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Letters to the Editor

Sheep (*Ovis aries*) as a model for cardiovascular surgery and management before, during, and after cardiopulmonary bypass

Dear Editor,

We read with great interest the article by Louis DiVincenti and colleagues.[2] The authors described the relevant sheep cardiovascular anatomy and physiology as well as animal management with cardiopulmonary bypass (CPB). In our opinion the authors presented excellent, detailed descriptions of surgical technique, pre- and intraoperative care, and anesthetic management. We highly recommend this article to any group interested in research involving CPB procedures in a sheep model. We have written this letter to discuss only a few points of operative procedure and to emphasize the importance of postoperative care. This article comes at an opportune time given the increasing number of scientific publications using a sheep model in cardiovascular disease research due to similarities with humans in the molecular basis of cardiac contraction and in coronary anatomy. Currently sheep are used as a model for cardiomyopathy of different etiologies, valve surgery, congenital defects, artificial heart implantation, etc.[1,4,5]

Surgical procedure

The vast majority of pre- and intraoperative procedures were expertly detailed and we learned a great deal from the authors' descriptions. For this section, we wanted to note four points and alternatives that we believe add to the superb operative procedure.[2] We use a median sternotomy rather than a thoracotomy to greatly improve access to the heart and to allow for bicaval cannulation. We believe this option decreases procedure time and increases quality, overcoming the invasive drawbacks of a sternotomy.[4] For bypass we cannulate the right or left carotid artery (instead of the femoral artery as the authors suggest) using a 12- or 14-F cannula which allows adequate arterial inflow. We then are able to monitor arterial pressure via a 16-gauge catheter in the femoral artery connected to an arterial line. Each of these cannulations is removed and repaired after chest closure.[1] While the authors present an excellent discussion of the advantages of cardioplegia, we would like to provide a further endorsement of its effectiveness. We always use antegrade cardioplegia (del Nido solution) with cannulation of the aortic root, yielding unmatched cardiac protection through the strenuous CPB process.[5] Contrary to the authors' claim, aortic cross clamping is quite feasible; it requires removal and retraction of fatty tissue in the aortic root, and is likely much more accessible by median sternotomy than by thoracotomy due to the very short ascending aorta. Our procedure includes average aortic cross-clamp times over 70 min, with average bypass times over 140 min. Beyond these 4 points, our operative protocol is extremely similar to that of the authors and we have experienced very high survival.

Postoperative care

The most important difference between our CPB protocol and that described by the authors is the rigor of certain aspects of postoperative care. Survival in large animal surgery with CPB depends highly on the quality of immediate critical care.[3,5,7] Surgical procedures such as valve replacement or repair, CABG, etc. are well known and described, yet only a small body of literature is devoted to large animal ICU management, tissue perfusion, blood transfusion, or inflammatory response, especially after surgery with CPB. These issues may contribute more to the high reported mortality than does poor surgical execution. We believe proper ICU management is much more cost-effective at generating data than increased surgery volume. Because of this, we would like to detail aspects of our postoperative protocol for any groups interested in CPB in large animals.

The primary component of acute postoperative care is the maintenance of adequate oxygen delivery to tissues. Appropriate monitoring provides valuable information on the animal's viability. We have created a postoperative protocol with the help of Dr Rose Nolen-Walson (University of Pennsylvania) which we have used for the past 5 y (Table 1). A key aspect of this protocol is the constant supervision of the animal for the first 36 h after surgery.

Before closing the chest we always perform transepicardial echocardiography and in case of low cardiac output we continue to give inotropic support and measure arterial pressure. We also place bilateral chest thoracostomy tubes and connect these to pleur-evac with wall suction. The thoracostomy tubes must be disconnected from the pleur-evac and Heimlich valves or Jackson–Pratt suction used when the animal is returned to the colony. We then remove the thoracostomy tubes 24 to 36 h postoperatively. The proper use of thoracostomy tubes helps reduce the incidence of pleural effusions. However, delayed pleural effusions may still occur, requiring insertion of new thoracostomy tubes for 24 h.

Postoperatively, we monitor central venous pressure (CVP) via triple lumen catheter in the jugular vein with a CVP manometer, which may remain in place and functional for up to 3 d without infection if properly flushed with heparin. CVP monitoring (target: 4 to 10 mm H_2O) provides important information regarding blood volume which may allow for life saving interventions with Lasix or fluids. We have found that CVP catheters provide the best balance of safety and monitoring compared to a Swan–Ganz catheter which presents a danger if left in an active animal. We also make use of arterial access via the auricular artery of either ear if possible. We obtain an

Table 1. Posteroperative Care Protocol: First 24-36 Hours

Monitoring	Medication	Fluids and Feed
Vital signs every 4 hours	O_2 : nasal 5/Lmin, decrease when stable	Hetastarch IV
SpO_2 monitoring until stable	Antibiotics	Normosol-R + dextrose + KCl until active
CVP every 4 hours	Furosemide if CVP >10 cm H_2O	Water when alert, hay 6 hours postop
EKG every 4-8 hours	K, Ca, Mg depending on ABG/VBG	Add 5% dextrose to fluids if still NPO next morning, care for BG level
ABG or VBG every 2-3 hours	HCO3 for metabolic acidosis	
Chest tube drainage every hour	Inotropes in case of low CO	
Urination	Amiodarone in case of arrhythmia	
	Normosol-R 50-80 mL/hr if CVP <4 cm H_20	

Table 2. Mortality and Morbidity for CPB procedures

Surgical Record	
Total Cases	25
Surgical Recoveries	21
Mortalities	4
% Survival	84%
Mortalities	4
Renal Failure	1
Brain Hypoperfusion	1
Bleeding Complications	1
Ventricular Fibrillation	1
Morbidities	
Without Complications	13
With Complications	8
Postsurgical Complications	**8**
Pneumonia	1
Pleural Effusions	2
Metabolic Acidosis	1
Impaired Liver Function	1
Transient Renal Function	2
Wound Infections	1

arterial blood gas sample every 2 to 4 h for the first 12 h to evaluate acid-base level and electrolytes—especially potassium, calcium and magnesium. In the absence of arterial access, venous samples from the jugular CVP catheter are used instead. Arterial pressure measurements are obtained via noninvasive cuff. We also monitor EKG every 4 to 8 h to determine the need for antiarrhythmic drugs.

Our first antibiotic regime includes procaine penicillin G 22,000 IU/kg IV and gentamicin 6.6 mg/kg IV, given 1 h prior to incision. Postoperatively, procaine penicillin G is provided every 6 h and gentamicin every 24 h for 4 to 5 d**.** If a second line treatment is required, we use ceftiofur sodium 2.2 mg/kg IM and enrofloxacin 5 mg/kg IM daily for 5 d. In the early postoperative period, we administer a fluid mixture composed of half crystalloid Normosol-R and half colloidal hydroxyethyl starch at a rate of up to 100 mL/h depending on CVP. It is not recommended to routinely transfuse blood that has been obtained from another donor sheep due to the possible risk of complications such as renal dysfunction, febrile reactions, etc. In practice, however, the majority of our sheep receive 1 to 2 units of donor blood, particularly in cases of low hematocrit and hypovolemia after CPB.

Our protocol has resulted in a survival of >80% and reduced complications for our last 25 animals undergoing CPB (Table 2)**.**

In summary, we find this article very helpful to all researchers who use sheep as a model for cardiovascular disease and must perform CPB. We hope our discussion may help such groups further increase their rates of survival.

Sincerely,
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Response to Dr Katz's Letter to the Editor:

We appreciate Dr Katz and his colleagues' comments regarding our manuscript describing care of sheep experiencing cardiovascular surgery and cardiopulmonary bypass. We agree that more research and reports describing the intricate care required for this model to be successful should continue to be published. We would like to respond to a few points that were made in their review of our manuscript.[1]

In regard to the surgical procedure, we feel that while a sternotomy may provide improved exposure to the vena cavae and/or enable simultaneous exposure of the left and right heart, the decreased morbidity of a thoracotomy incision may outweigh this benefit. For our specific purposes, the left thoracotomy via fifth intercostal space provided adequate exposure to the great vessels and left heart. Moreover, given that the sheep heart lies more in the left thorax, right-sided heart structures were easily exposed via a left thoracotomy in the third to fourth intercostal space. We discourage the routine use of a sternotomy when a thoracotomy will provide access to the sites of interest, and would prefer a thoracotomy even if it modestly lengthened surgery time. In our opinion, the severe post-operative pain associated with a sternotomy can be difficult to manage in quadripedal animals. Even with a combination of high dose of continuous opioid infusions, intrapleural therapy with local anesthetics, and nonsteroidal antiinflammatories, these animals still experience significant pain that, in the interests of animal welfare and the humane use of animals in research, should be avoided if a thoracotomy can provide sufficient access.

Second, in regard to cannulation strategy, we do not routinely cannulate the femoral artery, but rather the descending aorta, which is easily identified and accessed via a left thoracotomy incision. We do use a femoral cannula, which provides optimum

arterial flow. Third, we routinely cross-clamped the aorta to improve visualization during mitral valve repairs. It is safe to cross clamp the aorta for periods of up to 15 min followed by 5 min of intermittent reperfusion. We recognize the benefits of cardioplegia, also noted in our manuscript, but since we did require longer aortic cross-clamp times, we elected not to use cardioplegia. Instead, we used fibrillatory arrest and hypothermia to decrease the myocardial metabolic demands during cross-clamping.

With regard to postoperative care, we agree that the first 2 to 6 h between cessation of anesthesia and having the animal stand is a very critical period during which the animal is most at risk. Investing in this period will improve outcomes. However, in many academic institutions, 36 h of continuous supervision after surgery may not be feasible. We argue that based on our experience, this extended period of constant supervision may not be necessary if the animal is stabilized prior to being left unsupervised and the proper equipment and facilities are in place and the animal. We individualize postoperative monitoring and therapy (for example, frequency of diagnostics, continuous infusions of inotropes and/or chronotropes, fluid therapy, duration of antibiotic therapy) to a degree depending on how each animal recovers. We encourage research teams to rely heavily on their veterinarian for management during this period.

The judicious use of antibiotics as multidrug resistant bacteria continue to emerge is an important public health concern that laboratory animal veterinarians and biomedical researchers should share. Dr Katz and his colleagues describe an aggressive antibiotic regimen for their research animals. While it may seem reasonable to include more broad-spectrum antibiotic coverage for surgical prophylaxis in farm animals, our experience dictates that perioperative cefazolin provides adequate prophylactic antimicrobial therapy for these surgeries. All animals received 24 to 48 h of cefazolin preoperatively unless a known break in aseptic technique occurred or an infection was present. We have had no incidences of surgical site infections at our facility with this regimen. While we have used them as indicated, we also feel that the use of crystalloids and colloids postoperatively may not be necessary if the animal is eating and drinking independently, which occurs much more rapidly after a thoracotomy than a sternotomy. The nature of Dr Katz's research, coupled with a sternotomy approach, may account for less stable postoperative animals in his lab. We share Dr Katz's views on blood transfusion in this species, and have found that sheep do well even with moderate anemia postoperatively. Transfusing all blood left in the cardiopulmonary bypass pump back into the animal after bypass termination is another useful method of improving postoperative hemodynamics.

We thank Dr Katz and his colleagues for contributing to this dialogue, and share their goal of more communication and descriptions of the intricacies required to successfully manage these complex research protocols. Additional publications and collaboration can improve research outcomes and animal welfare in this growing area.

Sincerely,

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Reference

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