10, TNF-α, IFN-γ
Harvest superficial epigastric vein
Ex vivo: gene therapy & MSCs to re-endothelialize
Coronary Artery Bypass Grafting
Coronary angiogram and OCT at 3, 6, 12 months
Regular EKG, ECHO; Blood Draw for CRP and Lipid panel
Euthanasia at 12 months, harvest vein graft and coronary arteries
mRNA/protein SMC proliferation
Histologic and morphometric studies

A Porcine Model of Barrett's Esophagus and Esophageal Adenocarcinoma (under construction)

Uses Of Cardiac Ultrasound:

Myocardial Contrast Echocardiography (MCE); Microbubble Clot Dissolution

Laboratory of Thomas R. Porter

University of Nebraska Medical Center

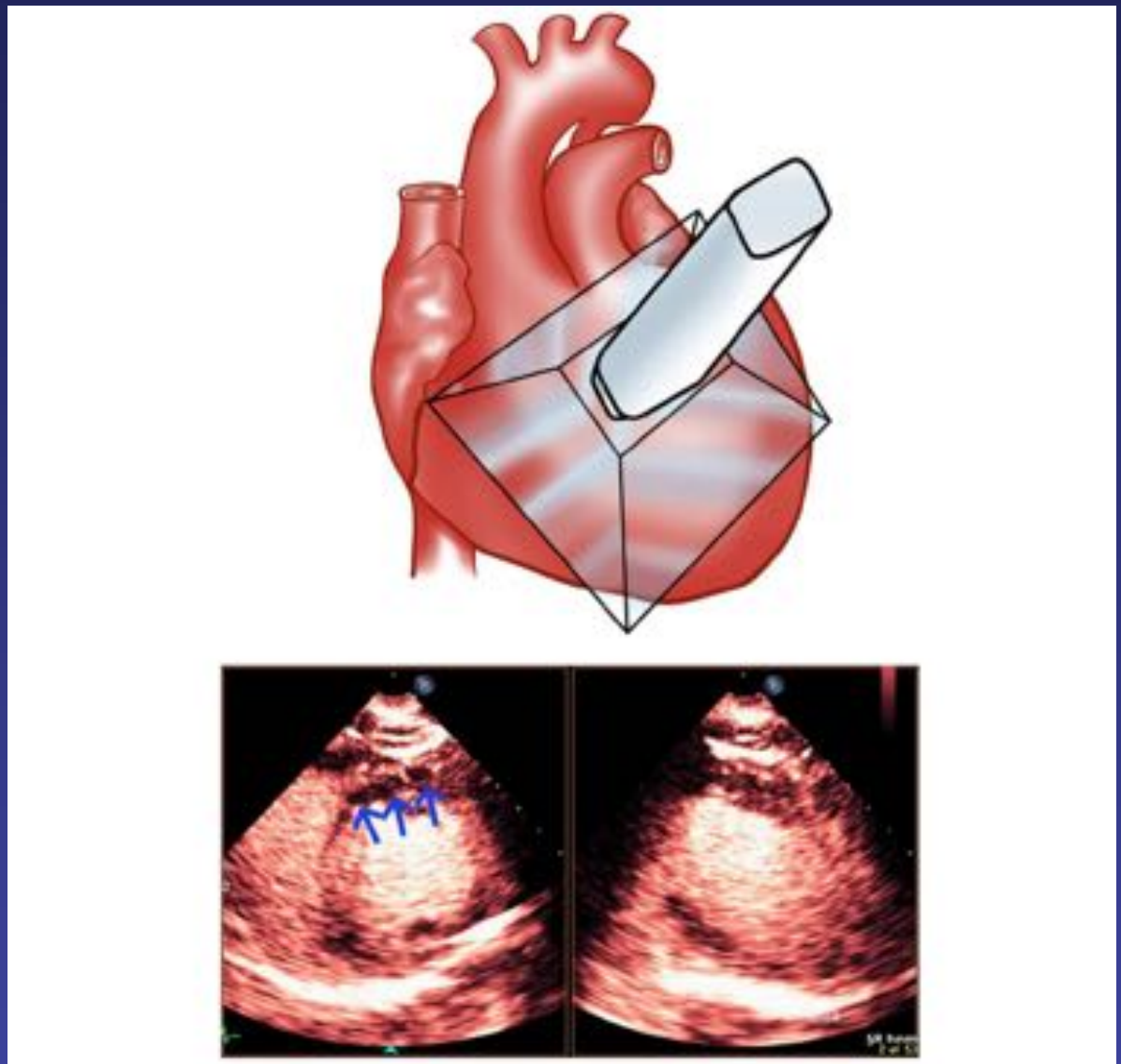


Figure 1.
Depiction of the transthoracic coverage of the 3D transducer used for the application of guided high mechanical index impulses following acute left anterior descending thrombotic occlusion. The blue arrows depict the hypoperfused zone identified with biplane low MI imaging following left anterior descending thrombotic occlusion.

Cardiovascular Research (2009) 83, 636–642
doi:10.1093/cvr/cvp206

Review

The utilization of ultrasound and microbubbles for therapy in acute coronary syndromes

Thomas R. Porter*

University of Nebraska Medical Center, 982265 Nebraska Medical Center, Omaha, NE 68198-2265, USA

Received 3 February 2009; revised 22 May 2009; accepted 1 June 2009; online publish-ahead-of-print 18 June 2009

Improvements in Cerebral Blood Flow and Recanalization Rates With Transcranial Diagnostic Ultrasound and Intravenous Microbubbles After Acute Cerebral Emboli

Shunji Gao, MD, Yan Zhang, PhD,† Juefei Wu, MD,* William T. Shi, PhD,‡ John Lof, MS,* Francois Vignon, PhD,‡ Lucas Drvol, BS,* Feng Xie, MD,* David Muirhead, MSA,§ Jeffry E. Powers, PhD,|| Robin High, MBA, MA,¶ Matthew L. White, MD,† and Thomas R. Porter, MD**

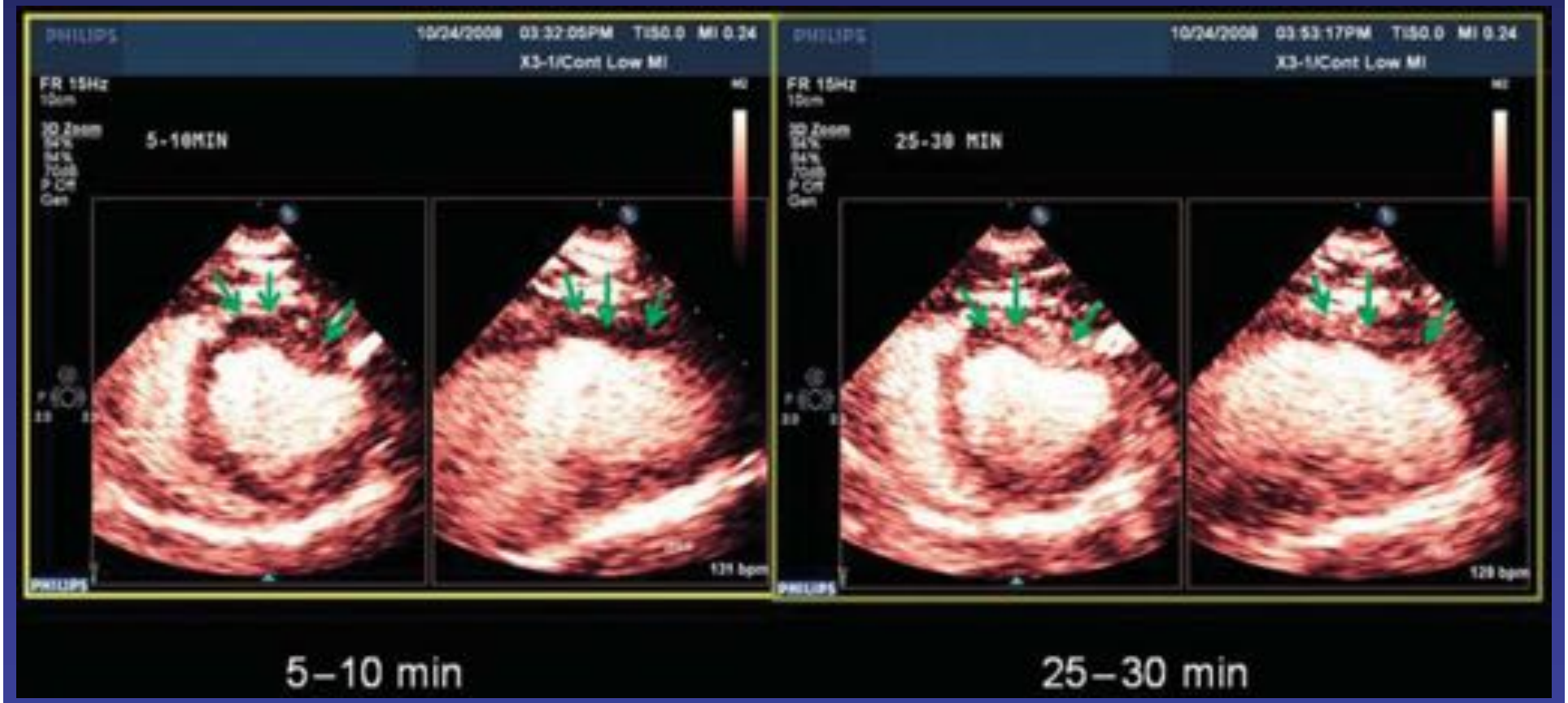


Figure 4 An example of replenishment within the risk area of a pig (arrows) with an acute thrombotic occlusion of the left anterior descending artery using a guided three-dimensional high mechanical index approach. The images represent the plateau intensity during a continuous infusion of microbubbles early on in the treatment period (left panel), and after 20–25 min of treatment in the right panel. Note the microcirculation within the risk area replenishes at 20–25 min into treatment. This pig also exhibited epicardial recanalization at 60 min into treatment.

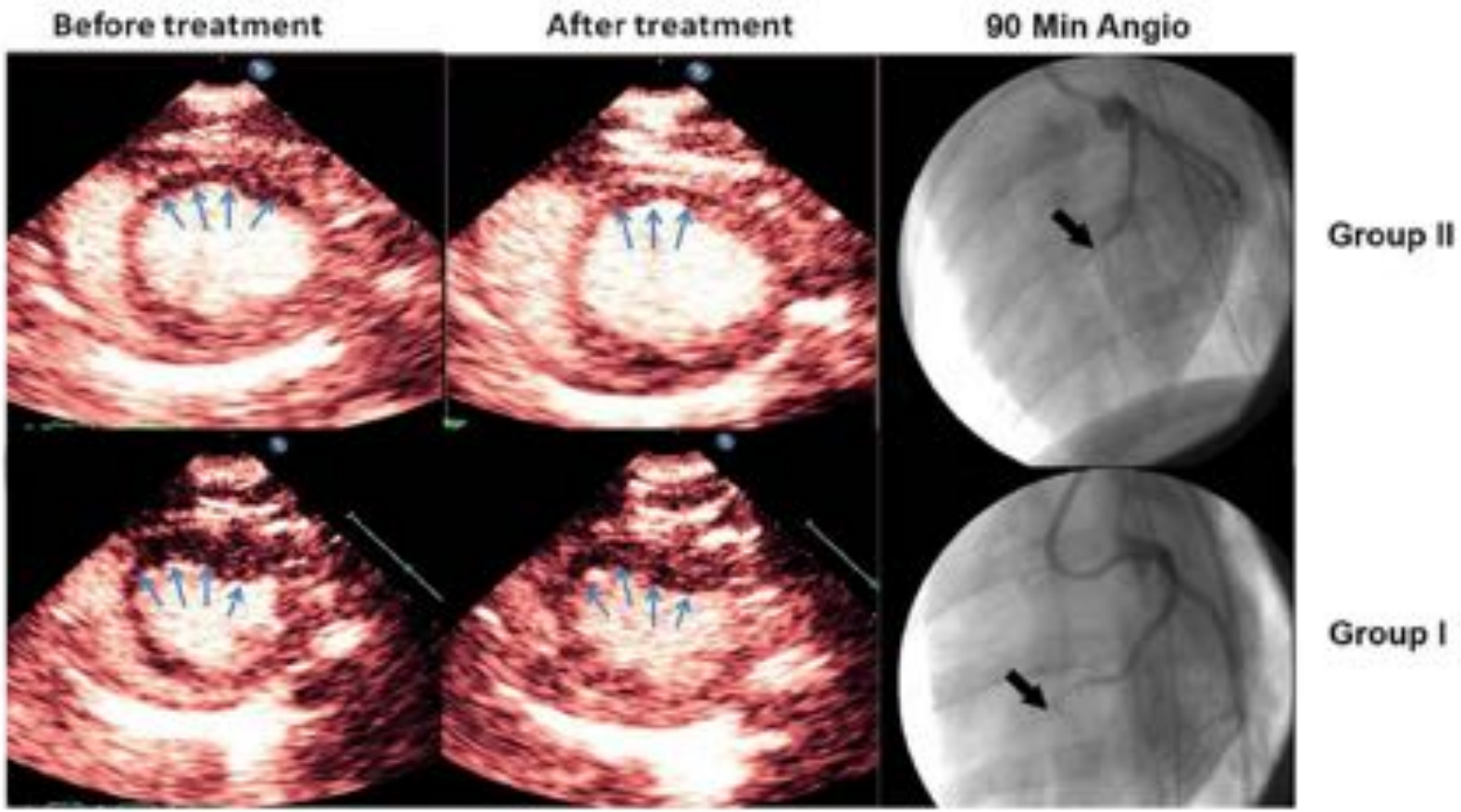


Figure 4.
Changes in plateau intensity defect size (ultimate infarct size) in a normal pig treated with 3D/MB with ½ dose TNK (Group II) versus TNK alone (Group I). Note the reduction in contrast defect size (arrows) in the 3D/MB treated pig (top panels), compared to the pig treated with TNK alone (arrows; bottom panels).

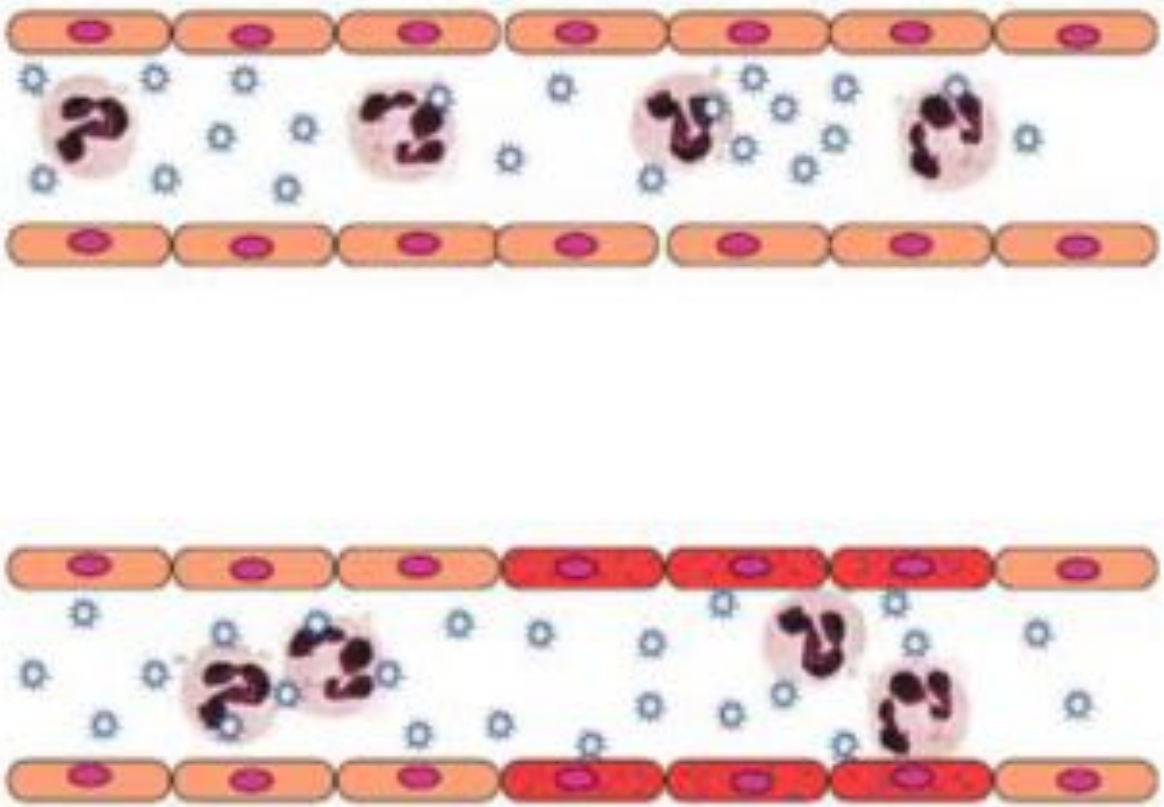


Figure 2. *Demonstration of how albumin-coated perfluorocarbon microbubbles containing drugs attached to their surface selectively adhere to sites of endothelial dysfunction (darker shaded cells in the lower panel). In the absence of endothelial dysfunction (upper panel), note that drug-bearing microbubbles stay in the blood pool and are not adherent to the endothelium.*

Development Of Robotic Surgical Devices

Laboratory of Dmitry Oleynikov

University of Nebraska Medical Center

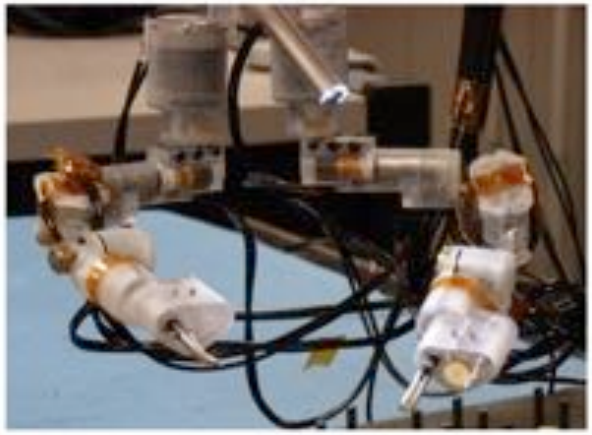


Fig. 1 Multifunctional surgical robot

Fig. 2 Remote surgeon interface

Fig. 3 Description of six degrees of freedom

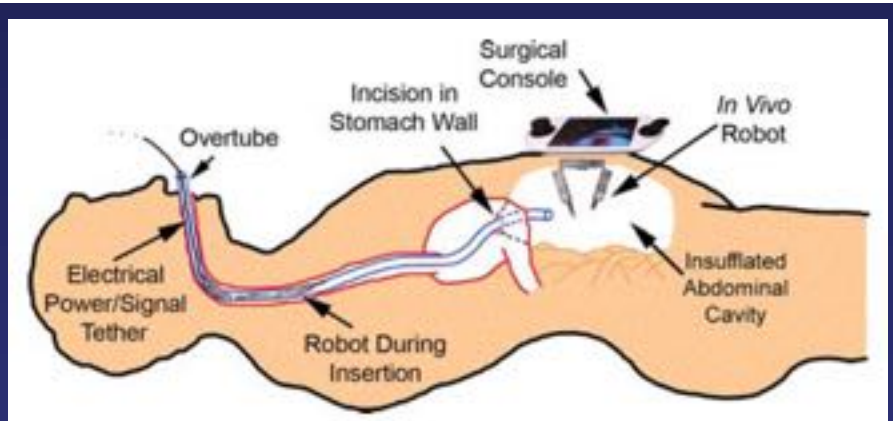
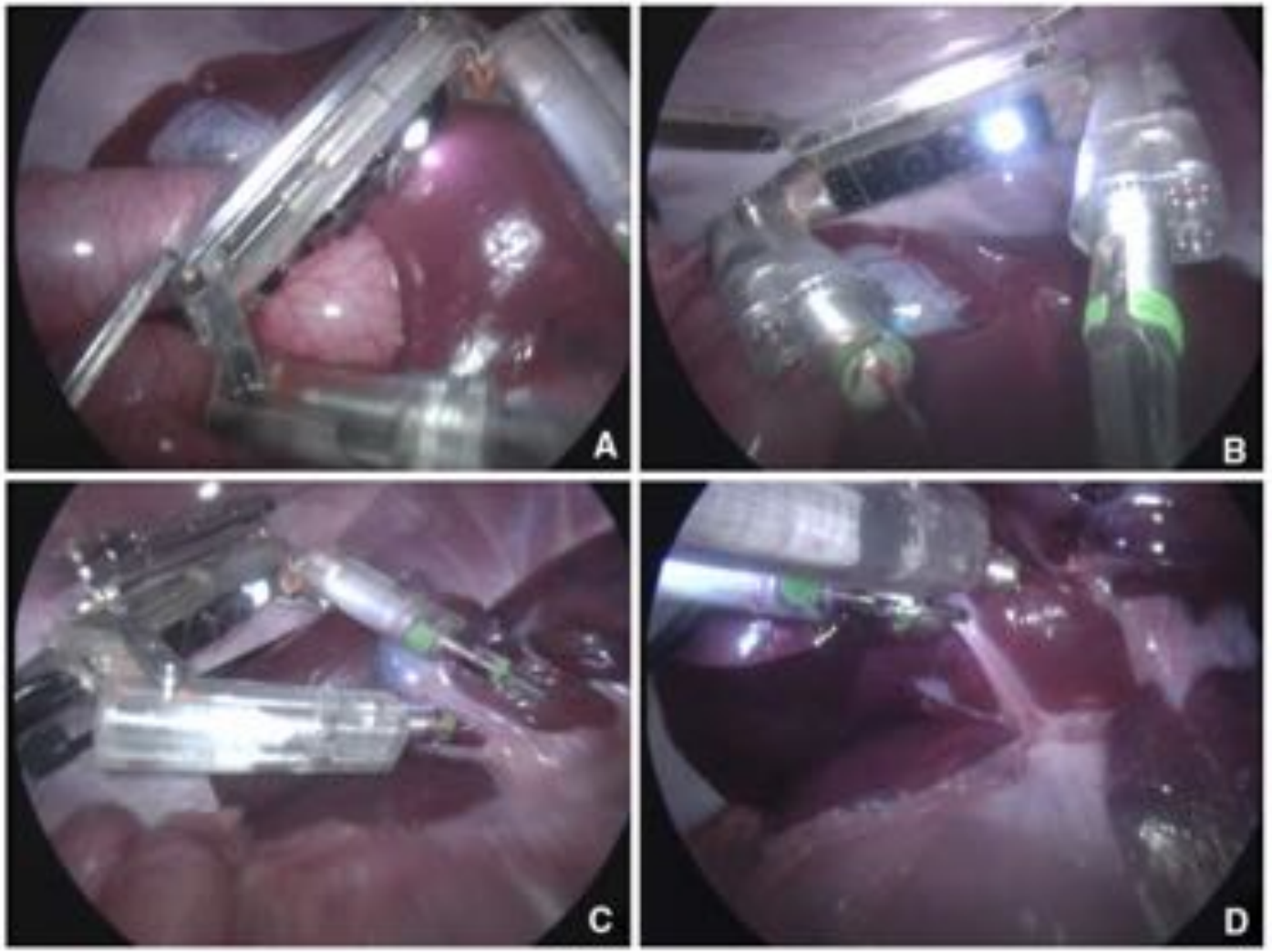


Fig. 1 Natural orifice surgery using a miniature in vivo robot platform

Robotic Cholecystectomy In Swine

Fig. 5 Laparoscopic view of the robot attachment (A and B) and positioning (C and D) using magnetic coupling with the external magnetic handle during the third animal model procedure



Surgical Robotics

- Hemostasis
- Cancer
- Skin Replacement
- Peripheral Arterial Disease

Carlson Laboratory

University of Nebraska Medical Center —

Omaha VA Medical Center

Hemostasis Of Severe Hemorrhagic Injury (Department of Defense)

Fibrin sealant made from recombinant clotting factors

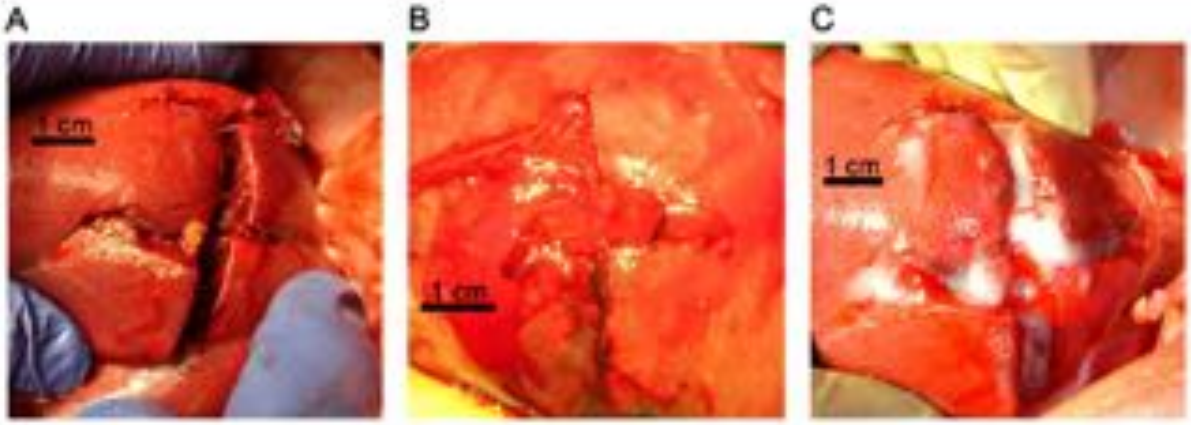


Figure 9. Evaluation of hemostatic and wound adhesive properties of rFI in a grade V central liver laceration model in swine. (A) Laceration without fibrin sealant. (B) Laceration after application of pdFI-based tissue sealant. (C) Laceration after application of rFI-based tissue sealant. Scale bars represent 1 cm.

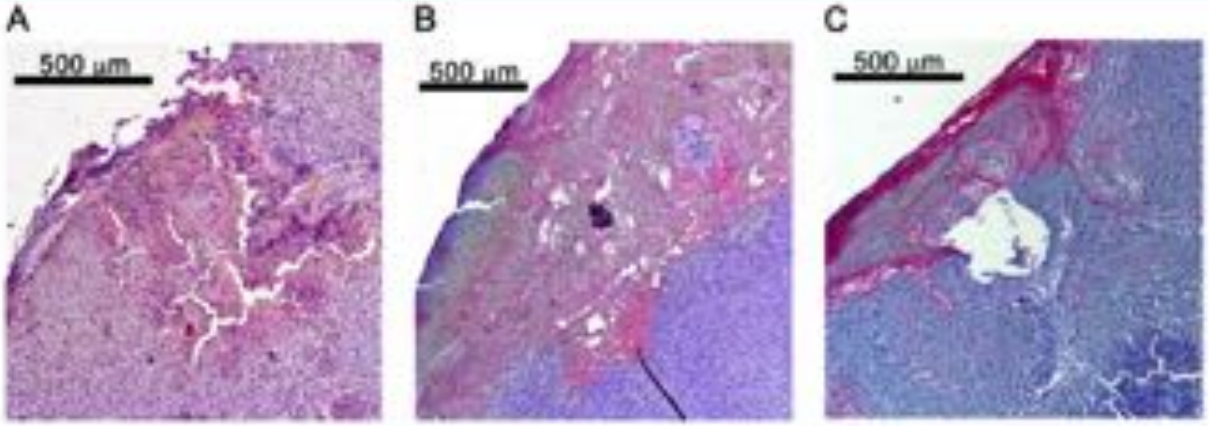
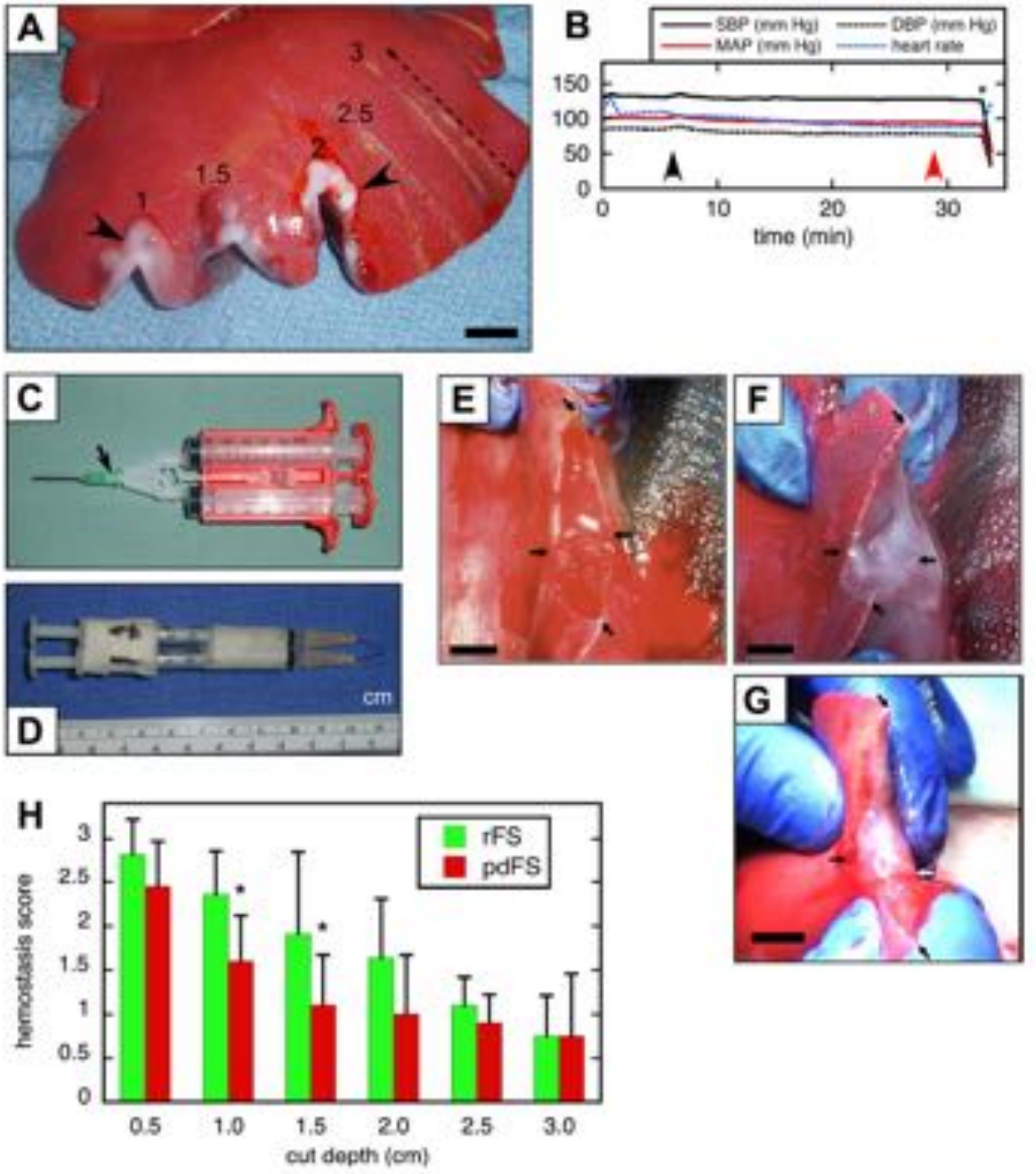


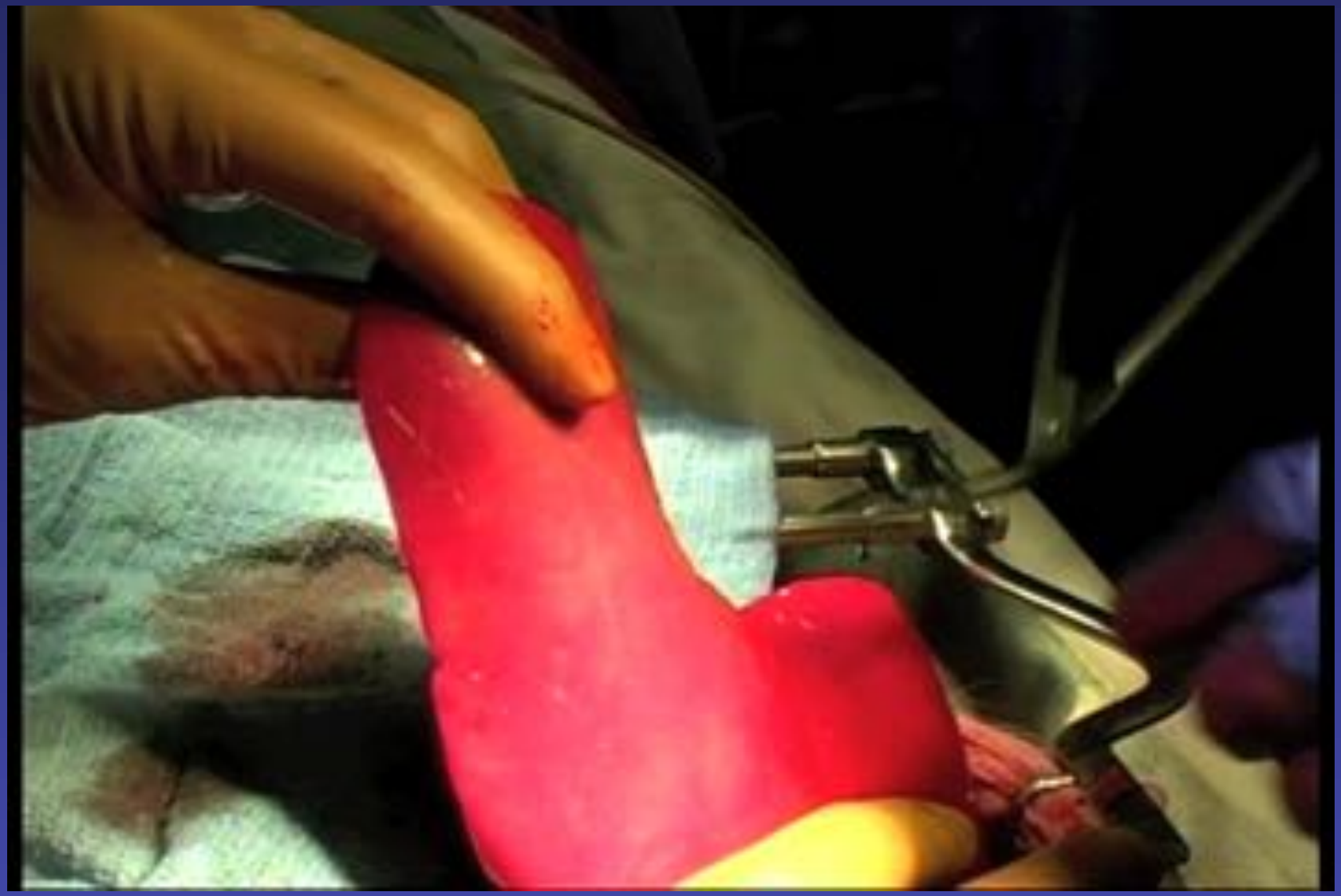
Figure 10. Histologic evaluation of the fibrin–wound interface in a liver lobe wedge excision model in swine. No fibrin sealant (A) and pdFI (B) and rFI (C) sealant treated wedge excisions were incubated with antiporcine FI antibody stained with DAB+ (brown) and antihuman FI antibody stained with Permanent Red (pink). Scale bars represent 500 μm.

Fibrin sealant made from recombinant clotting factors

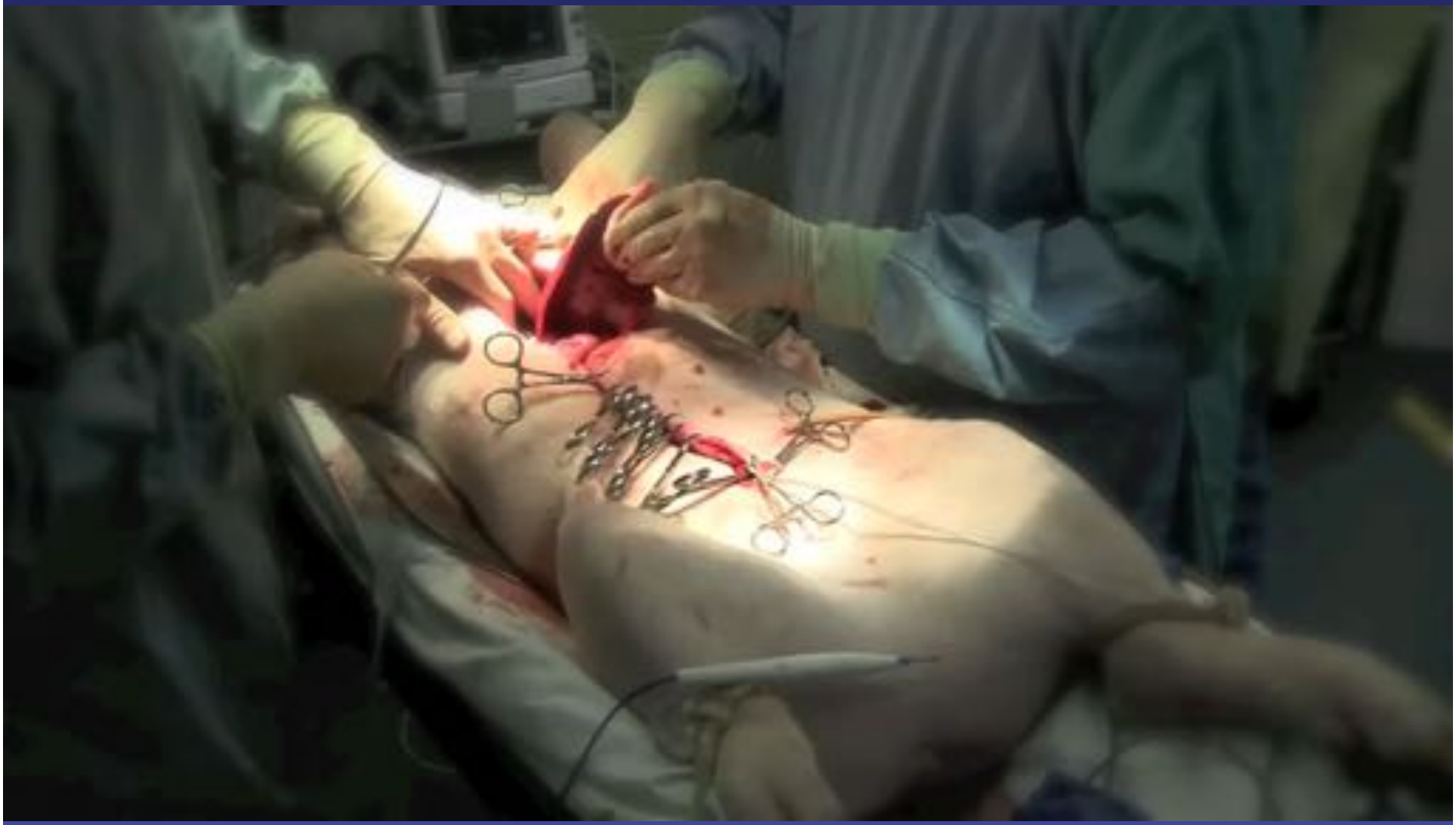
JOURNAL OF SURGICAL RESEARCH 187 (2014) 334–342



Liver Resection: Synthetic Bandage With Clotting Factors



Porcine Noncompressible Hemorrhage Model



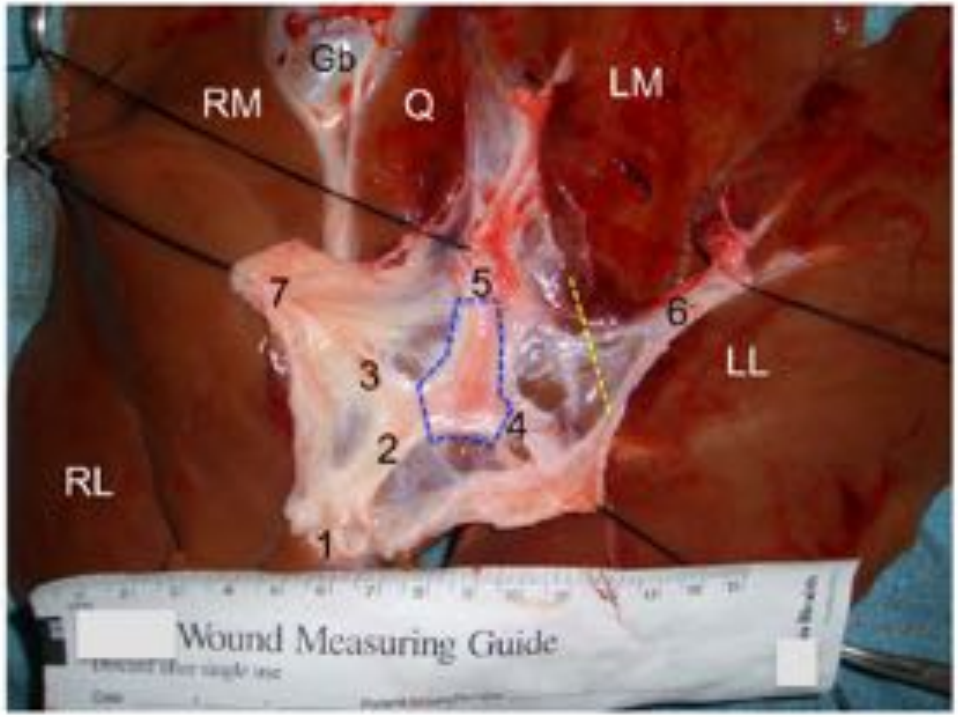
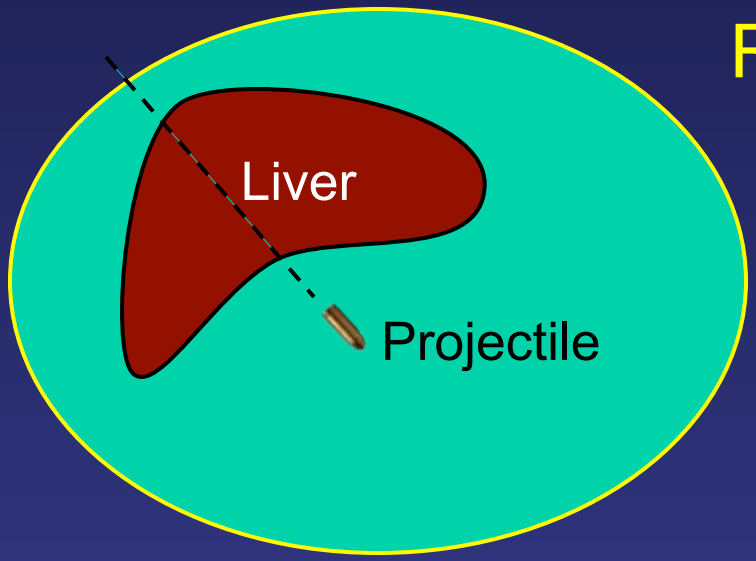


Figure 1. Dissection demonstrating the anatomy of the porcine intrahepatic portal venous system. *Ex vivo* porcine liver, inferior aspect (scale in cm). The soft tissues overlying the portal venous system have been dissected and retracted with silk stay sutures. RL = right lateral lobe; RM = right medial lobe; LM = left medial lobe; LL = left lateral lobe; Q = quadrate lobe; Gb = gallbladder; 1 = cut orifice of main portal vein; 2 = intrahepatic portal vein; 3 = RM lobe portal vein branch; 4 = 1st LL lobe portal vein branch; 5 = cut orifice of 2nd LL lobe portal vein branch (proximal end); 6 = distal end of structure 5; 7 = pedicle containing the common bile duct and hepatic artery (reflected laterally by stitch). In this dissection the 2nd LL lobe portal vein branch was transected (the two ends are labeled as 5 and 6). The hepatic veins were not exposed in this dissection. The dashed blue polygon indicates the portion of the portal vein that was resected for the PVR injury mechanism. The dashed yellow line indicates where the cut was made across base of LL lobe for the LLLH injury mechanism. Scale = cm. [201 words].

doi:10.1371/journal.pone.0108293.g001

Rx: noncompressible hemorrhage



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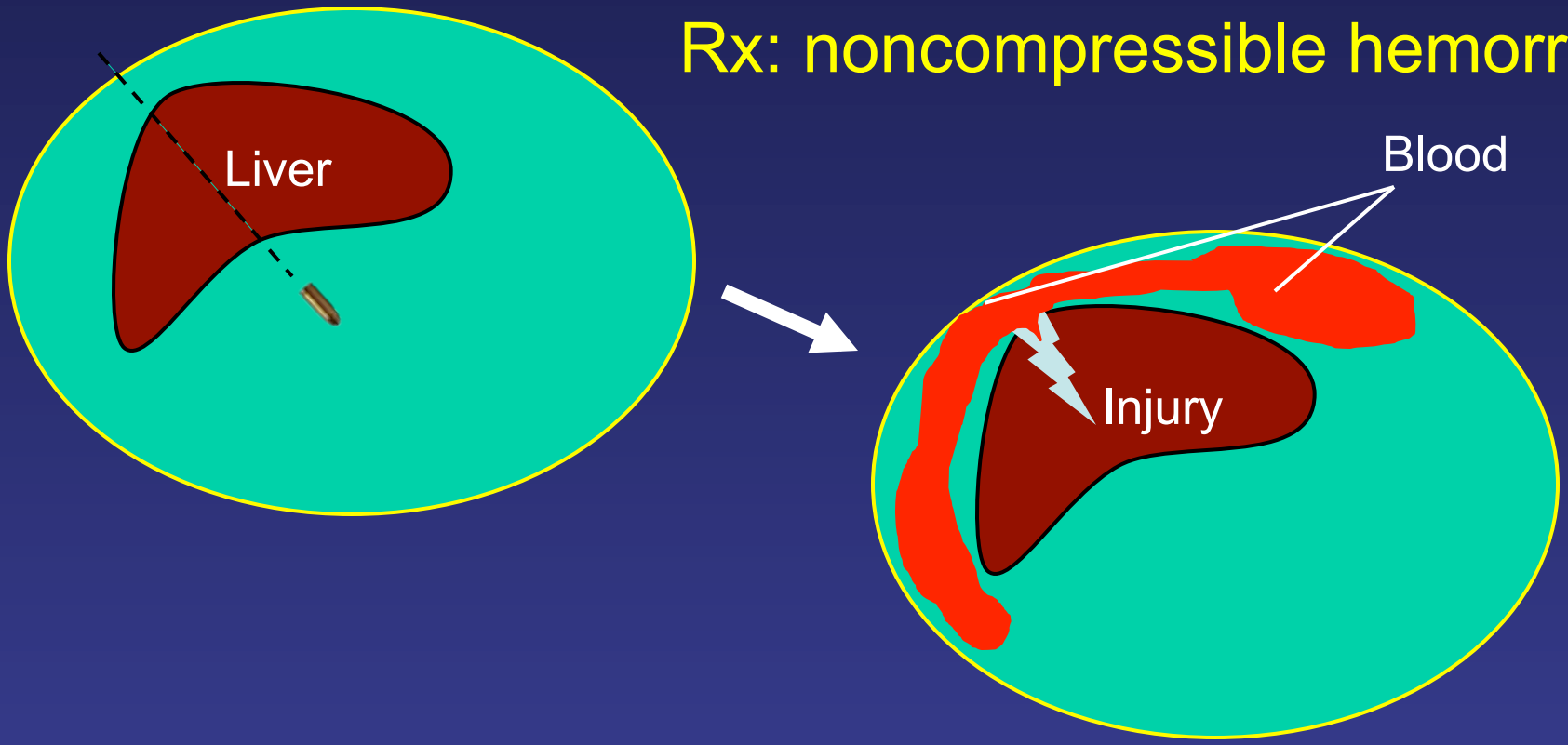

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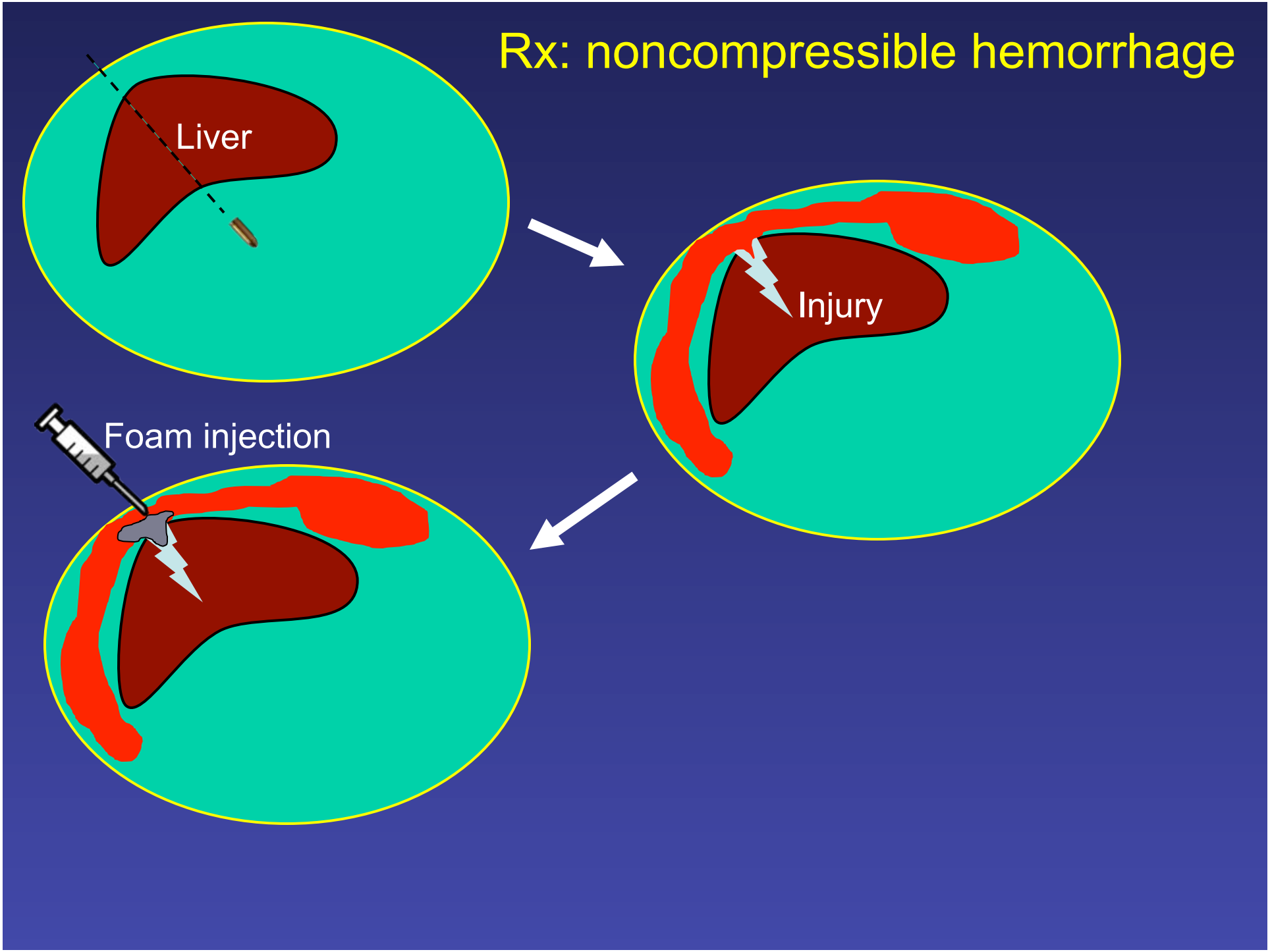
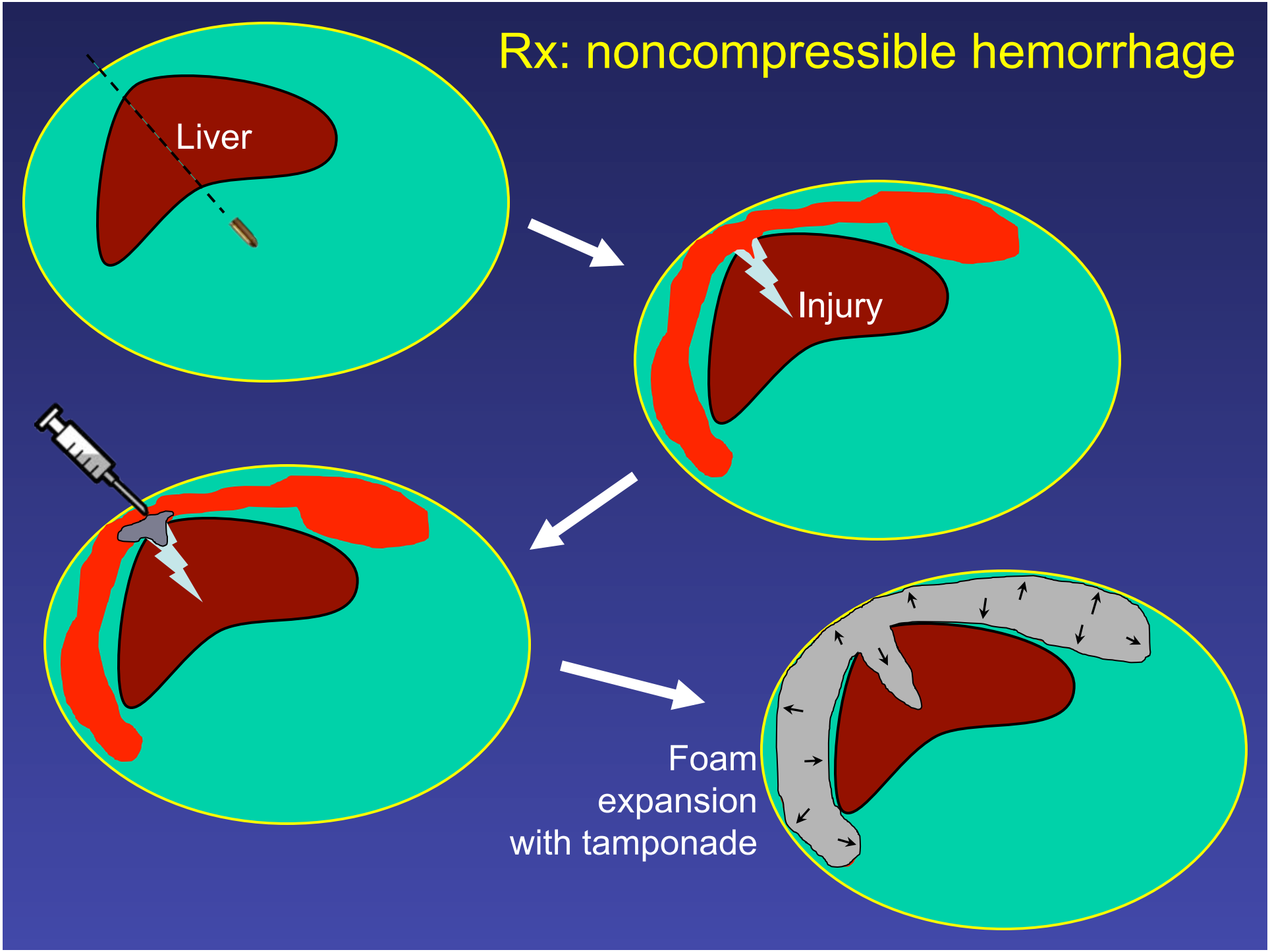
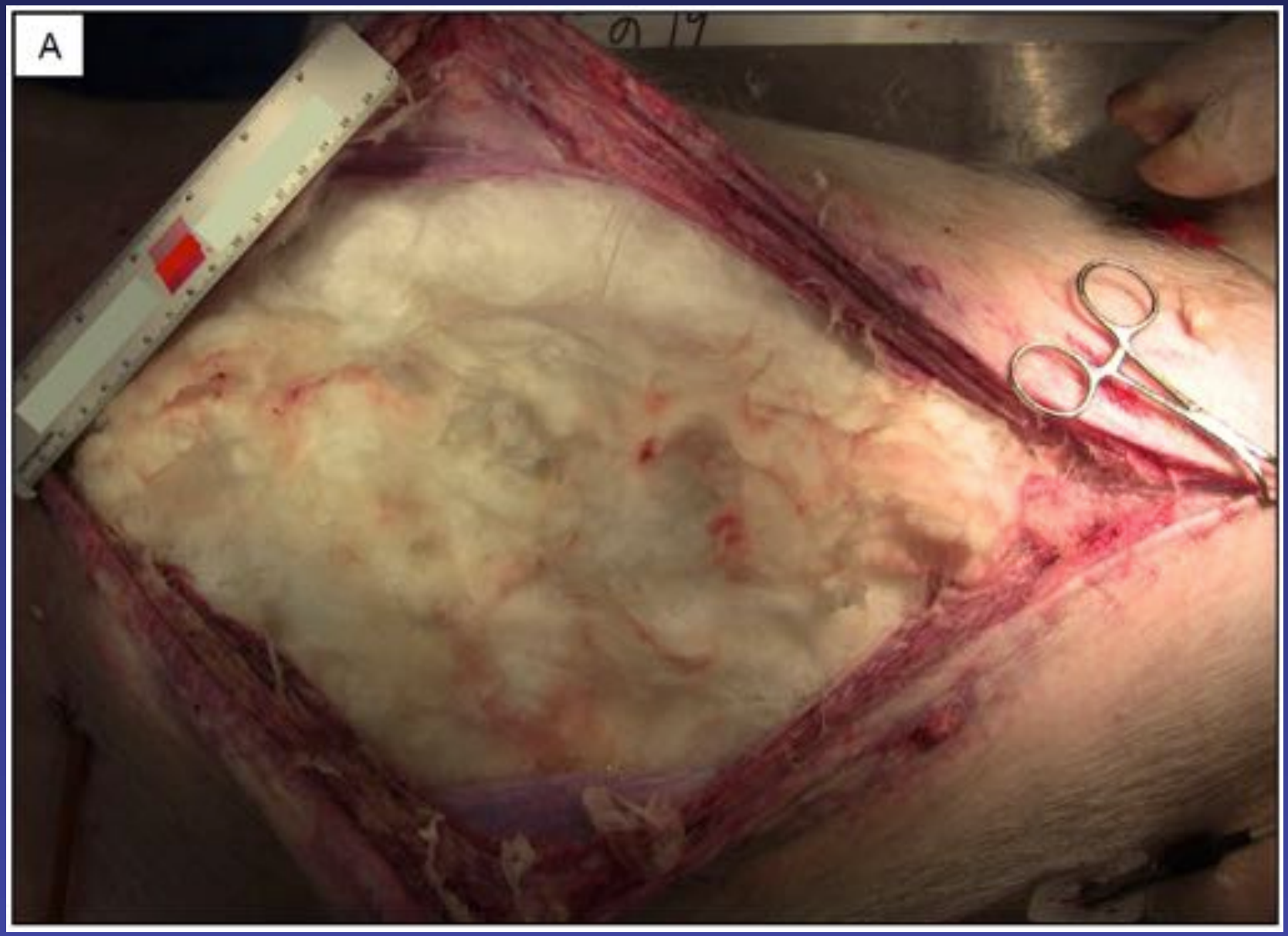


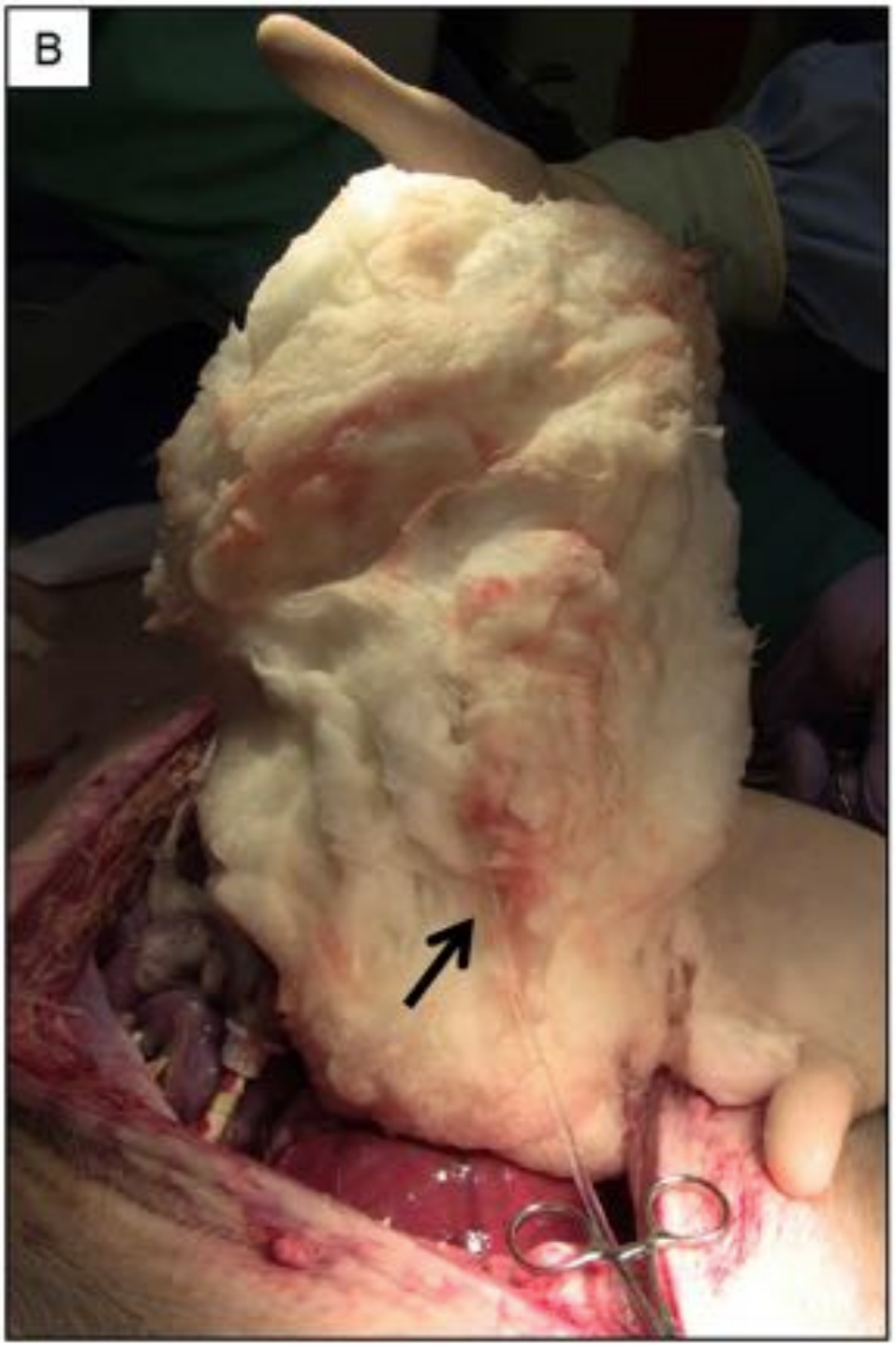
Rx: noncompressible hemorrhage

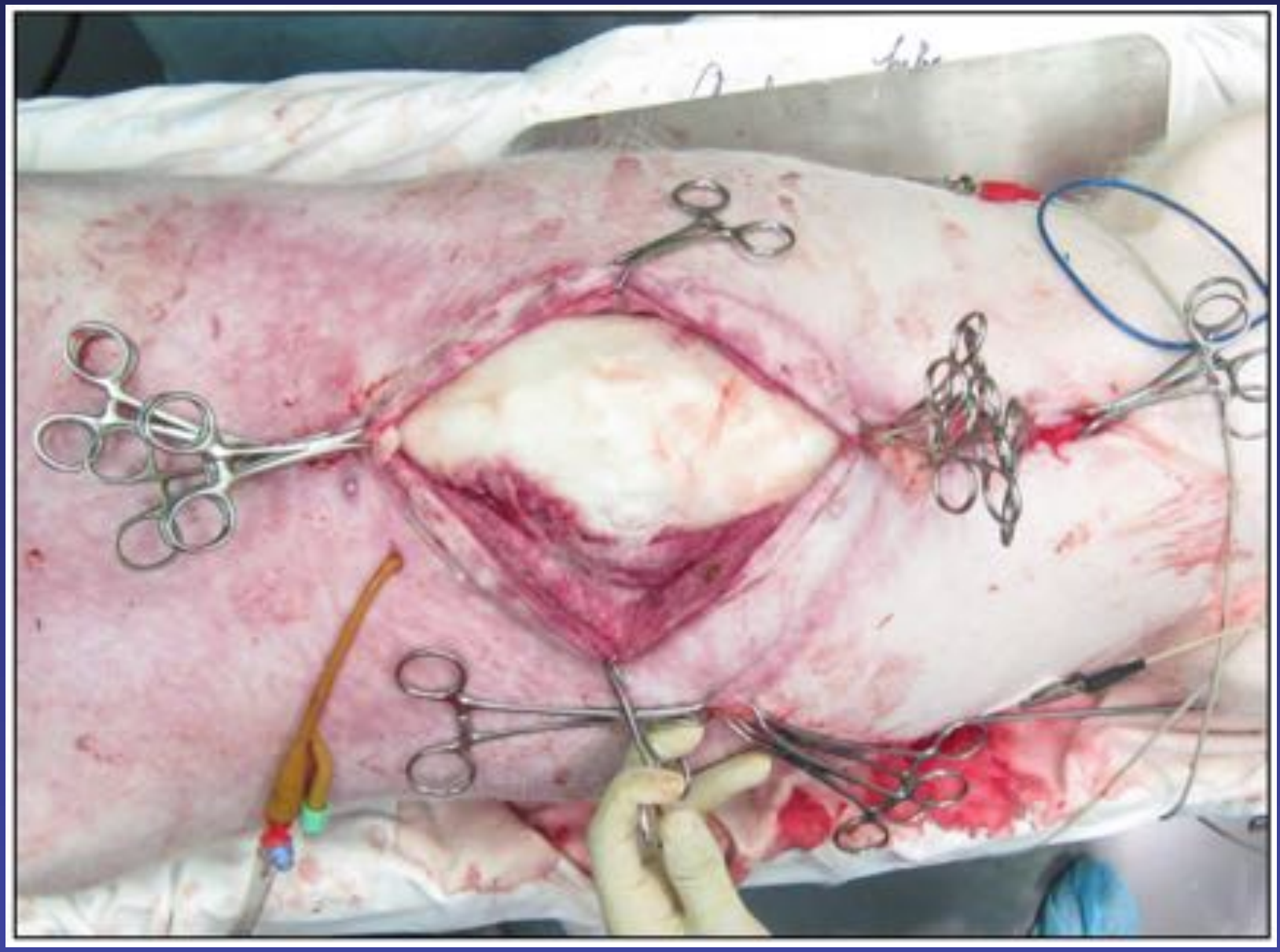




Calcium Alginate Foam At Necropsy

Calcium Alginate Foam At Necropsy





Incision Re-opening At Necropsy

Porcine Cancer Models

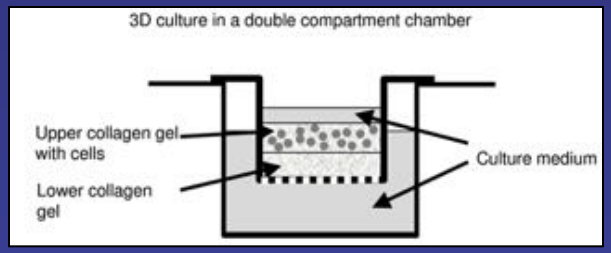
Cancer Research

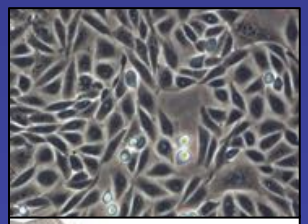
Clinical studies

Mouse models

Large Animal?

3D cell culture models





Monolayer cell culture

… a continuum

Mouse:

Reasonable model for *homo sapiens*, but…

Table 1. Murine *vs.* human phenotype for select mutations.

Mutated gene	Murine phenotype	Human phenotype
APC	Small intestine polyps	Colorectal cancer
CFTR	Intestinal disease	Cystic fibrosis
RB1	pituitary tumors	Retinoblastoma
FANC	low weight	Fanconi anemia

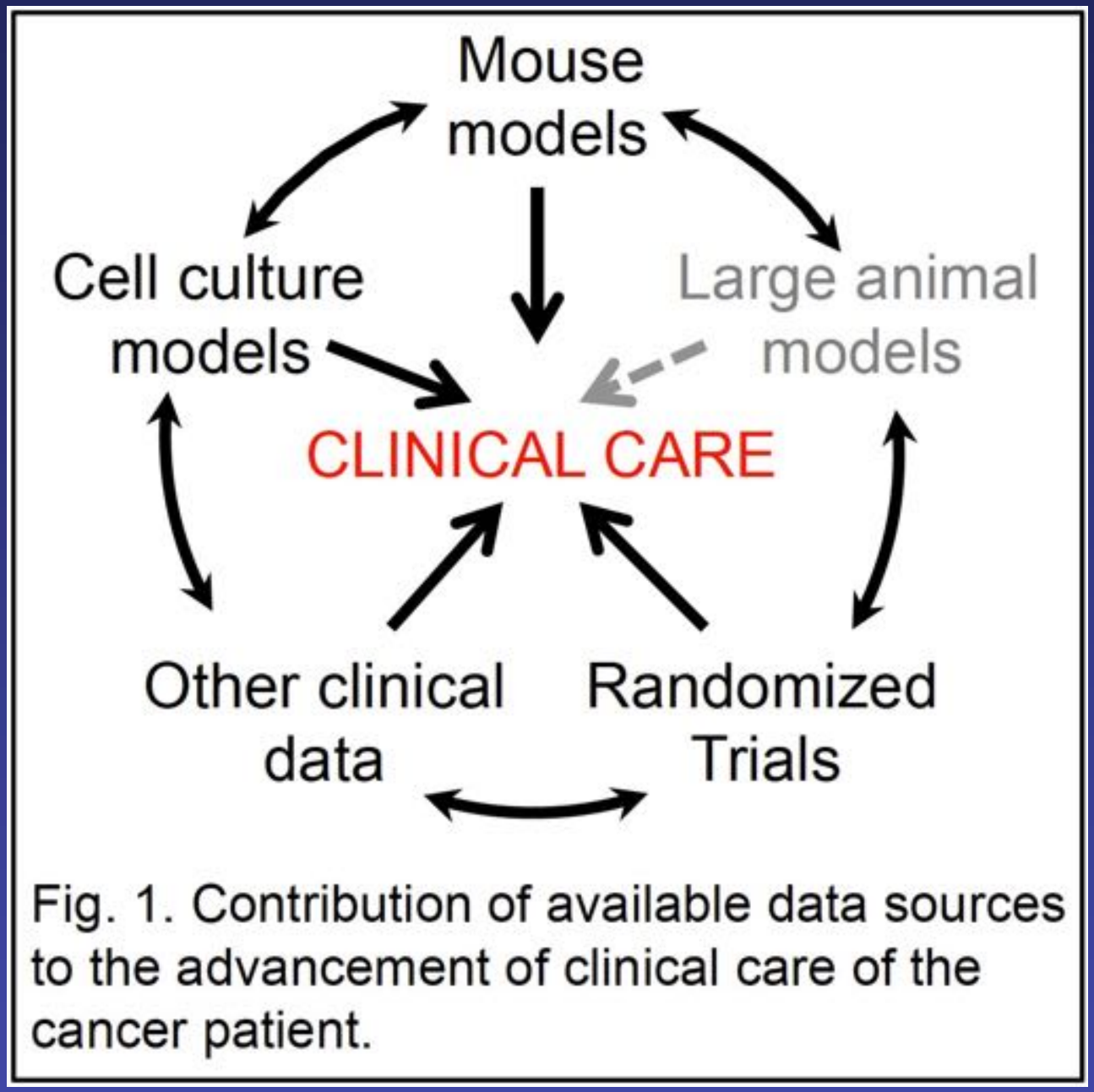


Fig. 1. Contribution of available data sources to the advancement of clinical care of the cancer patient.

WT

APC^{WT}/APC^{1311}

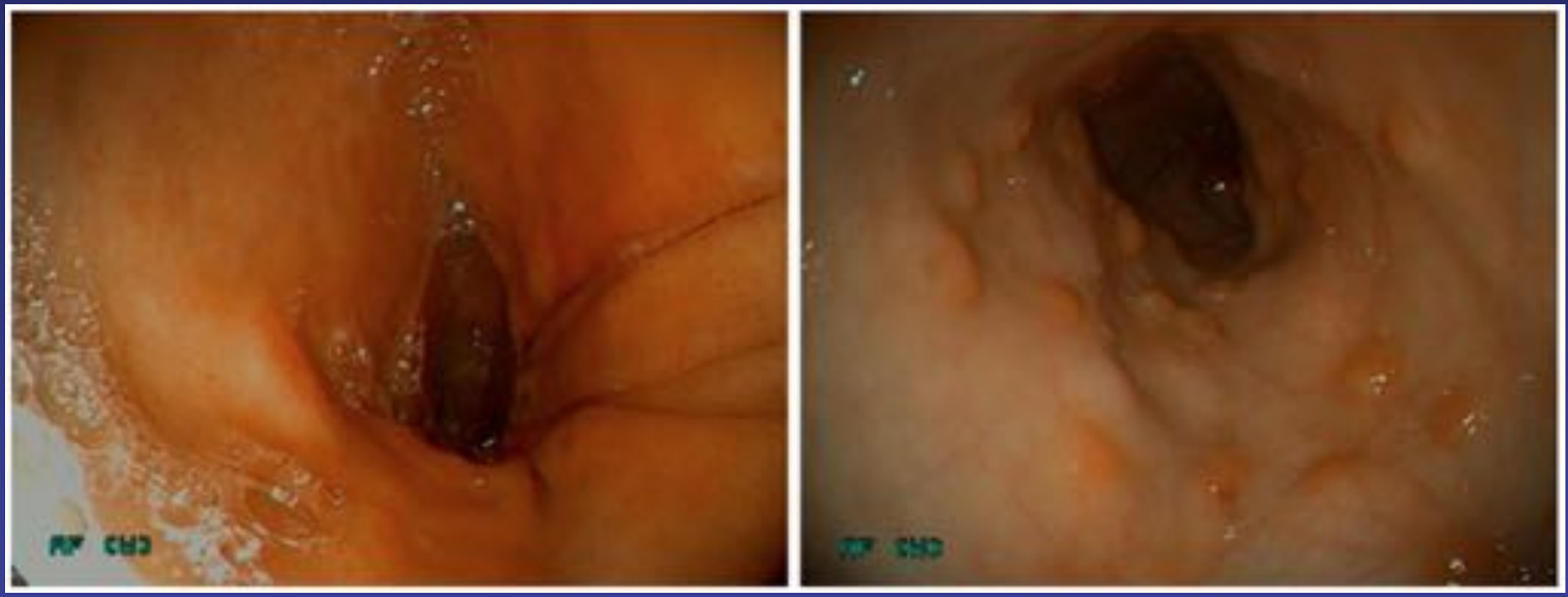


Fig. 1 Endoscopic imaging of the rectums of two sibling F1 generation male pigs at 7 months old. The animal on the *left* is wild type, the animal on the *right* carries the APC^{1311} mutation in heterozygous form

Flisikowska et al., *Transgen Res* 2013;22:673.

UNL transgenic swine (GFP pig)

Giving cancer to pigs:
Germline manipulation (transgenic swine)

KRAS/p53 “Onco-Pig” (NSRRC, Columbia MO)

- New, unpublished transgenic swine model
- *KRAS*^{G12D} & *p53*^{R167H} cassette
- Floxed termination signal
- Local treatment with AdCre (e.g., lung, pancreas)
- Local KRAS activation and p53 inhibition
- Monitor for tumor formation

Activating oncogenes *in vivo*

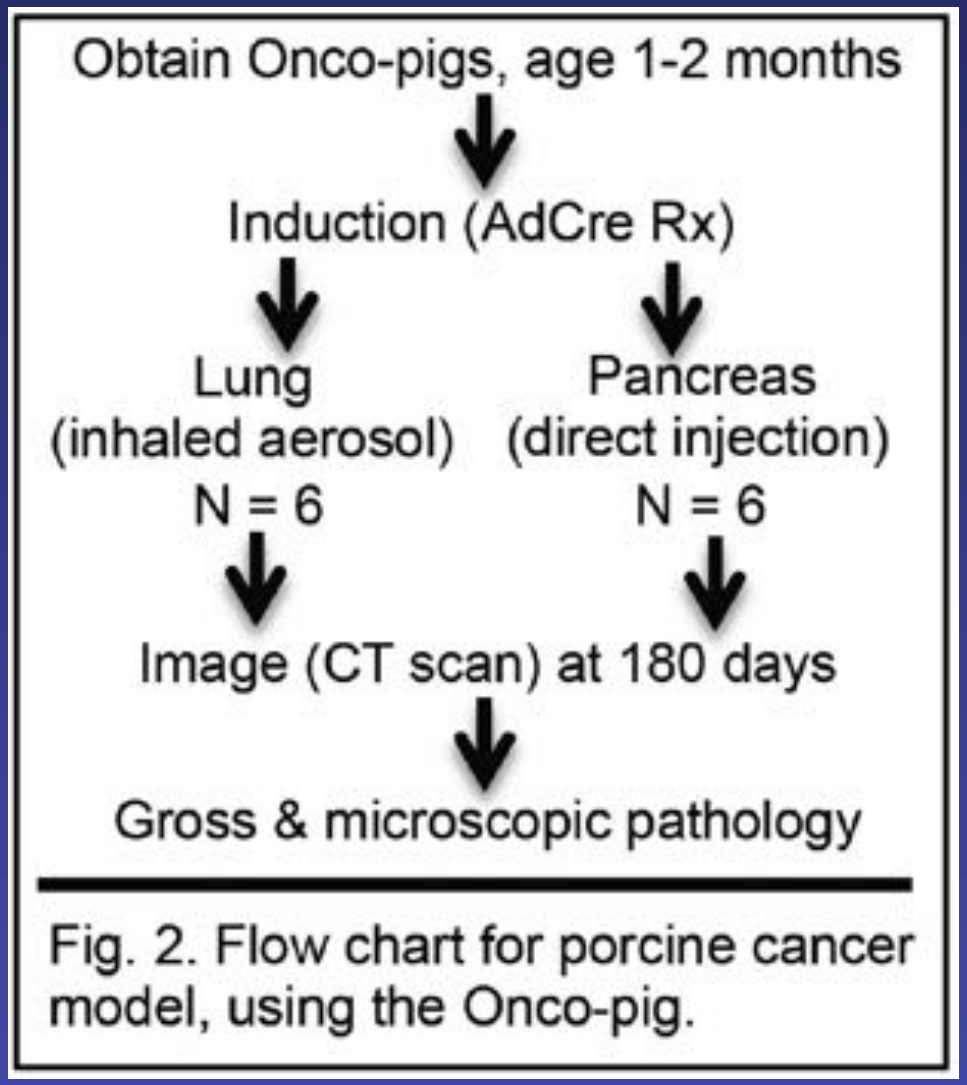


Fig. 2. Flow chart for porcine cancer model, using the Onco-pig.

In vitro transformation, then implantation

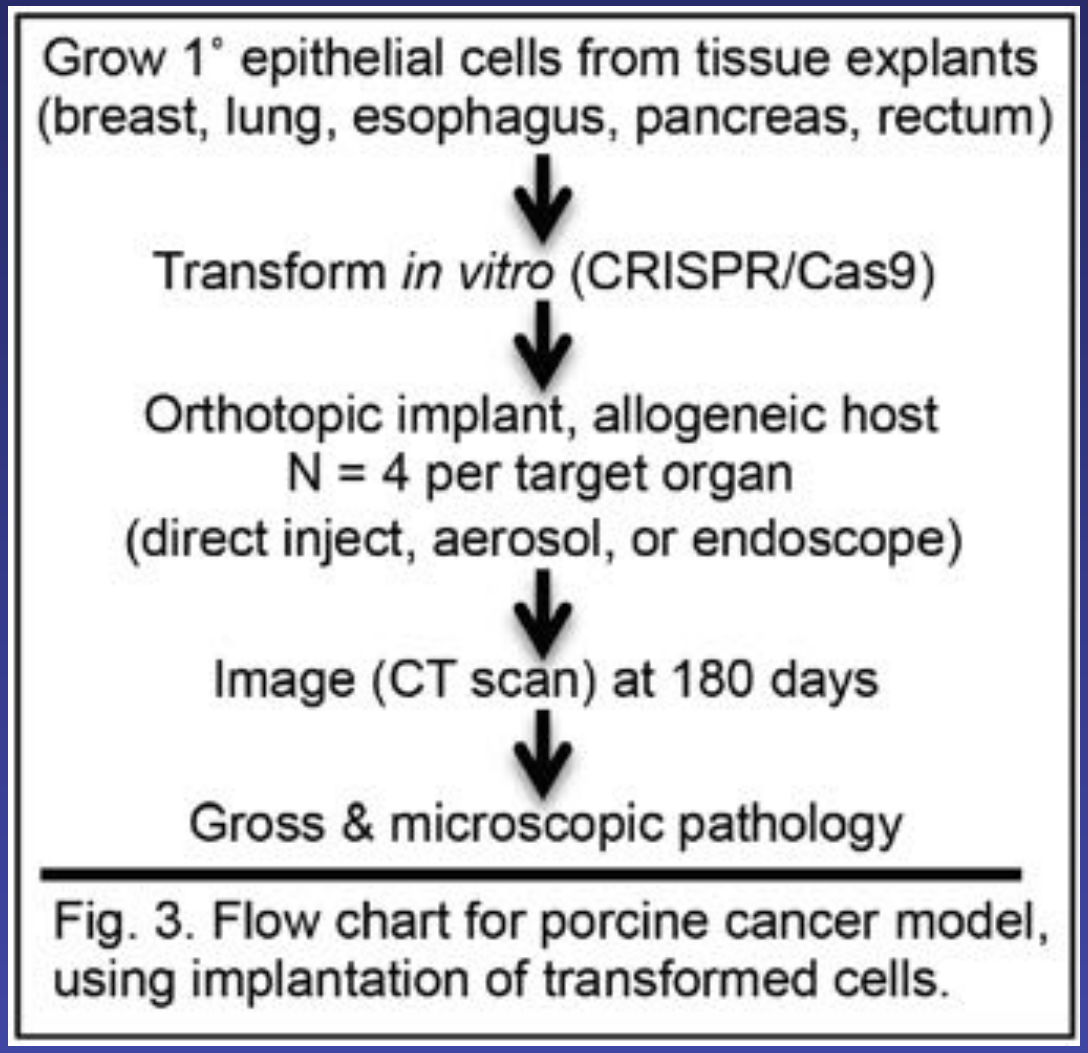
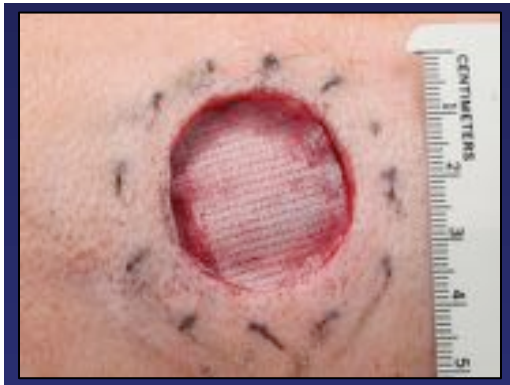
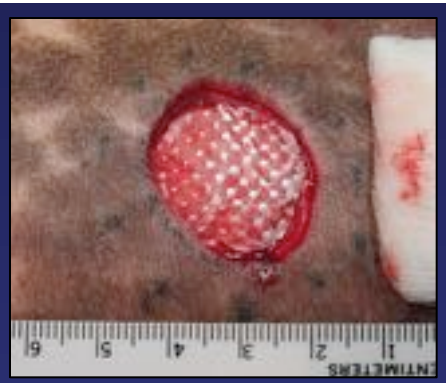


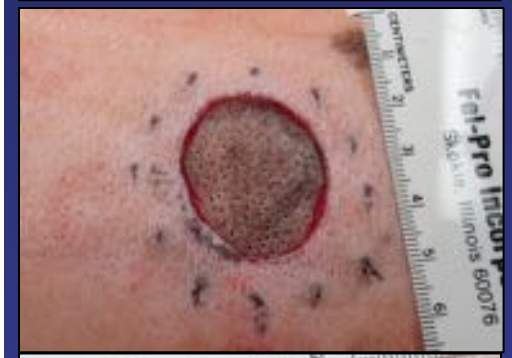
Fig. 3. Flow chart for porcine cancer model, using implantation of transformed cells.

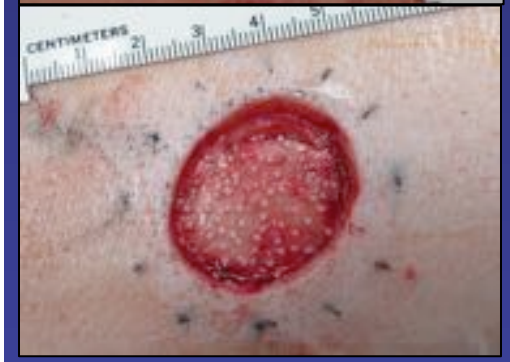
Tissue Engineering:

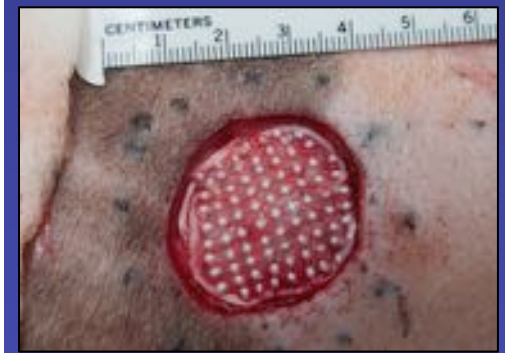
Skin Replacement



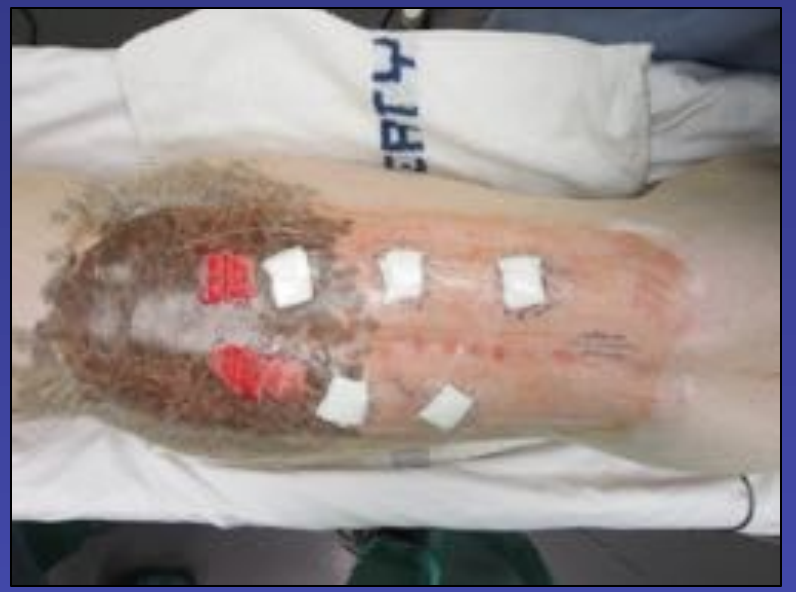








Nanoengineered synthetic matrices embedded with autologous microskin grafts for treatment of full-thickness skin defects



Porcine Model of Peripheral Arterial Disease

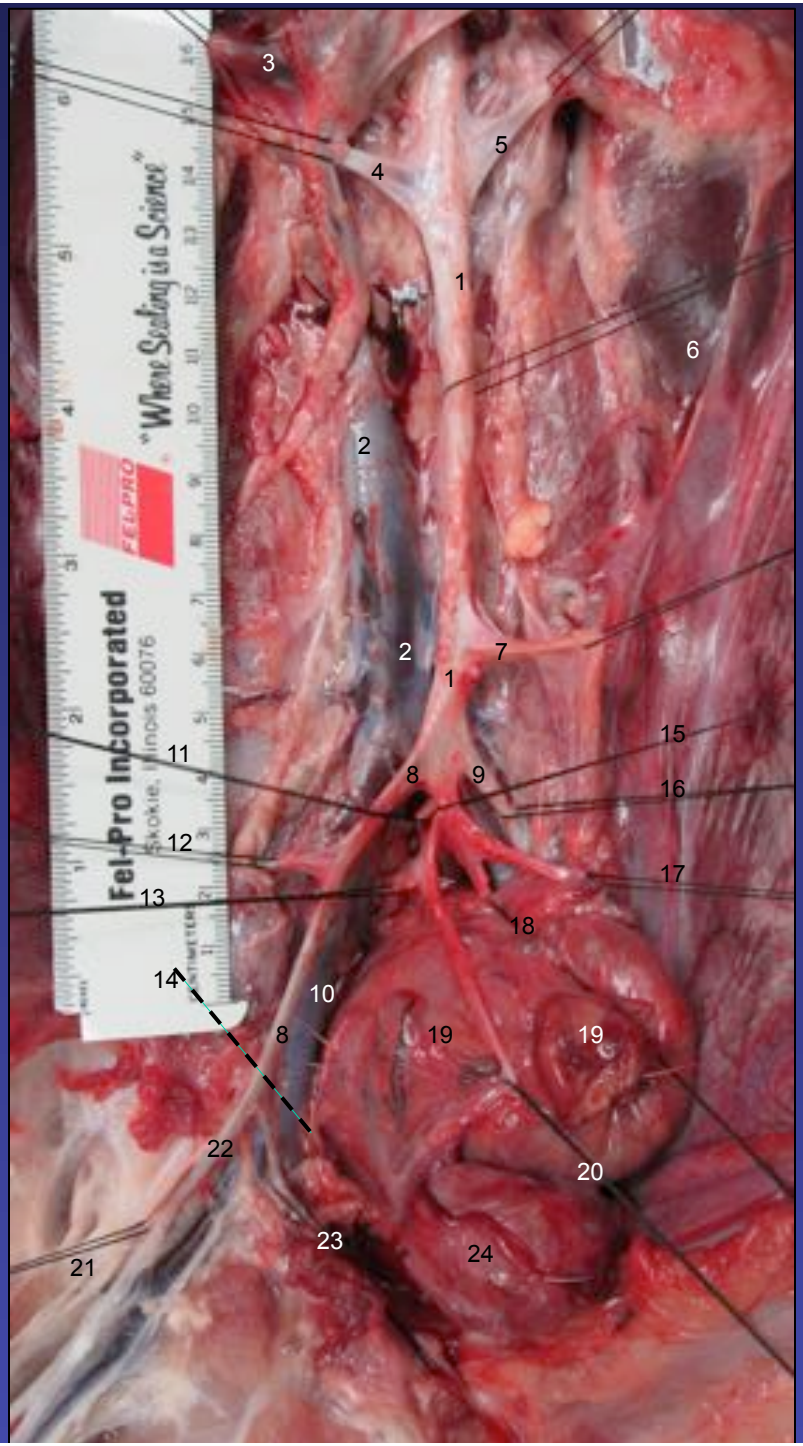


Figure 1. View of lower porcine abdomen in a postmortem subject from IACUC protocol #00760. Subject underwent euthanasia prior to commencing this dissection. Cephalad is at top; viscera have been removed in order to better visualize arterial anatomy. Dissection was performed to expose infrarenal vasculature.

Key to structures:

1. Infrarenal aorta
2. Inferior vena cava
3. Right renal vein
4. Right renal artery
5. Left renal artery
6. Lower pole left kidney
7. Inferior mesenteric artery
8. Right external iliac artery
9. Left external iliac artery
10. Right external iliac vein
11. Unknown on silk stay
12. Circumflex artery on silk stay
13. Right internal iliac artery on silk stay
14. Approximate level of inguinal ligament
15. Sacral artery on silk stay
16. Left external iliac artery on silk stay
17. Rectal branch on silk stay
18. Left internal iliac artery on silk stay
19. Rectal stump
20. Vesicular branch on silk stay
21. Lateral circumflex branch silk stay
22. Superficial femoral artery
23. Profunda femoris on silk stay
24. Urinary bladder

Questions?

UNMC

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