Journal of the American Association for Laboratory Animal Science
Vol 53, No 5
September 2014
Pages 439–448

Sheep (*Ovis aries*) as a Model for Cardiovascular Surgery and Management before, during, and after Cardiopulmonary Bypass

Louis DiVincenti Jr,[1,*] Robin Westcott,[2] and Candice Lee[3]

Because of its similarity to humans in important respects, sheep (*Ovis aries*) are a common animal model for translational research in cardiovascular surgery. However, some unique aspects of sheep anatomy and physiology present challenges to its use in these complicated experiments. In this review, we discuss relevant anatomy and physiology of sheep and discuss management before, during, and after procedures requiring cardiopulmonary bypass to provide a concise source of information for veterinarians, technicians, and researchers developing and implementing protocols with this model.

Abbreviations: ACT, activated clotting time; CPB, cardiopulmonary bypass; CVP, central venous pressure; MAP, mean arterial pressure; RAP, retrograde autologous priming.

The recent focus on rapidly translating findings in the laboratory to clinical applications has resulted in a shift to large animal models that closely correlate with human anatomy and physiology. The field of cardiac surgery is perhaps the most apparent beneficiary of animal research.[6] Sheep (*Ovis aries*) have emerged as a widely used model for cardiovascular surgical device testing, especially that involving cardiac valves.[30,42,62] Despite the widespread use of sheep in the research setting, there are no comprehensive reviews of the relevant anatomy and surgical approaches, animal preparation, anesthetic protocols and monitoring, and postoperative care. The unique physiology of sheep coupled with the complexity of cardiovascular surgical research results in significant morbidity and mortality in this animal model.[42]

After IACUC approval, our institution undertook investigations of several novel cardiac devices and interventions in sheep, thus requiring the development of a conditioning and management protocol for animals before, during, and after major cardiac surgery involving cardiopulmonary bypass (CPB). Because neither a thorough review of sheep anatomy and physiology nor an adequate discussion of all the layers of anesthetic management during these complex procedures existed in the literature, our procedures were developed through a review of published literature, extrapolation from humans, clinical experience, and continued refinements as outcomes were evaluated. In this review, we aim to provide a comprehensive overview of relevant literature and share our experience from studies conducted in our AAALAC-accredited, NIH-assured institution to provide a concise source for researchers and veterinarians who are developing cardiovascular surgical protocols in sheep and to minimize adverse events during their implementation.

Received: 12 Dec 2013. Revision requested: 07 Jan 2014. Accepted: 05 Mar 2014.
[1]Department of Comparative Medicine, [2]Division of Laboratory Animal Medicine, and [3]Department of Surgery, University of Rochester Medical Center, Rochester, New York.
[*]Corresponding author. Email: louis_divincenti@urmc.rochester.edu

Relevant Cardiovascular Anatomy and Physiology

Anatomic and physiologic similarities between sheep and humans make sheep an accepted animal model for cardiovascular research. A detailed review of cardiovascular anatomy and physiology is beyond the scope of this overview, but clinically relevant aspects are discussed briefly. The cardiovascular system consists of 2 circulations, the systemic and pulmonary, joined in series by the heart. Heart rate and intracardiac pressure of sheep approximate the normal values measured in humans.[5] The systemic circulation is a high-pressure system with a mean blood pressure of approximately 100 mm Hg in the large systemic arteries. In contrast, the pulmonary circulation has much lower pressure due to the low flow resistance of the lung capillary bed, and the mean pressure in the pulmonary artery is 15 mm Hg.[38] Systole (ventricular contraction) and diastole (ventricular relaxation) occur cyclically. An adequate cardiac output depends on preload (for example, blood volume filling the heart), afterload (for example, systemic vascular resistance), myocardial contractility, and heart rate.[38]

In addition to arterial pressures, central venous pressure (CVP) is frequently measured in cardiac patients or as a measure of intravascular volume deficit to guide fluid therapy and reflects right atrial pressure. In animals, CVP is most commonly measured in the cranial vena cava via catheterization of the external jugular vein, and measurements in awake sheep have been reported to be approximately 3 to 4 cm H_2O, with no difference in standing or laterally recumbent animals, but approximately 7 cm H_2O in anesthetized sheep.[73] Similarly, the normal CVP range in humans has been reported to be 4 to 11 cm H_2O.[39] Decreases in CVP indicate hypovolemia, vasodilation, or high cardiac output, whereas an elevated CVP indicates hypervolemia, right heart failure, or pulmonary hypertension.[28]

Similarities in body weight and cardiac output make sheep a more appropriate model for cardiac assist devices than smaller animals, such as rats and dogs.[13] Large animals other than sheep, such as pigs and cows, are useful models for cardiovascular surgical device testing, and readers are referred to more comprehensive explorations of animal models of cardiovascular research for more information.[26] However, being quadripedal animals, sheep do have significant anatomic variations from humans. In our view, the use of cadaver hearts or the dissection of carcasses should be the first step for a new research team prior to attempting procedures in live animals. Sheep have 13 pairs of ribs, 8 of which are associated with sternebrae.[17] The very narrow sternum of the sheep makes a median sternotomy technically difficult, given that the longitudinal dissection may result in deviation toward the intercostals.[2] The heart itself lies to the left of midline, but less significantly in sheep (and other quadripedal animals) than in people. In the standing sheep, the heart lies between the second and fifth intercostal spaces and is covered by the forelimbs. For this reason, an echocardiogram in the standing animal is best accomplished by pulling the left forelimb forward and applying the probe to the exposed axilla. The sheep heart is more ventrally tilted along its long axis than is the human heart and has a relatively blunt apex formed entirely by the left ventricle.[34] Whereas the normal heart weight:body weight ratio in adult humans has been reported to be 5 g/kg,[37] the ratio in sheep is 3 g/kg.[40] Because the base of the heart lies dorsally in the chest, a left lateral thoracotomy may provide improved surgical exposure to the left atrium and descending aorta than does a median sternotomy, a surgical approach that also is associated with more postoperative complications than is a lateral intercostal thoracotomy.[67] As previously reported,[67] we have found that a left lateral thoractomy at the fifth intercostal space provides adequate access to the left side of the heart and great vessels when the sheep is in right lateral recumbency. In contrast, a right lateral thoracotomy at the fourth intercostal space provides better access to the right side of the heart and venae cavae.

The valvular anatomy of sheep is similar to that of humans in terms of size and function.[63] The mitral valve has 2 leaflets (anterior and posterior) that are attached to anteriolateral and posteriomedial papillary muscles by chordae tendinae. The intervalvular, or membranous, septum, a fibrous continuity between the mitral valve and the aortic valve is present in humans but absent in sheep.[34] The aortic valve leaflets have been described as especially thin and fragile compared with those of humans.[63] In terms of approach, the pulmonary and aortic valves are at the levels of the third and fourth ribs, respectively, approximately 10 cm dorsal to the costochondral junctions, whereas the tricuspid and mitral valves are at the levels of the fourth and fifth ribs. The moderator band, a prominent cross-chamber muscular band, is usually present within the right ventricle of most sheep and is highly prevalent in the left ventricle as well.[34]

Finally, a discussion of the great vessels and blood supply to the heart is important. The coronary venous systems are fairly similar between sheep and humans, with the major exception of the left azygous vein. In sheep, the left azygous vein drains the dorsal part of the left chest and crosses cranial to the root of the left lung and winds over the surface of the left atrium before entering the right atrium at the coronary sinus.[17] A right azygous vein is also present but is relatively insignificant. In contrast to that in humans, the ascending aorta in the sheep is short and immobile, which has implications during cannulation prior to cardiopulmonary bypass (CPB). Whereas pigs and people have a right coronary type circulation, sheep (and dogs) have a left coronary type circulation, in which the majority of the myocardium receives its blood supply via branches of the left coronary artery.[48] Sheep do not have a significant coronary collateral network, similar to the situation in pigs and people, and so may be useful in myocardial ischemia research.[65] Infarction models can readily be created in sheep through ligation of the left anterior descending coronary artery, which is accessible through a left thoractomy via the fourth intercostal space.[41] Although considerable literature describes either very general or very specific aspects of mammalian cardiac anatomy, little quantitative, truly comparative research has been done, so researchers using animal models of cardiovascular disease should continue to publish their findings to add to the literature to allow such translation to human interventions.

Preoperative Care

The care of sheep during the preoperative period, from arrival through surgery, is critical to the success of research projects using this animal model. Housing and husbandry procedures must take into account the 'prey mentality' of sheep and provide the animals with social companionship whenever possible.[19] Depending on the specific protocol, Dorset sheep ranging in age from neonates to adults were acquired from a conventional farm and arrived in our facility at least 1 wk prior to the surgical procedure for acclimation and conditioning. SPF sheep are available but were unnecessary for the type of research performed. Sheep were housed in pens that provided approximately 20 to 40 ft^2 per animal, depending on the census, and that contained straw or woodchip bedding. Whenever possible, sheep were housed in social groups consisting of at least 2 animals, but when only one animal was housed, mirrors were used to provide visual enrichment. Environmental conditions in the room were: temperature, 21 ± 1 °C; humidity, 30% to 70%; light cycle, 12:12 h. Veterinary staff evaluated each animal to ensure overall health and performed routine husbandry and diagnostic procedures as indicated to characterize the animal's fitness for surgery. At minimum, diagnostic evaluation prior to cardiovascular surgery included a CBC, a chemistry panel, and Q fever serology. After characterizing parasite load associated with particular vendors by using routine fecal examinations, anthelmintic selection was based on historic results, and animals were treated prophylactically on arrival with either fenbendazole (50 mg/kg PO) or ivermectin (0.2 mg/kg SC). Vendors that provided heavily parasitized animals were excluded. In addition, sheep were treated with florfenicol (20 mg/kg IM every 48 h for 1 wk) for prophylactic treatment of subclinical mycoplasmosis. During this time, the animals had free access to water and were fed pelleted feed as well as high-quality hay to encourage normal rumen function. Although the potential for grain overload warrants caution, sheep undergoing these major recovery procedures may have increased metabolic demands and may require additional grain to meet their nutritional requirements, especially during times of decreased hay quality.

The large, multicompartmental stomach characteristic of ruminants requires careful consideration to avoid hemodynamic and respiratory compromise during periods of recumbency. In addition, regurgitation and aspiration of gastric contents during anesthesia is more common in ruminants than other animals. Although fasting has little effect on the volume of digesta present, it may reduce the incidence of rumenal tympany.[22] Reduction of the weight of the gastrointestinal tract through withholding of food and water ameliorates the negative effects on cardiorespiratory function that occur in recumbent animals during anesthesia.[71] Water should also be restricted because continued water intake in a fasting animal would increase the liquid contents of the rumen and increase the risk of regurgitation. In our facility, food was withheld from adult animals for 24 h and water was withheld for 8 to 12 h prior to surgery. Suckling lambs were used for some studies, but as previously reported, these animals were allowed to nurse until the time of anesthesia.[12]

Anesthetic Management

Table 1 provides an overview of drugs, dosages, and indications useful for cardiac surgery in sheep. The goal of premedication prior to anesthesia is to minimize the stress that the animal experiences. In our experience, the placement of an intravenous catheter on the day prior to surgery in animals that could be manually restrained easily greatly facilitated induction on surgery day. In most cases, sheep were compliant, and minimal restraint was necessary once the animal was turned on its hindquarters in the rumping position. A single-lumen, 18-gauge central venous catheter (J1041, Jorgensen Laboratories, Loveland, CO) was aseptically placed in an external jugular vein, and the site bandaged to protect it from the animal. Alternatively, double-lumen catheters were used in some cases to allow for measurement of CVP simultaneously with fluid or drug administration intraoperatively. If preplacing catheters was not possible, intravenous drug delivery through the jugular vein was used for anesthetic premedications to minimize preanesthetic distress. Although the choice of specific drugs is a matter of preference and depends on the experience and comfort level of the staff, an anesthetic regimen that rapidly induces sedation and recumbency in the housing room provides for a more stable induction and early anesthetic period. We have found that ketamine (4 mg/kg IV; Ketamine hydrochloride, 200 mg/mL, Wildlife Pharmaceuticals, Windsor, CO) combined with midazolam (0.4 mg/kg IV; Midazolam, 50 mg/mL, Wildlife Pharmaceuticals)[71] and glycopyrrolate (0.004 mg/kg IV) combined in the same syringe and administered into the external jugular vein to be the most rapid and reliable drug regimen, with concentrated formulations resulting in a small volume for rapid injection. A combination of intravenous midazolam (0.5 mg/kg) and fentanyl (0.005 mg/kg) has also been described,[71] and we have used this regimen in our facility without complication for nonsurgical procedures requiring recumbency. Both of these regimens result in lateral recumbency.

Use of anticholinergics in ruminants is controversial; however, we have found the use of glycopyrrolate to be beneficial with no observed adverse effects. The continuous salivation of ruminants coupled with the increased salivation observed after ketamine administration can prevent clear visualization of the larynx during intubation.[58] Suction may be a useful aid in visualization of the larynx, but anticholinergics can also be effective when suction is insufficient. Although anticholinergics reportedly do little to affect the volume of secretions of ruminants,[57] we have observed marked reduction in salivation resulting in improved intubation efficiency. For cardiovascular surgery, anticholinergics generally are avoided due to concerns with cardiac arrhythmias. However, the rapid removal of glycopyrrolate from serum after IV administration makes it likely that the drug is mostly excreted prior to surgical manipulation;[57] however, pharmacokinetic data regarding glycopyrrolate metabolism and excretion in sheep are not available. We have observed no changes in heart rhythm with the addition of glycopyrrolate to our anesthetic protocol. Finally, the potential for anticholinergics to cause gastric stasis and ileus is a significant consideration for their use in ruminants, in which rapid return to normal gastrointestinal function is imperative for anesthetic recovery. We observed no decrease in gastrointestinal function, based on return of normal rumination, appetite, and fecal production, after surgery for which glycopyrrolate was added to our anesthetic regimen.

After heavy sedation in the housing room, sheep were quickly transported to the preparatory area in either left lateral or sternal recumbency. As soon as practical, each animal received respiratory support with 100% oxygen via face mask. Supplementation with isoflurane or another gas anesthetic was occasionally necessary during this time if a catheter had not been preplaced. The animal then was shifted to sternal recumbency to prepare for intubation, and general anesthesia was induced with propofol (2 to 4 mg/kg IV). Assuring that the animal was anesthetized sufficiently prior to attempting intubation increased the likelihood of success,[71] and in our experience, injectable induction agents have been much more efficient at producing sufficient anesthesia than are inhalant anesthetics delivered via face mask. Propofol has emerged as a first-choice induction agent in a variety of species for a variety of surgical procedures. When dosed to effect, propofol's short half-life and rapid metabolism facilitate rapid induction and recovery with minimal effects on cardiac function. Administering the drug slowly until sufficient jaw relaxation occurs reduces likelihood of significant cardiorespiratory depression. In sheep, visualization of the epiglottis is limited due to the narrow oral opening, but the use of a laryngoscope with a long blade (> 20 cm) and an endotracheal tube stylet is helpful. The animal was retained in sternal recumbency until the endotracheal tube was secured and the cuff inflated. Anesthesia then was maintained with 1% to 2% isoflurane in 100% oxygen.

Intermittent positive-pressure ventilation was used as soon as the endotracheal tube was secured. In the laterally recumbent animal, the large mass of abdominal organs places pressure on the diaphragm, so use of intermittent positive-pressure ventilation is essential to prevent serious respiratory compromise and acidosis. The baseline settings used for the ventilator in our facility were as follows: tidal volume, 10 to 12 mL/kg; inspiratory:expiratory ratio, 1:2; respiratory rate, 12 to 15 breaths per minute; and positive end-expiratory pressure, 5 to 10 cm H_2O. Tidal volume was adjusted up or down to generate inspiratory pressures of approximately 15 to 20 cm H_2O. Although usually unnecessary, the respiratory rate was increased when intermittent positive-pressure ventilation was first initiated to capture the animal's spontaneous respirations, and then adjusted to maintain an end-tidal CO_2 of 35 to 45 mm Hg. Many veterinary ventilators cannot provide positive end-expiratory pressure, which minimizes atelectasis associated with long surgical procedures and recumbent positions by maintaining positive pressure even during expiration.[64] However, we have observed and at least one study has reported significant improvement in lung aeration and gas exchange with the use of positive end-expiratory pressure in mechanically ventilated sheep.[68]

Next, an orogastric tube was placed to minimize free-gas bloat and rumenal tympany.[22] The sheep then received carprofen (3 mg/kg IV, Rimadyl , Zoetis Animal Health, Florham Park, NJ) for preemptive analgesia. Carprofen, a cyclooxygenase-2-specific NSAID, was the first veterinary NSAID to be labeled specifically for postoperative pain. In addition, a single intravenous dose reportedly provides analgesia in sheep for as long as 3 d with minimal adverse effects.[70] Sheep also received cefazolin (22 mg/kg IV) for antimicrobial prophylaxis during surgical preparation. Few controlled clinical trials in sheep are available to guide antimicrobial prophylaxis regimens, so our regimen was adopted from recommendations for human cardiovascular surgery. A first-generation cephalosporin, cefazolin provides adequate coverage against bacteria most likely to cause surgical site infections and is considered the first choice

Table 1. Drugs used during cardiovascular surgery

	Dosage	Indication
Anesthetic premedication and induction		
Ketamine	4 mg/kg IV	As a part of a premedication cocktail to induce lateral recumbency
Midazolam	0.4 mg/kg IV	As part of a premedication cocktail to induce lateral recumbency
Glycopyrolate	0.004 mg/kg IV	As part of a premedication cocktail to reduce salivary secretions
Propofol	2–4 mg/kg IV to effect	Anesthetic induction
Preoperative		
Heparin	5000 U SC	To reduce risk of venous thrombus formation during surgical preparation
Cefazolin	22 mg/kg IV	Prophylactic antimicrobial therapy
Carprofen	3 mg/kg IV	Preemptive analgesia
Intraoperative		
Heparin	250 U/kg IV, repeated as 5000- to 10,000-U boluses as needed	To induce anticoagulation prior to CPB
Phenylephrine	100–200 µg IV 1 µg/kg/min IV	To increase systemic vascular resistance in response to hypotension
Lidocaine	2–4 mg/kg IV	To prevent or treat ventricular fibrillation
Epinephrine	2–8 µg IV 2–10 µg/kg/min IV	To provide inotropic support in cases of ventricular dysfunction
Dobutamine	5–10 µg/kg/min IV	To provide inotropic support in cases of ventricular dysfunction
Calcium chloride	0.5–1 g IV	To treat persistent hypocalcemia induced by CPB
Potassium chloride	5–10 mEq IV slowly	To treat hypokalemia ($K^+ < 3.5$ mEq/L) induced by CPB
Magnesium sulfate	1–2 g IV	To treat hypomagnesmia induced by CPB
During surgical closure		
Bupivicaine	Up to 2 mg/kg perineurally	To provide local anesthesia
Furosemide	0.5–1.0mg/kg IV	To induce diuresis to minimize potential postoperative pulmonary edema
Buprenorphine	0.005 mg/kg IV	To provide postoperative analgesia
Albuterol	Via nebulization	To induce bronchodilation to increase air flow
Postoperatively		
Buprenorphine	0.005 mg/kg IV or IM TID or BID	To provide analgesia if needed
Cefazolin	22 mg/kg IV BID	Continued antimicrobial coverage for 24–48 h after surgery

for antimicrobial prophylaxis for clean and clean–contaminated surgeries in humans.[46] Cefazolin was readministered every 2 to 3 h throughout the procedure and continued twice daily for 24 to 48 h after surgery, unless a known break in aseptic technique warranted additional infection control measures. Although antimicrobial treatment that extends beyond the surgical period does not reduce the incidence of postoperative infection,[36] we believe that this 24 to 48 h period of antimicrobial control was important as the animal still had a central venous line in place that represented a possible source of microbial exposure.

After provision of preemptive analgesia and prophylactic antimicrobials, an arterial catheter (no. 498107, Argon Medical

Devices, Athens, TX) was placed in the radial artery of the forelimb by using a cutdown procedure. Although other sites for arterial catheter placement such as the auricular, carotid, and femoral arteries are available, we have found that the artery in the forelimb site is the most consistently successfully catheterized and maintained. Because the sheep was in left lateral recumbency during preparation and the artery could readily be palpated on the caudomedial aspect of the limb, the arterial catheter was placed in the left (down) forelimb. Standard aseptic techniques for arterial catheter placement were used. Immediately after placement, an arterial blood sample was taken for assessing baseline acid–base status, electrolytes, hematocrit, hemoglobin, and activated clotting time (ACT). A transducer with blood sampling ports (DT4812, DTX Plus, Argon Medical Devices) was used to allow simultaneous invasive blood-pressure monitoring and arterial blood sampling during the procedure. Finally, the sheep was moved into the position required for surgery, and the surgery site and an area on the rump for the electrocautery ground pad were clipped and cleaned. Once in the operating room, the animal was positioned appropriately, and the head was elevated to reduce regurgitation of rumen contents. ECG, pulse oximetry, capnography, invasive blood pressure, and rectal temperature were monitored by a dedicated anesthetist continuously throughout the procedure.

Intraoperative Care and CPB

Systemic heparinization is required to prevent thrombosis of the CPB circuit or the animal's organs during CPB, and the ACT is the standard method of assessing anticoagulation status intraoperatively. Although our institution uses 480 s as the target for heparin therapy in human CPB patients, the minimal ACT to prevent thrombosis in humans and animals remains unknown.[20] During our protocol development, we noted a wide variation in responses to heparin dosing and complications associated with both prolonged bleeding and thrombus formation. The actual heparin doses required to attain acceptable ACT actually paralleled the dose range (200 to 400 U/kg) reported in the literature for sheep models[9,14,50,75] and reports of heparin response variability and resistance in human patients.[7,8] In response to a lethal pulmonary embolism that developed in one of our animals, potentially secondary to embolization of a deep-vein thrombosis, we adopted a protocol of preoperative administration of 5000 U heparin subcutaneously in adult sheep immediately on arrival to the surgical preparatory space. In human surgical patients, subcutaneous heparin therapy reduces the incidence of thrombosis without inducing clinically significant bleeding.[23] Because venous stasis is likely present in the sheep's extremities during the preparatory and operative phases, we believe that early subcutaneous heparin therapy reduces the risk of thrombus formation without causing bleeding complications during surgery. After exposure of the heart, each sheep received an additional bolus of heparin (250 U/kg IV). Because heparin induces its maximal effect within 5 min of administration,[25] ACT was measured at that point. ACT measurements were repeated every 45 to 60 min throughout the procedure, and heparin boluses of 5,000 to 10,000 U were given as needed to maintain sufficient ACT.

Like others,[9,67] we have not used protamine to reverse heparin. A protein derived from salmon sperm, protamine neutralizes heparin through ionic binding but may result in anaphylaxis and a terminal pulmonary vasoconstriction syndrome, especially in sheep.[45] However, several others have used protamine (maximum, 2 mg/kg) in sheep without encountering these adverse effects.[13,50] The rate of protoamine administration apparently is the primary factor in protamine sensitivity, and adverse effects can be eliminated by administering the dose over a 30-min period.[50] Using these data, we were prepared to administer protamine if clinically indicated, but we have yet to note clinical bleeding at the time of wound closure that warranted heparin reversal.

Although we have not found it necessary for the types of procedures performed in our facility, neuromuscular blockade is common in human cardiac surgery[32] and has been used in ovine cardiac surgery.[66] Sheep can be intubated and mechanically ventilated without the use of neuromuscular blocking agents, so, as in humans, the use of these agents should be restricted to situations where clinical neuromuscular paralysis is necessary.[32] Vecuronium and atracurium dosed as 25-μg/kg and 500-μg/kg IV boluses, respectively, have been used in sheep to facilitate mechanical ventilation and produce paralysis indicated for surgery.[10,68] Vecuronium reaches its peak effect approximately 3.4 min after intravenous injection and persists for 40 min.[10] Atracurium, with a more rapid onset and shorter duration, may be a more appropriate neuromuscular blocking agent when a brief blockade is desired. The effective dose of vecuronium in sheep is reportedly a maximum of one third that in humans, so extrapolation of dosages from human medicine should be done with caution.[47] Cardiac dysrrhythmias have not been reported in sheep treated with vecuronium, atracuruim, or mivacurium.[10] Neuromuscular blockage can be reversed with edrophonium (500 μg/kg) or neostigmine (50 μg/kg) and atropine 80 μg/kg.[11] When neuromuscular blocking agents are used, return of voluntary muscle function must be confirmed before the sheep leaves the operating room.

Shifting to a discussion on the surgical aspects of this model, sheep have a relatively short ascending aorta, so arterial cannulation of the ascending aorta and placement of an aortic cross clamp may not be feasible. The descending aorta is well exposed and can be accessed easily through a left thoracotomy via the fifth intercostal space. We performed arterial cannulation via the descending aorta with an 18-French femoral arterial cannula (Fem-Flex II, Edwards Lifesciences, Irvine, CA). In larger animals, a small-sized aortic cannula (18 to 21 French) can be used in place of a femoral cannula. When choosing an aortic cannula, consider the aortic diameter at the cannulation site and the maximum flow permitted through the cannula. The aortic cannula should be small enough that it will not impede normal aortic blood flow around the cannula when inserted, but it should be large enough to provide adequate flow without excessive pressure drop across the cannula to minimize shearing forces. The femoral arterial cannula that we chose provides a maximal flow of 4.25 L/min, which is more than adequate to provide optimal CPB flow rates (2.2 to 2.5 L/min-m^2) in adult sheep.[52]

The CPB circuit was primed with 1200 to 1500 mL Plasma-Lyte A (Baxter Healthcare, Deerfield, IL); however, retrograde autologous priming (RAP) routinely was used to minimize hemodilution. After insertion of the arterial cannula, arterial blood was pulled retrograde through the CPB circuit to replace the priming solution in the arterial line, venous reservoir, and filter–oxygenator. At the start of CPB, blood was taken back through the venous tubing to clear Plasma-Lyte A from the venous lines, leaving only 100 to 200 mL Plasma-Lyte A remaining in the circuit. Although the use of RAP has been shown to decrease intraoperative and postoperative blood transfusion requirements, it has not clearly been shown to improve patient outcomes.[60,69] Nevertheless, RAP can be an extremely useful tool to offset intraoperative blood losses and hemodilution during instances where blood transfusions are not readily available. If the surgical team elects to use crystalloid priming solutions in place of RAP, plasma expanders (for example albumin, mannitol, dextran, and hetastarch) can be added to the priming solution to minimize hemodilution and pulmonary edema.[49] In human patients at our institution, 150 mL 15% mannitol is typically added to 1050 mL Plasma-Lyte A to make 1200 mL priming solution for CPB.

Venous cannulation was performed with a 24-French venous catheter (DLP Single Stage Venous Cannula, Medtronic, Minneapolis, MN) placed in the main pulmonary artery and directed toward the pulmonary valve. If pulmonary surgery is planned, right atrial venous cannulation can be performed via the right atrial appendage, which is accessible through a left thoractomy incision in the third or fourth intercostals space. The superior and inferior vena cava, however, are not easily accessed from a left thoractomy incision. If access to right-sided heart structures is required, then a right thoracotomy via the fourth or fifth intercostal space or a median sternotomy is preferable. Bicaval venous cannulation and constriction will provide more efficient venous drainage and improve visualization during the repair of right-sided heart structures.

Immediately after initiation of CPB, systemic arterial hypotension occurs secondary to the acute intravascular hypovolemia and hemodilution that occur during RAP of the circuit. Although hemodilution improves organ blood flow through a reduction in blood viscosity during CPB, excessive hemodilution (Hgb < 5 to 7 g/dL) can be detrimental inducing a variety of complications including hypotension, pulmonary edema, renal failure, and stroke.[27] To minimize hemodilution, intravenous fluid administration should be conservative prior to bypass and cease after initiation of CPB. Decreases in Hct of 20% to 40% are common and significantly reduce blood viscosity, reducing systemic vascular resistance and secondarily mean arterial pressure (MAP). A target MAP of 50 to 60 mm Hg has generally been considered to be appropriate during CPB, but recent data are suggesting that MAP greater than 70 mm Hg improves outcomes in high-risk patients.[52] The lower limit of this interval is based on data suggesting that cerebral autoregulation is disrupted below 50 mm Hg, but more recent data indicate that cerebral autoregulation may be maintained at MAP as low as 20 to 30 mm Hg in anesthetized humans during hypothermic CPB.[52] In our experience, sheep typically maintained a MAP of 70 to 80 mm Hg in a surgical plane of anesthesia prior to CPB, and MAP fell to 35 to 40 mm Hg immediately after initiation of CPB. After stabilization of pump settings and occasional pharmacologic support, MAP stabilized to 40 to 50 mm Hg throughout the procedure.

Phenylephrine, a selective α1-adrenergic agonist with no effects on β receptors, has emerged as the best choice for pharmacologic treatment of CPB-induced hypotension given that its principal effect is increasing systemic vascular resistance through vasoconstriction. Phenylephrine boluses of 100 to 200 μg can be given directly into the pump or into the central venous line to counteract moderate to severe hypotension associated with CPB initiation. The short half-life of phenylephrine (about 4 min) in sheep requires repeated boluses or continuous rate infusion at 1 to 3 μg/kg/min to sustain the effect.[24,55] As a result of the absence of sufficient data to provide recommendations for appropriate MAP during CPB, it is important to recognize that pump flow rate may affect systemic oxygen delivery more than MAP. Thus, increasing the flow rate on the CBP machine may be more beneficial in terms of oxygenation than are pharmacologic options to increase blood pressure, with an optimal flow rate of 2 L/min in most situations.[56] In this regard, pH is a useful bedside measurement in determining adequate perfusion because it can indicate the adequacy of total blood flow, given that lactic acid produced by anaerobic metabolism will lower pH.[16] In any case, the anesthetist should communicate with the perfusionist to address CPB-induced hypotension.

During CPB, a surgical plane of anesthesia was maintained by using isoflurane at 1% to 2% delivered directly into the circuit. When ventilation did not interfere with surgical exposure, lung ventilation continued throughout the procedure, to minimize atelectasis. It is important to optimize cardioprotection yet maintain a bloodless working field during the procedure. The mainstays of cardioprotection during cardiac surgery are cardioplegia, hypothermia, and prevention of cardiac distention. Cardioplegia is designed to rapidly induce electromechanical cardiac arrest, thereby decreasing metabolic demands as well as preventing degenerative and ischemic changes during CPB.[29,49] These benefits limit the degree of myocardial injury during aortic cross clamping[29] and aid the surgeon by allowing surgery to be performed on a flaccid heart. If the surgeon elects not to use cardioplegia, the heart may be fibrillated and moderate hypothermia (approximately 25 °C) used. Moderate hypothermia has been shown to protect the myocardium by decreasing cell metabolism, oxygen demand, and cell damage and death.[72] During periods of mechanical or functional cardiac arrest, monitoring and preventing cardiac distention is key to reduce myocardial oxygen demand (from increased myocardial wall tension), reduce the risk of air emboli, and improve surgical visualization.[33] To prevent cardiac distention in our sheep, the left side of the heart was vented by using a CPB suction catheter placed through the mitral valve into the left ventricle during ventricular fibrillation.

Distortion of the left ventricle and left ventricular outflow tract during surgical manipulation can cause aortic insufficiency, so intermittent aortic cross clamping was used to inhibit back-bleeding of blood into the surgical field. Prolonged aortic cross clamping is associated with increased postoperative morbidity and mortality, including increased 30-d mortality, risk of stroke, renal insufficiency, and myocardial dysfunction.[53] However, intermittent aortic cross-clamping on fibrillated hearts under mild–moderate hypothermia (32 to 34 °C) for periods up to 15 min alternating with periods of perfusion for 5 min has been shown to provide equivalent myocardial protection as cardioplegic arrest, with comparable postoperative morbidity.[64] Aortic cross-clamping in the fibrillated heart not only facilitates visualization but also can reduce the risk of air emboli. In addition, the use of CO_2 insufflations minimizes the risk of air embolism. CO_2 is more than 25 times as soluble in tissue and blood as in air and is 50% heavier than air; these characteristics assist in the displacement of air from the cardiothoracic cavity. Before initating cardiac repair, CO_2 was flooded into the intrathoracic surgical field through a 10-French Jackson–Pratt drain tucked adjacent to the cardiotomy. Prior to release of the aortic cross clamp and rewarming, lidocaine (2 mg/kg) was given directly into the CPB circuit to reduce the risk of reperfusion ventricular fibrillation and improve cardioconversion back into sinus rhythm.[4] Pediatric internal paddles connected to a manual defibrillator (HeartStart MRx, Philips Medical Systems, Andover, MA) were used to defibrillate the animals back into sinus rhythm when they did not spontaneously revert after rewarming and lidocaine.

Once sinus rhythm was restored and echocardiography confirmed adequate cardiac function, sheep were weaned off CPB. At this point, ventilation was restored for cases in which ventilation had not been continuous throughout the procedure.

During this period, significant complications can occur, requiring effective communication among all members of the surgical, anesthesia, and perfusion team. Prior to weaning, normothermia was achieved by using the heat exchanger on the bypass machine and a Bair hugger or other warming device. The animal's anesthetic plane was monitored carefully during this period, because the reduction in anesthetic requirement resulting from induced hypothermia and slowed metabolism reversed as the animal's temperature increased. We administered buprenorphine (0.005 mg/kg IV) at this time to reduce the requirements for isoflurane, a cardiodepressant, and to provide immediate postoperative analgesia.

In addition, arterial blood analysis was performed to assess the proximity of electrolytes (especially K^+ and Ca^{2+}), acid–base status, and glucose to their normal ranges. Any abnormalities were corrected prior to attempts at bypass weaning. Human clinical studies indicate that ionized calcium and magnesium decrease in all cases, whereas potassium decreases after CPB in as many as 40% of patients.[1,18,59] Because myocardial contractility is dependent on calcium,[38] clinical decreases in ionized calcium should be corrected if the hypocalcemia is sustained during rewarming.[61] However, the routine administration of calcium to human patients in the absence of hypocalcemia and ventricular dysfunction is not advised, because excess calcium may worsen reperfusion injuries.[61]

Although magnesium is not often evaluated in veterinary patients, correction of hypomagnesemia is important, given that it predisposes to cardiac arrhythmias.[1] Magnesium decreases systemic vascular resistance, potentially inducing hypotension, so blood pressure should be closely monitored during administration. In addition, magnesium can potentiate neuromuscular blocking drugs, so the timing of magnesium administration should be sufficiently prior to extubation to allow monitoring for these effects. Magnesium repletion should be considered when cardiac arrhythmias, especially atrial fibrillation, occur during CPB weaning. Furthermore, hypokalemia predisposes to ventricular arrhythmias and can result in generalized muscle weakness.[76] The perfusionist can administer 5 to 10 mEq KCl over 1 to 2 min, 0.5 to 1 g calcium chloride or gluconate, or 1 to 2 g magnesium sulfate (or their combinations) directly into the CPB pump.

Simultaneously with electrolyte replacement, oxygenation and mechanical ventilation was reset, and the lungs were reinflated manually by using sustained pressures of 40 to 50 mm Hg. Ventilating the lungs throughout the procedure, even while the animal is on CPB, will prevent significant atelectasis and improve oxygenation postoperatively. Alternatively, intraoperative alveolar recruitment with sustained insufflations until visual confirmation of the absence of atelectasis throughout most of the lungs also improved postoperative oxygenation[77] and reduced recovery time, in our experience.

Apparent hypotension as measured by the arterial catheter may develop at the end of CPB, especially during rewarming. This reduction represents a relatively common artifact, in which radial artery pressures underestimate central aortic pressure by as much as 30% to 40% and can continue for as long as 30 min after discontinuation of CPB.[16] The physiologic basis of this mismatch is not fully understood, but radial arterial pressures should be confirmed by obtaining either central aortic or even a noninvasive pressure measurement before aggressive therapy is initiated. Hypotension at the time of bypass cessation is often a result of reduced preload and underfilling of the heart, which can be assessed visually by the surgeon.[43] We have found that autotransfusion of blood from the cardiotomy reservoir improved cardiac output in these situations. Other research groups report having donor whole blood available for transfusion when necessary.[9] Judicious use of crystalloids and colloids (for example, hetastarch) may also be used to increase intravascular volume. In cases in which hypotension occurred secondary to ventricular dysfunction in a sheep with a normal electrolyte status, inotropic support was helpful. β-agonists are typically the first choice in these cases in people, and epinephrine is used often, given that it has both inotropic and chronotropic effects.[61] When necessary, epinephrine was given as 2- to 8-µg boluses or as an infusion at 2 to 10 µg/min.

Once these physiologic measures (temperature, cardiac function, ventilation, and acid–base and electrolyte status) were normalized and the surgeon controlled all major sources of bleeding, weaning and disconnection from CPB ensued. Blood volume from the cardiotomy reservoir was gradually emptied into the animal, paying close attention to its ability to accommodate the increased preload by increasing stroke volume and maintaining MAP. After successfully weaning the sheep from CPB, the venous cannula was removed. Blood from the CPB machine was still given via the arterial cannula during this time. After sufficient blood volume had been returned with adequate MAP, the arterial cannula was removed. Both venous and arterial cannulation sites were checked for hemostasis before closure.

During closure, bupivicaine intercostal nerve blocks (maximal dose, 2 mg/kg) were performed at the thoracotomy rib space as well as the rib spaces cranially and caudally to provide local anesthesia to the surgical site. Albuterol, a selective β2-agonist, was administered via neubulization directly into the endotracheal tube to prevent bronchoconstriction–bronchospasm and to increase air flow into the lungs.[9,57] Furosemide (0.5 mg/kg IV) was administered to treat reactive pulmonary edema after cardiac surgery and CPB.[15] Although outcomes were not improved in a clinical trial assessing routine use of diuretics in human cardiac patients,[44] we have observed improvements in postoperative ventilation after the routine addition of furosemide to our recovery phase. Electrolyte replacement and autotransfusion continued as needed throughout this period. Once the surgeon had completed the procedure, a final arterial blood sample was taken to assess acid/base status. If no abnormalities were noted, the arterial catheter was removed, the artery ligated, and the site bandaged. Concurrently, the anesthetist began weaning the sheep from the ventilator by slowly decreasing both isoflurane concentration and respiratory rate until spontaneous respiration occurred. Once spontaneous respiration returned, the sheep was transported to the recovery area. In the recovery area, the animal was shifted to sternal recumbency as soon as possible to allow normal eructation to occur. Pulse oximetry was used to monitor heart rate and oxygen saturation, and a rectal thermometer used to monitor temperature. Heat supplementation, including warm fluids and a Bair hugger, were provided, and 100% oxygen was administered into the endotracheal tube until the animal began chewing. The endotracheal tube was removed with the cuff partially inflated to remove any aspirated ingesta from the trachea.

Postoperative Care

Immediately after extubation, the sheep was moved from the table to a Panepinto sling. In our experience, the ability to maintain the animal in sternal recumbency until standing has been critical to recovery. We have observed that hypoxia, hypothermia, electrolyte deficiencies (especially hypokalemia and hypocalcemia), and pain markedly hinder rapid recovery. As discussed, these factors should be addressed prior to the discontinuation of anesthesia to provide maximal benefit. In terms of oxygenation, using positive pressure and sustained lung insufflations to eliminate atelectasis intraoperatively improves oxygenation.[35] Providing 100% oxygen via face mask and maintaining the animal in sternal recumbency to shift the weight of the abdominal organs away from the diaphragm also improved the animal's oxygenation. To address hypothermia, the CPB machine's heat exchanger was used to allow rapid rewarming of the animal at the end of bypass, but this technique also results in a heat debt. This heat debt manifests as a rebound hypothermia in the postoperative period, referred to in the human literature as 'afterdrop.'[16] We have observed sheep that leave the operating room at near normothermic (102 °F) and that subsequently become moderately hypothermic (96 to 98 °F) within 30 min of transfer to the recovery area, necessitating the use of warming blankets and other aggressive warming techniques (warmed intravenous fluids and so forth) to prevent such hypothermia. We also have noted moderate to severe hypokalemia (2.8 to 3.2 mmol/L) and hypocalcemia (0.9 to 1.1 mmol/L) during the postoperative period and occasionally persisting for as long as 24 h after surgery. Potassium rapidly self-corrected once the animal began eating, but calcium required supplementation with 0.5 to 1 g calcium gluconate or calcium chloride intravenously in many cases. Hypocalcemia was so severe in some sheep that we believe it resulted in generalized weakness that hindered recovery. After calcium supplementation in these animals, a prolonged recovery rapidly ended, with the animal being able to stand and eat within just a few minutes of intravenous calcium supplementation. Because venous blood gas values can be used in lieu of arterial samples for evaluation of acid–base and oxygenation status in sheep,[54] the indwelling central venous catheter provided an essential tool for evaluating both acid–base and electrolyte status in the recovery period.

Assessment of pain in sheep can be difficult given that they are relatively stoic prey animals that may not display obvious signs of pain or distress.[21] Signs of pain may be subtle, such as shifting, reluctance to move, and mild tachycardia, or more significant, such as pawing at the surgical site or anorexia. Early in the recovery process, pain may be manifested simply as a prolonged recovery in the absence of electrolyte, temperature, or oxygenation abnormalities. Despite the difficulties in assessing pain in ruminants, multimodal preemptive analgesia is the current standard of veterinary care in companion animals[31] and should be used for research sheep undergoing surgery even in the absence of significant signs of pain. As described, sheep are preemptively administered carprofen, a NSAID indicated for the treatment of postsurgical pain that has been reported to provide analgesia for as long as 3 d in sheep.[70] Although other drug classes are useful to reduce the intensity of postoperative pain, NSAID are unique in their ability to treat the underlying processes (that is, inflammation) that result in pain.[3] In addition to preemptive NSAID administration, we use nerve blocks with local anesthetics, the most commonly used analgesics in food animals,[51] to provide immediate postoperative pain relief. In the case of thoracic surgery in quadrupeds in which considerable body weight is maintained on the ribs and sternum, such local immediate pain relief improves the animal's ability to rapidly regain a normal posture after surgery. Opioids are more effective in small ruminants such as sheep than cattle, and the use of buprenorphine as an analgesic in sheep after surgery is well described.[9,12,67] Buprenorphine's long half-life compared with other opioids makes its use more attractive in the laboratory animal setting. In our facility, sheep receive carprofen (3 mg/kg IV) prior to surgery and buprenorphine (0.005 mg/kg IV) during skin closure. Buprenorphine (0.01 mg/kg IM or IV) was repeated 6 h later and then every 12 h, and carprofen (2 to 4 mg/kg IM or IV) was repeated as often as once daily for 5 to 7 d as needed. We have used an 'all of the above' approach to analgesia, using low doses of multiple agents, including local anesthetics, NSAID, and opioids, to provide sufficient postoperative pain relief without adverse effects.

Each sheep was maintained in the recovery area until it had normal respirations, pink mucous membranes and normal oxygen saturation without any oxygen therapy, was able to stand with minimal ataxia, and no longer tolerated the Panepinto sling. On average, sheep spent approximately 2 to 4 h in the recovery area. During this time, animals were observed continuously and monitored as discussed so that timely intervention could be provided when necessary. Once the animal was standing, it was returned to the housing room and allowed nose-to-nose contact with conspecifics but was singly housed. After return to housing, sheep were monitored for 1 to 2 h longer until complete anesthetic recovery occurred. By this time, animals were ambulating normally and eating.

In the 7 d after surgery, each sheep was observed daily for evidence of surgical site infection, pain, cardiovascular compromise, and electrolyte status. This observation included at minimum a brief physical exam, observation of the surgical site, assessment of attitude, activity, appetite, and fecal production, measurement of temperature, pulse rate, respiratory rate, thoracic auscultation, and oxygen saturation via pulse oximetry. When necessary, blood pressure measurements, electrocardiography, or echocardiography (and their combinations) were performed. Laboratory analyses of WBC count and electrolytes were performed daily for 1 to 2 d after surgery and whenever animals appeared weak or lethargic. However, WBC count in animals after CPB should be interpreted with caution, because CPB initiates a systemic inflammatory response through complement activation, cytokine release, leukocyte activation, and production of oxygen free radicals and other inflammatory mediators.[74] No surgical site infections have been observed, although a local infection at the site of the arterial catheter occurred in 2 animals. In animals that were eating within 6 to 12 h of surgery, electrolyte replacement therapy was not necessary beyond the immediate perioperative period. However, some animals did require potassium and calcium supplementation for 1 to 2 d after surgery. Sheep were returned to social housing with conspecifics within 48 h of surgery (after removal of central venous catheters), and analgesics were continued as needed for 5 to 7 d after surgery.

Conclusion

Cardiovascular surgery in sheep presents the research team with significant challenges, but sheep provide a robust animal model for the investigation of human cardiovascular disease and novel interventions. Through careful management, attention to the sheep's unique anatomic and physiologic characteristics, and effective collaboration among a research team including surgeons, veterinarians, anesthetists, and perfusionists, the sheep model is reproducibly successful for translating research findings into novel interventions for human cardiovascular patients.

References

1. **Aglio LS, Stanford GG, Maddi R, Boyd JL 3rd, Nussbaum S, Chernow B.** 1991. Hypomagnesemia is common following cardiac surgery. J Cardiothorac Vasc Anesth **5:**201–208.
2. **Ali ML, Kumar SP, Bjornstad K, Duran CMG.** 1996. The sheep as an animal model for heart valve research. Cardiovasc Surg **4:**543–549.

3. **Anderson DE, Muir WW.** 2005. Pain management in ruminants. Vet Clin North Am Food Anim Pract **21:**19–31.
4. **Baraka A, Kawkabani N, Dabbous A, Nawfal M.** 2000. Lidocaine for prevention of reperfusion ventricular fibrillation after release of aortic cross-clamping. J Cardiothorac Vasc Anesth **14:**531–533.
5. **Barnhart GR, Jones M, Ishihara T, Chavez AM, Rose DM, Ferrans VJ.** 1982. Bioprosthetic valvular failure. Clinical and pathological observations in an experimental animal model. J Thorac Cardiovasc Surg **83:**618–631.
6. **Bianco RW, Gallegos RP, Rivard AL, Voight J, Dalmasso AP.** 2009. Animal models for cardiac research. In: Iazzo PA, editor. Handbook of cardiac anatomy, physiology, and devices, 2nd ed. New York (NY): Springer.
7. **Bull BS, Huse WM, Brauer FS, Korpman RA.** 1975. Heparin therapy during extracorporeal circulation. II. The use of a dose-response curve to individualize heparin and protamine dosage. J Thorac Cardiovasc Surg **69:**685–689.
8. **Bull BS, Korpman RA, Huse WM, Briggs BD.** 1975. Heparin therapy during extracorporeal circulation. I. Problems inherent in existing heparin protocols. J Thorac Cardiovasc Surg **69:**674–684.
9. **Carney EL, Clark JB, Myers JL, Peterson R, Wilson RP, Weiss WJ.** 2009. Animal model development for the Penn State pediatric ventricular assist device. Artif Organs **33:**953–957.
10. **Clutton RE, Glasby MA.** 1998. A comparison of the neuromuscular and cardiovascular effects of vecuronium, atracurium, and mivacurium in sheep. Res Vet Sci **64:**233–237.
11. **Clutton RE, Glasby MA.** 1998. A comparison of edrophonium and neostigmine for the reversal of mivacurium-induced neuromuscular blockade in sheep. Res Vet Sci **64:**265–266.
12. **Clutton RE, Murison PJ, Funnell OD.** 1998. Anaesthesia for lmabs undergoing spinal surgery: a case series. Lab Anim **32:**414–421.
13. **Collan R.** 1970. Anesthetic and paraoperative management of sheep for total heart replacement. Anesth Analg **49:**336–343.
14. **Connell JM, Khalapyan T, Al-Mondhiry HA, Wilson RP, Rosenberg G, Weiss WJ.** 2007. Anticoagulation of juvenile sheep and goats with heparin, warfarin, and clopidogrel. ASAIO J **53:**229–237.
15. **Culliford AT, Thomas S, Spencer FC.** 1980. Fulminating noncardiogenic pulmonary edema. A newly recognized hazard during cardiac operations. J Thorac Cardiovasc Surg **80:**868–875.
16. **Davis RF, Kurusz M, Conti VR.** 2008. Conduct of cardiopulmonary bypass. In: Gravlee GP, Davis RF, Stammer AH, Ungerleider RM, editors. Cardiopulmonary bypass: principles and practice, 3rd ed. Philadelphia (PA): Lippinott, Williams and Wilkins.
17. **Dyce KM, Sack WO, Wensing CJG.** 2002. Textbook of veterinary anatomy, 3rd ed. Philadelphia (PA): Saunders.
18. **Ebert PA, Jude JR, Gaertner RA.** 1965. Persistent hypokalemia following open-heart surgery. Circulation **31:**137–143.
19. **Federation of Animal Science Societies.** 2010. Guide for the care and use of agricultural animals in research and teaching, 3rd ed. Champaign (IL): Federation of Animal Science Societies.
20. **Finley A, Greenberg C.** 2013. Heparin sensitivity and resistance: management during cardiopulmonary bypass. Anesth Analg **116:**1210–1222.
21. **Fitzpatrick J, Scott M, Nolan A.** 2006. Assessment of pain and welfare in sheep. Small Rumin Res **62:**55–61.
22. **Flecknell P.** 2009. Laboratory animal anesthesia, 3rd ed. Burlington (MA): Academic Press.
23. **Gallus AS, Hirsh J, Tuttle RJ, Trebilcock R, O'Brien SE, Carroll JJ, Minden JH, Hudecki SM.** 1973. Small subcutaneous doses of heparin in prevention of venous thrombosis. N Engl J Med **288:**545–551.
24. **Geddes LA, Hinds M, Babbs CF, Tacker WA, Schoenlein WE, Elabbady T, Saeed M, Bourland JD, Ayers GM.** 1996. Maintenance of atrial fibrillation in anesthetized and unanesthetized sheep using cholinergic drive. Pacing Clin Electrophysiol **19:**165–175.
25. **Gravlee GP, Angert KC, Tucker WY, Case LD, Wallenhaput SL, Cordell AR.** 1988. Early anticoagulation peak and rapid distribution after intravenous heparin. Anesthesiology **68:**126–129.
26. **Gross D.** 2009. Animal models in cardiovascular research, 3rd ed. New York (NY): Springer.
27. **Habib RH, Zacharias A, Schwann TA, Riordan CJ, Durham SJ, Shah A.** 2003. Adverse effects of low hematocrit during cardiopulmonary bypass in the adult: should current practice be changed? J Thorac Cardiovasc Surg **125:**1438–1450.
28. **Haskins SC.** 1999. Perioperative monitoring. In: Paddleford RR, editor. Manual of small animal anesthesia, 2nd ed. Philadelphia (PA): Saunders.
29. **Hausenloy DJ, Boston-Griffiths E, Yellon DM.** 2012. Cardioprotection during cardiac surgery. Cardiovasc Res **94:**253–265.
30. **Hecker JF.** 1983. The sheep as an experimental animal. London (UK): Academic Press.
31. **Hellyer P, Rodan I, Downing R, Hagedorn JE, Robertson SA.** 2007. AAHA-AAFP pain management guidelines for dogs and cats. J Am Anim Hosp Assoc **43:**235–248.
32. **Hemmerling TM, Zaouter C.** 2010. Neuromuscular blockage and outcome in cardiac anesthesia. Ann Card Anaesth **13:**189–191.
33. **Hessel EA 2nd.** 2008. Circuitry and cannulation techniques. In: Gravlee GP, Davis RF, Stammer AH, Ungerleider RM, editors. Cardiopulmonary bypass: principles and practice, 3rd ed. Philadelphia (PA): Lippincott, Williams and Wilkins.
34. **Hill AJ, Iaizzo PA.** 2009. Comparative cardiac anatomy. In: Iaizzo PA, editor. Handbook of cardiac anatomy, physiology, and devices, 2nd ed. New York (NY): Springer.
35. **Holmberg DL, Olsen DB.** 1987. Anesthesia and cardiopulmonary bypass technique in calves and sheep. Vet Surg **16:**463–465.
36. **Howe LM, Boothe HW.** 2006. Antimicrobial use in the surgical patient. Vet Clin North Am Small Anim Pract **36:**1049–1060.
37. **Hughes HC.** 1986. Swine in cardiovascular research. Lab Anim Sci **36:**348–350.
38. **Kittleson MD, Kienle RD.** 1998. Small animal cardiovascular medicine. St Louis (MO): Mosby.
39. **Klingensmith ME, Chen LE, Glasgow SC.** 2008. The Washington manual of surgery. Philadelphia (PA): Lippincott, Williams and Wilkins.
40. **Lee JC, Taylor FN, Downing SE.** 1975. A comparison of ventricular weights and geometry in newborn, young, and adult mammals. J Appl Physiol **38:**147–150.
41. **Lee LS, Ghanta RK, Mokashi SA, Coelho-Filho O, Kwong RY, Bolman RM, Chen FY.** 2010. Ventricular restraint therapy for heart failure: the right ventricle is different from the left ventricle. J Thorac Cardiovasc Surg **139:**1012–1018.
42. **Leroux AA, Moonen ML, Pierard LA, Kolh P, Amory H.** 2012. Animal models of mitral regurgitation induced by mitral valve chordate tendineae rupture. J Heart Valve Dis **21:**416–423.
43. **Licker M, Diaper J, Cartier V, Ellenberger C, Cikirikcioglu M, Kalangos A, Cassina T, Bendjelid K.** 2012. Clinical review: management of weaning from cardiopulmonary bypass after cardiac surgery. Ann Card Anaesth **15:**206–223.
44. **Lim E, Ali ZA, Attaran R, Cooper G.** 2002. Evaluating routine diuretics after coronary surgery: a prospective randomized controlled trial. Ann Thorac Surg **73:**153–155.
45. **Lowenstein E, Johnston WE, Lappas DG, D'Ambra MN, Schneider RC, Daggett WM, Akins CW, Philbin DM.** 1983. Catastrophic pulmonary vasoconstriction associated with protamine reversal of heparin. Anesthesiology **59:**470–473.
46. **Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR; The Hospital Infection Control Practices Advisory Committee.** 1999. Guidelines for prevention of surgical site infection. Am J Infect Control **27:**97–134.
47. **Martin-Flores M, Pare MD, Campoy L, Gleed RD.** 2012. The sensitivity of sheep to vecuronium: an example of the limitations of extrapolation. Can J Anaesth **59:**722–723.
48. **Michaelsson M, Ho SY.** 2000. Congenital heart malformations in mammals: an illustrated text. River Edge (NJ): Imperial College Press.
49. **Molina JE.** 2009. Cardiopulmonary bypass and cardioplegia. In: Iazzo PA, editor. Handbook of cardiac anatomy, physiology, and devices, 2nd ed. New York (NY): Springer.
50. **Morel DR, Costabella PM, Pittet JF.** 1990. Adverse cardiopulmonary effects and increased plasma thromboxane concentrations following the neutralization of heparin with protamine in awake sheep. Anesthesiology **73:**415–424.
51. **Muir WW, Hubbell JAE, Skarda R, Bednarksi RM.** 1995. Local anesthesia in cattle, sheep, goats and pigs. In: Muir WM, Hubbell

JAE, Skarda R, Bednarksi RM, editors. Handbook of veterinary anesthesia, 2nd ed. St Louis (MO): Mosby.
52. **Murphy GS, Hessel EA, Groom RC.** 2009. Optimal perfusion during cardiopulmonary bypass: an evidence-based approach. Anesth Analg **108:**1394–1417.
53. **Nissinen J, Biancari F, Wistbacka JO, Peltola T, Loponen P, Tarkianen P, Virkkila M, Tarkka M.** 2009. Safe time limits of arotic cross-clamping and cardiopulmonary bypass in adult cardiac surgery. Perfusion **24:**297–305.
54. **Onmaz AC, Gunes V, Atalan G, Gelfert CC, Atalan G.** 2009. Comparison of arterial and venous blood gas values in sheep before and during isoflurane anaesthesia. Revue Med Vet **160:**356–361.
55. **Papich MG, editor.** 2007. Saunder's handbook of veterinary drugs, 2nd ed. St Louis (MO): Saunders.
56. **Plochl W, Orszulak TA, Cook DJ, Sarpal RS, Dickerman DL.** 1999. Support of mean arterial pressure during tepid cardiopulmonary bypass: effects of phenylephrine and pump flow on systemic oxygen supply and demand. J Cardiothorac Vasc Anesth **13:**441–445.
57. **Plumb DC, editor.** 2011. Plumb's veterinary drug handbook, 7th ed. Ames (IA): Blackwell Publishing.
58. **Prassinos NN, Galatos AD, Raptopoulos D.** 2005. A comparison of propofol, thiopental or ketamine as induction agents in goats. Vet Anaesth Analg **32:**289–296.
59. **Robertie PG, Butterworth JF 4th, Royster RL, Prielipp RC, Dudas L, Black KW, Cole LR, Zaloga GP.** 1991. Normal parathyroid hormone responses to hypocalcemia during cardiopulmonary bypass. Anesthesiology **75:**43–48.
60. **Rosengart TK, DeBois W, O'Hara M, Helm R, Gomez M, Lang SJ, Altorki N, Ko W, Hartman GS, Isom OW, Krieger KH.** 1998. Retrograde autolgous priming for cardiopulmonary bypass: a safe and effective means of decreasing hemodilution and transfusion requirements. J Thorac Cardiovasc Surg **115:**426–438.
61. **Royster RL, Thomas SJ, Davis RF.** 2008. Termination of cardiopulmonary bypass. In: Gravlee GP, Davis RF, Stammer AH, Ungerleider RM, editors. Cardiopulmonary bypass: principles and practice, 3rd ed. Philadephia (PA): Lippinott, Williams and Wilkins.
62. **Salerno CT, Droel J, Bianco RW.** 1998. Current state of in vivo preclinical heart valve evaluation. J Heart Valve Dis **7:**158–162.
63. **Sands MP, Rittenhouse EA, Mohri H, Merendino KA.** 1969. An anatomical comparison of human, pig, calf, and sheep aortic valves. Ann Thorac Surg **8:**407–414.
64. **Scarci M, Fallouh HB, Young CP, Chambers DJ.** 2009. Does intermittent cross-clamp fibrillation provide equivalent myocardial protection compared to cardioplegia in patients undergoing bypass graft revascularization. Interact Cardiovasc Thorac Surg **9:**872–878.
65. **Schaper W, Flameng W, DeBrabander M.** 1972. Comparative aspects of coronary collateral circulation. Adv Exp Med Biol **22:**267–276.
66. **Schauvliege S, Narine K, Bouchez S, Desmet D, Parys VV, Van-Nooten G, Gasthuys F.** 2006. Refined anaesthesia for implantation of engineered experimental aortic valves in the pulmonary artery using a right heart bypass in sheep. Lab Anim **40:**341–352.
67. **Shofti R, Zaretzki A, Cohen E, Engel A, Bar-El Y.** 2004. The sheep as a model for coronary artery bypass surgery. Lab Anim **38:**149–157.
68. **Staffieri F, Driessen B, Monte VD, Grasso S, Crovace A.** 2010. Effects of positive end-expiratory pressure on anesthesia-induced atelectasis and gas exchange in anesthetized and mechanically ventilated sheep. Am J Vet Res **71:**867–874.
69. **Sun P, Ji B, Zhu X, Liu J, Long C, Zheng Z.** 2013. Effects of retrograde autologous priming on blood transfusion and clinical outcomes in adults: a meta-analysis. Perfusion **28:**238–243.
70. **Swindle MM, Vogler GA, Fulton LK, Marini RP, Popilskis S.** 2002. Preanesthesia, anesthesia, analgesia, and euthanasia. In: Fox JG, Anderson LC, Loew FM, Quimby FW, editors. Laboratory animal medicine, 2nd ed. New York (NY): Academic Press.
71. **Valverde A, Doherty TJ.** Anesthesia and analgesia of ruminants. In: Fish RE, Brown MJ, Danneman PJ, Karas AZ, editors. Anesthesia and analgesia in laboratory animals, 2nd ed. New York (NY): Academic Press.
72. **Vazquez-Jimenez JF, Qing M, Hermanns B, Klosterhalfen B, Woltje M, Chakapurakal R, Schumacher K, Messmer BJ, vonBernuth G, Seghaye MC.** 2001. Moderate hypothermia during cardiopulmonary bypass reduces myocardial cell damage and myocardial cell death related to cardiac surgery. J Am Coll Cardiol **38:**1216–1223.
73. **Vesal N, Karimi A.** 2006. Evaluation of central venous pressure in ruminants. Vet Archiv **76:**85–92.
74. **Wan S, LeClerc JL, Vincent JL.** 1997. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. Chest **112:**676–692.
75. **Wei X, Li T, Sanchez P, Wakins A, Li S, DeFilippi C, Lu ZL, Griffith BP.** 2013. Initial experience with a juvenile sheep model for evaluation of the pediatric intracorporeal ventricular assist services. ASAIO J **59:**75–80.
76. **Weiner ID, Wingo CS.** 1997. Hypokalemia – consequences, causes, and correction. J Am Soc Nephrol **8:**1179–1188.
77. **Whalen FX, Gajic O, Thompson GB, Kendrick ML, Que FL, Williams BA, Joyner MJ, Hubmavr RD, Warner DO, Sprung J.** 2006. The effects of the alveolar recruitment maneuver and positive end-expiratory pressure on arterial oxygenation during laparoscopic bariatric surgery. Anesth Analg **102:**298–305.