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Perioperative Evaluation of Arterial and Venous Whole Blood in the Lamb (*Ovis aries*) Fontan Model

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Whole blood analysis can evaluate numerous parameters, including pH, pCO_2, pO_2, HCO_3^-, base excess, glucose, electrolytes, lactate, blood urea nitrogen, creatinine, bilirubin, and hemoglobin. This valuable tool enables clinicians to make more informed decisions about patient care. However, the current body of literature describing perioperative whole blood analysis in Dorset sheep (*Ovis aries*) is small, so clinicians lack adequate information to guide their decision-making when evaluating test results. We evaluated arterial and venous whole blood pH, bicarbonate, pCO_2, lactate, creatinine, and blood urea nitrogen before and for the first 24 hours after surgery in 2 cohorts of male and female *Ovis aries* undergoing one of 2 major cardiovascular procedures, a Single-Stage Fontan or an inferior vena cava to pulmonary artery extracardiac conduit implantation (IP-ECC). The cohort undergoing a Single-Stage Fontan, which is the more complex procedure, exhibited greater deviation from baseline measurements than did the cohort undergoing the IP-ECC for lactate, bicarbonate, and creatinine. The cohort undergoing the IP-ECC showed no significant deviation from baseline for any parameters, potentially indicating a better safety margin than expected when compared with the Single-Stage Fontan. Together, these results indicate the clinical value of arterial and venous whole blood measurements in perioperative management of sheep and can provide a reference for clinicians managing sheep after significant cardiovascular procedures.

Abbreviations and Acronyms: AKI, acute kidney injury; IP-ECC, inferior vena cava to pulmonary artery extracardiac conduit implantation; IVC, inferior vena cava; PA, pulmonary artery; SSF, Single-Stage Fontan; SVC, superior vena cava

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Introduction

Measurement of whole blood components is a rapid, effective, commonly used tool that enables clinicians to evaluate acid-base balance, gas exchange, and respiratory and metabolic function in patients.[3,39,41] Measurable parameters include pH, pCO_2, pO_2, HCO_3^-, base excess, glucose, electrolytes, lactate, blood urea nitrogen (BUN), creatinine, bilirubin, and hemoglobin.[3] In the context of major cardiovascular surgery, whole blood measurements are particularly useful in characterizing pathologic deviations in respiratory, circulatory, and metabolic functions that may occur during and after surgery.[5,15,22,23,39] Arterial whole blood samples are preferred for whole blood analysis due to perceived superior accuracy to venous samples for many parameters including pH, bicarbonate, and pCO_2.[15,21,25,37] The validity of this idea has been challenged in multiple studies, but known minor differences in some parameters between arterial and venous blood increase with deviation from normal physiology.[3,4,14,21,25,28,30,37,39] However, venous blood is simpler and safer to collect than arterial blood, so understanding any meaningful differences in arterial and venous whole blood results is useful when determining how invasive one must be during sample collection.[3,6,25]

We have developed a sheep (*Ovis aries*) model for the Fontan procedure, a major cardiovascular operation used in the palliation of children born with single-ventricle heart disease.[36] This procedure creates significant alterations to blood circulation patterns; both the superior vena cava (SVC) and inferior vena cava (IVC) are detached from the right atrium and anastomosed to the pulmonary artery (PA), creating completely passive venous return to the pulmonary circulation (Figure 1A–C).[36] The Fontan can be completed in one stage (Single-Stage Fontan [SSF]), or in multiple stages (Staged Fontan) to reduce the operative burden on the patient. In SSF, the IVC and SVC are both anastomosed to the PA in one operation (Figure 1B). In a typical Staged Fontan in human patients with single-ventricle anatomy, the SVC is anastomosed to the PA in an initial procedure, and the IVC-to-PA anastomosis is completed in a second-stage procedure.[2]

In our experience, ovine subjects with native cardiac anatomy do not tolerate the standard staged clinical approach, thus necessitating a switch in procedure staging. Therefore, our first-stage procedure is an IVC to pulmonary artery extracardiac conduit implantation procedure (IP-ECC) in which only the IVC is anastomosed to the PA (Figure 1C), such that venous return to the pulmonary circulation is only partially passive; SVC-to-PA anastomosis is then completed during a second surgery.

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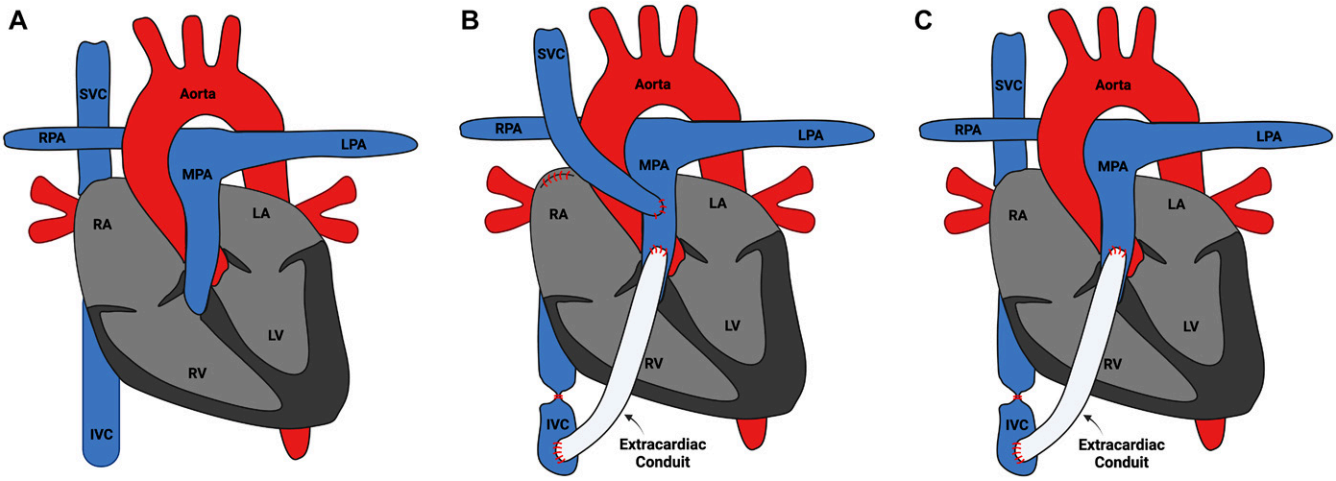

Figure 1. (A) Normal ovine cardiac anatomy; (B) ovine cardiac anatomy after completion of a Single-Stage Fontan, with both the superior vena cava (SVC) and inferior vena cava (IVC) anastomosed to the main pulmonary artery (MPA); and (C) ovine cardiac anatomy after completion of our IVC to pulmonary artery extracardiac conduit, with only the IVC anastomosed to the MPA. LA, left atrium; LV, left ventricle; LPA, left pulmonary artery; RA, right atrium; RPA, right pulmonary artery; RV, right ventricle (A–C created with BioRender.com).

The IP-ECC is shorter and induces less hemodynamic disruption than the SSF, reducing operative burden and improving survivability.[2,26] Postoperative patient survival in humans has steadily increased since the Fontan procedure was pioneered 50 years ago; today, 30-year survival is approximately 85%.[36] However, long-term survival with Fontan circulation has been associated with widespread and progressive downstream physiologic consequences.[8,36] Building on the work of others, our group has established a long-term sheep model of Fontan circulation to improve mechanistic understanding of these consequences and validate therapeutic strategies.[45]

Ovis aries are a popular animal model for cardiovascular research due to anatomic and physiologic similarities to humans.[10,12,38] However, despite the likelihood that whole blood analysis could provide useful information to guide peri- and postoperative management of ovine patients, there is a paucity of literature reporting pre- and postoperative reference values for parameters of clinical importance. Our review of the literature indicated that studies commonly describe baseline pH, pCO_2, and bicarbonate values for sheep (*Ovis aries*), but we found only 2 studies that reported baseline whole blood lactate values, and only one that reported baseline whole blood creatinine and urea values.[9,20,27,33,40,43] Furthermore, we found no studies that compared pre- and postoperative values in a panel of whole blood parameters. Aside from a single report on lactate in ovine oophorectomy, pre- to postoperative variation in whole blood components has not been described in the literature.[27] More specifically, no studies are available that evaluate whole blood parameters in sheep in the context of a major cardiovascular procedure. The absence of published peer-reviewed benchmarks complicates peri- and postoperative patient management because it diminishes the utility of whole blood monitoring as a guide for clinical decision-making.

We retrospectively analyzed whole blood measurements of ovine subjects that underwent major cardiovascular surgeries. Our primary goal was to characterize the immediate postoperative course of subjects undergoing one of 2 major cardiovascular procedures that differ in complexity and operation time. Currently, clinicians who want to use whole blood measurements to monitor ovine patients must interpret results using clinical gestalt instead of utilizing published data for guidance. The establishment of baseline (preoperative) and postoperative reference ranges for sheep may improve both patient outcomes and our understanding of the similarities and differences between sheep and humans. We predicted that sheep undergoing the SSF procedure would show more pronounced deviations from baseline than would sheep undergoing IP-ECC for all analyzed parameters including lactate, pH, bicarbonate, pCO_2, BUN, and creatinine due to the greater length and complexity of the SSF procedure.[2]

Materials and Methods

Animal subjects. Animal work was approved by the IACUC at the Abigail Wexner Research Institute and was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th edition) at an AAALAC-International-accredited facility.

Data from 22 Dorset sheep (*Ovis aries*) were used in this study. All subjects that had undergone either an SSF (n = 12) or an IP-ECC (n = 10) at our institution were included. One SSF sheep died intraoperatively and was excluded from the analysis. The analyzed SSF cohort (n = 11) consisted of 7 males and 4 females with an average age at surgery of 13 mo and a range of 3 to 28 mo. The analyzed IP-ECC cohort (n = 10) consisted of 6 males and 4 females with an average age at surgery of 13 mo and a range of 5 to 21 mo. Age at surgery was not significantly different (P = 0.9036) between the SSF and IP-ECC cohorts as shown by a Mann Whitney test (GraphPad Prism 9.4.1; GraphPad Software, Boston, MA).

Dorset sheep were purchased from either Archer Farms (Darlington, MD) or the Ohio Agricultural Research and Development Center (Wooster, OH). They were treated on arrival for parasites using anthelmintics such as ivermectin (0.4 mg/kg; Aspen Veterinary Resources, Liberty, MO) and moxidectin (0.2 to 0.3 mg/kg; Elanco, Greenfield, IN). Females of breeding age received an injection of lutalyse (5 to 10 mg/kg; Zoetis, Kalamazoo, MI) due to cohousing with males; males had been castrated by the vendor. All sheep were tested before arrival for *C. burnetii* using PCR and serological assays and were monitored for parasite loads using FAMACHA scores and fecal tests. Sheep with a positive PCR for *C. burnetii* were culled. Fecal floats or additional diagnostic tests were performed if specific parasites were suspected. Additional *C. burnetii* serology and

PCR were obtained before release from quarantine and subsequent imaging procedures.

Sheep were housed on a 12:12-h light:dark cycle and were housed in groups in collapsible caging except for the immediate postoperative period to protect catheters. Sheep were housed long-term on a floor with bedding after they had recovered from surgery. They were fed an alfalfa/clover mix twice daily and either Purina Lamb Grower Complete B30 (Land O'Lakes, St. Louis, MO) once daily or LabDiet Rumilab 5508 (Land O'Lakes) based on weight and age. Enrichment included a variety of fruits and vegetables, floor and hanging toys, and exercise in a facility hallway. Sheep were observed at least once daily by husbandry staff, as well as by veterinary staff in clinical cases. If concerns regarding appetite or weight loss developed during the postoperative period, fruits, vegetables, or additional grain were added to the diet.

Surgical procedures. ***Preoperative preparation and intraoperative management.*** Sheep were sedated with ketamine (up to 4 mg/kg; Hospira, Lake Forest, IL), butorphanol (0.1 mg/kg; Zoetis, Parsippany, NJ), and either midazolam (up to 0.5 mg/kg; Athenex, Buffalo, NY) or diazepam (up to 0.5 mg/kg; Hospira, Lake Forest, IL) based on drug availability. Induction was performed as an intravenous injection into the jugular vein. Once induced, sheep were intubated with an endotracheal tube ranging from 9.5 to 10 mm (Medtronic, Minneapolis, MN) and placed on isoflurane (Baxter International, Deerfield, IL). Mechanical ventilation (Aestiva/5 Anesthesia Machine; GE Healthcare, Madison, WI) was started on pressure mode, either positive pressure or pressure support ventilation with 50% to 100% oxygen and flow rate maintained at 1 to 3 L/min, and maintained throughout the procedure. Tidal volume was calculated as 8 to 10 mL/kg and adjusted based on $EtCO_2$ (normal range: 35 to 45 mmHg). Catheters (BD Angiocath; Becton Dickinson, Franklin Lakes, NJ) were placed in the posterior auricular artery (22 to 24 gauge), jugular vein (16 to 18 gauge), and saphenous vein (18 gauge). Surgical anesthesia was maintained with propofol (Fresenius Kabi, Lake Zurich, IL) as a constant rate infusion (CRI) at a rate of 25 to 40 mg/kg/h IV.

Sheep were monitored using SurgiVet Advisor Tech Vital Signs Monitors (Smiths Medical, St. Paul, MN) for the following: electrocardiography, invasive blood pressure via the posterior auricular artery, noninvasive blood pressure via a cuff on the hind limb (Technicuff Blood Pressure Cuff [9 to 25 cm], Leesburg, FL; or Medline Blood Pressure Cuff [13.8 to 21.5 cm], Northfield, IL), $EtCO_2$, SPO_2, and temperature. Temperature was maintained using heated intravenous fluids, a water-recirculating blanket (Stryker Medical, Kalamazoo, MI), and a Bair Hugger (3M, St. Paul, MN). Vital signs were recorded at 15-min intervals or as necessary to maintain stable anesthesia. Blood samples were collected and measured at 30- to 60-min intervals during surgery. Vasopressin (20 U/mL; American Regent, Shirley, NY), epinephrine (1 mg/mL; Par Pharmaceutical, Chestnut Ridge, NY), and phenylephrine (10 mg/mL; Westward Pharmaceutical, Eatontown, NJ) were used to maintain blood pressure and heart rate during the procedure. Sheep received approximately 100 mL/h of lactated Ringer solution (ICU Medical, San Clemente, CA) during the procedure. Fentanyl CRI (5 to 10 µg/kg/h; Hospira, Lake Forest, IL) was administered for pain management starting at the time of first incision. Sheep also received cefazolin (25 mg/kg; Qilu Antibiotics Pharmaceutical, Jinan, Shandong, China) every 90 min until the end of surgery. Bupivicaine (1 to 2 mg/kg; Hikma Pharmaceuticals, Berkeley Heights, NJ) and/or liposomal bupivicaine (Exparel; 5.3 mg/kg; Pacira BioSciences, Tampa FL) were used as local anesthetics and were administered after sterile preparations using a ring block technique around the surgical site. Additional doses of buprenorphine (0.01 to 0.03 mg/kg; Par Pharmaceutical; Chesnut Ridge, NY) and/or buprenorphine ER (0.01 to 0.3 mg/kg SQ; ZooPharm, Laramie, WY) were given to maintain adequate pain control.

Venous catheters (jugular and saphenous veins) were placed for delivery of fluids, CRI, and other medications to maintain stable anesthesia. Up to 2 arterial lines (one per ear, posterior auricular artery) were used for invasive blood pressure and whole blood monitoring. Sheep were positioned on right lateral recumbency. Wool was clipped at the surgical site and the skin was prepared using alternating chlorhexidine (Aspen Veterinary Resources, Loveland, CO) and isopropyl alcohol swabs (Cardinal Health, Dublin, OH).

SSF and IP-ECC study groups. Two surgical procedures were evaluated: IP-ECC and SSF. Our IP-ECC procedure refers to an operation that redirects flow from the IVC to the main PA (MPA) by connecting an 18-mm-diameter Gore-Tex synthetic conduit (W.O. Gore and Associates, Newark, DE) from the IVC to the MPA (Figure 1C).

In our subjects, the IP-ECC is followed later by the Glenn operation, which involves anastomosis of the SVC to the MPA. Notably, this is the opposite order of Staged Fontan procedures for human patients. In humans, the Glenn operation is conducted first and is followed later by the IP-ECC implantation. We reversed the staging of these procedures because our subjects did not survive procedures done in the typical order. An SSF includes an IP-ECC as described above in addition to the creation of a Glenn shunt during the same operation (Figure 1B). In our experience, the operative times for the SSF and IP-ECC groups were approximately 5 h and 3 h, respectively.

Glenn shunt (implemented in SSF patients). A right lateral thoracotomy was performed at the third intercostal space. The pericardium was incised to expose the right atrium and the PA. During the Glenn operation, the SVC and the right atrium were each cannulated with a venous cannula, and the SVC-right atrium bypass was initiated by connecting the SVC and right atrium cannulas. The SVC was clamped proximally and distally of the cannula and then transected below the cannula near the heart; the SVC end close to the heart was closed with a continuous running suture (2-0 silk; Perma-Hand; Ethicon, Cincinnati, OH). The cranial side of the PA was clamped via a side-biting technique and the SVC was anastomosed side-to-end to the PA using 6-0 polypropylene sutures (Prolene; Ethicon, Cincinnati, OH).

IVC to IP-ECC implantation. The IVC was partially clamped via a side-biting technique. An 18-mm-diameter Gore-Tex graft (W.O. Gore and Associates, Newark, DE) was then anastomosed end-to-side to the IVC. The IVC end close to the right atrium was tied with a 2-0 silk suture (Perma-Hand; Ethicon, Cincinnati, OH). The right side of the MPA was partially clamped, and the polytetrafluoroethylene graft was anastomosed to the MPA in an end-to-side fashion. The clamps and cannula were removed. Two chest tubes (PleurX Pleural Catheter; Becton Dickinson, Franklin Lakes, NJ) were placed (anterior and posterior or left and right) and connected to a J-vac system (Johnson and Johnson, New Brunswick, NJ).

Postoperative management. After weaning from the ventilator using pressure support ventilation, sheep were moved to a recovery room. They received oxygen by nasal cannula (Medline, Northfield, IL) during the immediate postoperative period (first 1 to 6 h) until recovered and ambulatory. Sheep received their daily ration of grain and hay on recovery. Sheep were monitored continuously for 24 h after surgery and potentially longer based on the animal's condition.

Blood samples were analyzed using the i-STAT point-of-care laboratory system (Abbott Point of Care, Princeton, NJ), hourly in the immediate postoperative window, then every 2 h, and then every 4 h. Pain score, food and water consumption, chest tube volume of air and fluid, and urination and defecation were monitored continuously and tracked in 24-h increments. Pain was scored using an established Sheep Grimace Scale.[16] Fluid levels were tracked by monitoring the volume of fluid intake and urine output. Hydration status was maintained via oral fluids mixed with electrolytes (PRANG) or via intravenous lactated Ringer solution (approximately 50 mL/h) or another crystalloid like 0.9% NaCl. After the initial 7 d, sheep were monitored at least 5 times daily for another 7 d.

Chest tubes were evaluated every 2 to 4 h in the immediate postoperative period depending on chest tube output. Chest tube output was tabulated at 8-h intervals. If output decreased or abnormalities in PaO_2, SpO_2, or TCO_2 were noted, the chest tube was evaluated for signs of a fibrin clot. Syringes were intermittently attached to J-vac tubing to dislodge any possible clots. If clots were noted, alteplase (2 mg; Genentech, South San Francisco, CA) was used. Chest tube output dropped as sheep recovered from surgery. Once output ceased, the tubes were removed (usually at 14 d after surgery). For treatment of metabolic acidosis (pH ≤ 7.3), one dose of bicarbonate (1 mEq/kg; Hospira, Lake Forest, IL) was administered. A repeat dose was given if the acidosis did not improve on repeat blood work. Calcium chloride (10 mg/kg; American Regent, Shirley, NY) was administered for hypocalcemia. Oral gabapentin (10 to 15 mg/kg SID-BID; Alkem Laboratories, Parsippany, NJ) was started on the first day after surgery, and individual doses of meloxicam (0.5 to 1 mg/kg; Cipla, Warren, NJ) could be given as needed (as clinical signs indicated). Clinical interventions were made in collaboration with a cardiologist, an ICU cardiologist, an anesthesiologist specializing in cardiothoracic surgery, and the veterinary staff.

Sheep whole blood sample acquisition Pre- and postoperative whole blood samples were collected from an arterial line or venous catheter (Figure 2). Preoperative samples were collected before surgery in the first 20 min after induction and intubation. Arterial samples were always collected from the posterior auricular artery, and venous samples were always collected from the internal jugular vein. Samples at all time points were either venous or arterial (the 2 sample types were never collected concurrently). The i-STAT point-of-care system (Abbott Point of Care, Princeton, NJ) was used to analyze whole blood samples. CG8+, CHEM8+, and CG4+ cartridges were used to measure parameters that included electrolytes, acid-base parameters, oxygen, hemoglobin, hematocrit, and lactate to monitor the animals' physiologic responses to the SSF or IP-ECC procedures.

Data collection and statistical analysis. A retrospective analysis of whole blood data was conducted for each of the 21 sheep that survived either a SSF or IP-ECC procedure. All measurements made during the first 24 h immediately after surgery were collated to characterize postoperative recovery. Due to the retrospective nature of the study, whole measurements had not been conducted at uniform postoperative time intervals because sample collection was dictated by clinical management of the sheep. To establish consistency across the cohort, data were grouped into 3-h intervals after surgery, as most patients had undergone at least one whole blood measurement every 3 h after surgery for the first 24 h (Figure 2). Therefore, for each parameter, each subject had a single data point at baseline (before surgery) and at postoperative time points of 0, 3, 6, 9, 12, 15, 18, 21, and 24 h. To report summary data from the 24-h intervals, we aggregated all postoperative data points (0 to 24 h) separately for each cohort and compared them with baseline. All sheep in this retrospective study had missing data points for multiple parameters at some time points, including baseline. This strategy standardized the number of data points that contributed to the overall cohort dataset from each patient. As such, sheep that had extremely active clinical management and more frequent blood collection were not overrepresented in the dataset as compared with sheep that needed less intense clinical management; all subjects that survived for 24 h were represented by the same number of data points.

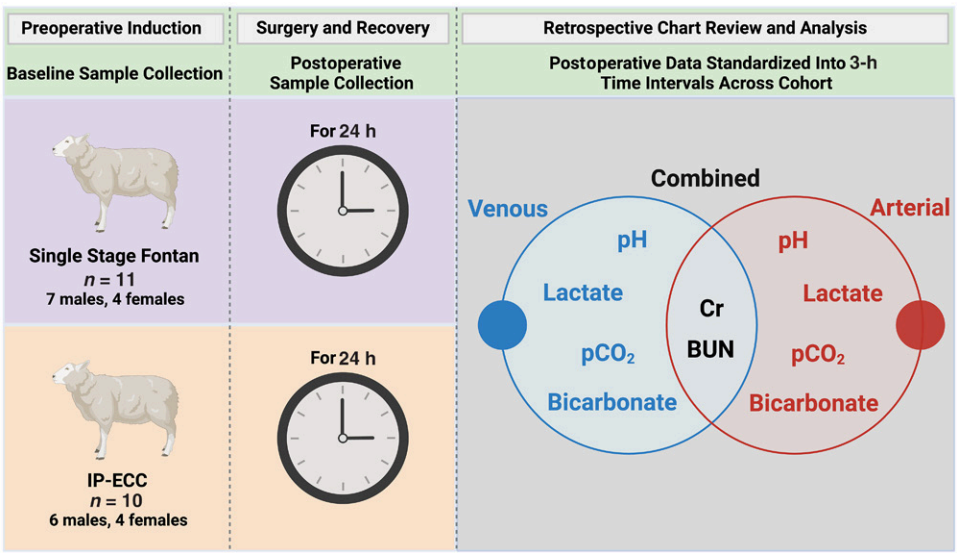

Figure 2. Diagram to demonstrate baseline and postoperative sample collection for each cohort. Preoperative samples were collected less than 20 min after induction of anesthesia, and postoperative samples were collected based on clinical need during the first 24 h after surgery. For postoperative samples, for each parameter, only one measurement per 3-h interval was included for each subject in our dataset. Therefore, for each parameter, each subject had a single data point at baseline (before surgery) and at postoperative time points of 0, 3, 6, 9, 12, 15, 18, 21, and 24 h. All subjects had missing data at some time points for some parameters. Arterial and venous values were combined for creatinine and BUN, and reported separately for pH, bicarbonate, pCO_2, and lactate.

During the first 24 h after surgery, arterial blood remained the source for whole blood analysis for different amounts of time between animals; after arterial blood was no longer viable to collect, venous blood was collected. This variability was due to differences in arterial line patency across the cohort; for some animals, it was practical to continue collecting arterial blood throughout the entire first 24 h, while for others, the arterial line was dislodged or lacked sufficient blood return sooner after surgery. Once the arterial line was no longer viable for a particular animal, only venous samples were collected. Therefore, whole blood data for the first 24 h after surgery is a combination of arterial and venous samples, and intersubject variability in the respective proportion of arterial compared with venous samples is due to differences in loss of arterial line patency. Due to this, we reported arterial and venous measurements of pH, bicarbonate, and pCO_2 separately due to known variability between arterial and venous measurements. Lactate was also reported as separate arterial and venous measurements due to a previous report of arterial-venous variation in sheep.[27] We combined arterial and venous measurements of creatinine and BUN into single profiles because these parameters are not expected to vary across sample types (Figure 2).

Data were collated, analyzed, and presented using GraphPad Prism 9.4.1 (GraphPad Software, Boston, MA). A P value of less than 0.05 was considered significant. A Kruskal-Wallis test with Dunn's multiple comparisons was used to evaluate differences between average values across the full 24-h postoperative interval and baseline (preoperative) for SSF and IP-ECC study groups for each parameter. A mixed-effects model for main effects only with a Geisser-Greenhouse correction to account for nonsphericity was used to assess the fixed effect of time after surgery on 24-h parameter trends. As the arterial lines of all but one subject remained patent until at least 3 h after surgery, we had too few data points for venous pH, venous bicarbonate, venous pCO_2, and venous lactate at the 0-h postoperative time point to implement a Geisser-Greenhouse correction. Therefore, we did not assess the effect of time after surgery for these sample groups.

Results

Preoperative and postoperative comparisons of whole blood parameters. Parameter measurements during the first 24 h after surgery were aggregated regardless of the specific time interval after surgery (Table 1 and Figures 3 and 4). Comparisons of baseline and postoperative measurements for both SSF and IP-ECC surgeries revealed several differences. SSF had higher creatinine ($P < 0.0001$, $P < 0.0001$) and venous lactate ($P = 0.0002$, $P = 0.0021$) than both the baseline and IP-ECC cohorts (Figure 4B and C). Arterial lactate in the SSF cohort was higher than baseline ($P = 0.0143$) but was not elevated relative to the IP-ECC cohort (Figure 4A). Arterial bicarbonate fell in SSF sheep as compared with the other 2 cohorts ($P = 0.0086$, $P < 0.0001$) (Figure 3B). BUN was higher in SSF sheep than in IP-ECC sheep ($P < 0.0001$) but was not significantly different from baseline (Figure 4D). Finally, there were no intercohort differences between SSF, IP-ECC, and baseline in arterial or venous pH, venous bicarbonate, or arterial or venous pCO_2 (Figure 3A, C, and D–F). Notably, the IP-ECC cohort did not deviate significantly from baseline measurements for any parameter, although IP-ECC BUN levels fell to a level that approached significance ($P = 0.0548$) as compared with baseline (Figure 4D).

Temporal profile of whole blood parameters after surgery. To further characterize changes in the serum parameters of SSF and IP-ECC sheep, we compared values obtained during 3-h intervals throughout the first 24 h after surgery (Figures 5 and 6). Arterial and venous pH values indicated immediate postoperative acidosis that quickly returned to baseline, with no differences between cohorts (Figure 5A and D). Arterial bicarbonate measurements peaked immediately after surgery and then declined over the next 24 h in both cohorts (Figure 5B). In contrast, venous bicarbonate values did not show an obvious pattern (Figure 5E). Arterial and venous pCO_2 peaked immediately after surgery in both cohorts, returning to baseline over the 24-h period (Figure 5C and F).

Arterial and venous lactate levels peaked during the 3- to 6-h interval after surgery in both cohorts and had not returned to baseline within 24 h (Figure 6A and C). This pattern was more apparent in venous as compared with arterial lactate measurements. The magnitude of the lactate peak was higher in the SSF cohort, corresponding with our 24-h aggregate findings (Figure 6A and C).

Creatinine showed a similar pattern, with a peak in the SSF cohort in the 15-h interval that had not returned to baseline by 24 h after surgery (Figure 6B). The IP-ECC cohort showed a similar pattern, but it was less obvious (Figure 6B). Again, the SSF cohort had a higher peak (Figure 6B).

Temporal patterns in BUN measurements for the SSF cohort were nearly identical to those of creatinine, with a slow postoperative elevation from baseline that peaked at around 15 h and had not returned to baseline by 24 h after surgery (Figure 6D). BUN measurements in the IP-ECC cohort did not show an obvious temporal pattern.

A mixed-effects model analysis was conducted for arterial pH, arterial bicarbonate, arterial pCO_2, arterial lactate, creatinine, and BUN. Of these variables, time from surgery reached significance as a fixed effect for arterial pH ($P = 0.0039$) and arterial pCO_2 ($P = 0.0055$). The fixed effect of time was not significant for arterial bicarbonate, arterial lactate, creatinine, or BUN ($P = 0.1589$, 0.1023, 0.1733, and 0.454, respectively).

Table 1. Average baseline and first 24-h postoperative whole blood measurements of pH, bicarbonate, pCO_2, lactate, creatinine, and BUN for the Single-Stage Fontan (Fontan) and inferior vena cava to pulmonary artery extracardiac conduit (IP-ECC) cohorts

	Baseline	Postoperative Fontan cohort	Postoperative IP-ECC cohort
Arterial pH	7.47 ± 0.05 (7.33, 7.54)	7.44 ± 0.07 (7.27, 7.56)	7.46 ± 0.08 (7.31, 7.57)
Venous pH	7.45 ± 0.04 (7.36, 7.51)	7.41 ± 0.11 (7.04, 7.54)	7.45 ± 0.06 (7.29, 7.53)
Arterial bicarbonate (mEq/L)	30.3 ± 3.1 (24.3, 39.0)	27.5 ± 3.1 (20.8, 37.2)	31.6 ± 4.6 (19.0, 39.7)
Venous bicarbonate (mEq/L)	29.8 ± 2.5 (24.0, 34.9)	29.4 ± 4.1 (21.9, 39.0)	31.2 ± 4.0 (21.7, 40.7)
Arterial pCO_2 (mmHg)	41.6 ± 5.9 (32.7, 55.1)	41.3 ± 10.6 (27.8, 73.2)	45.2 ± 10.6 (32.6, 73.9)
Venous pCO_2 (mmHg)	43.2 ± 5.4 (33.8, 52.6)	44.9 ± 7.8 (34.0, 66.5)	45.2 ± 7.4 (36.6, 72.8)
Arterial lactate (mmol/L)	1.08 ± 0.61 (0.40, 1.96)	4.10 ± 3.62 (0.46, 15.45)	2.54 ± 2.37 (0.30, 9.39)
Venous lactate (mmol/L)	0.93 ± 0.34 (0.41, 1.44)	6.45 ± 4.00 (0.88, 14.21)	2.48 ± 2.17 (0.40, 9.70)
Creatinine (mg/dL)	0.6 ± 0.1 (0.4, 0.7)	1.0 ± 0.4 (0.5, 2.0)	0.6 ± 0.2 (0.5, 1.7)
BUN (mg/dL)	17 ± 4 (11, 22)	20 ± 6 (9, 46)	14 ± 3 (8, 23)

Data are presented as mean ± SD (minimum, maximum).

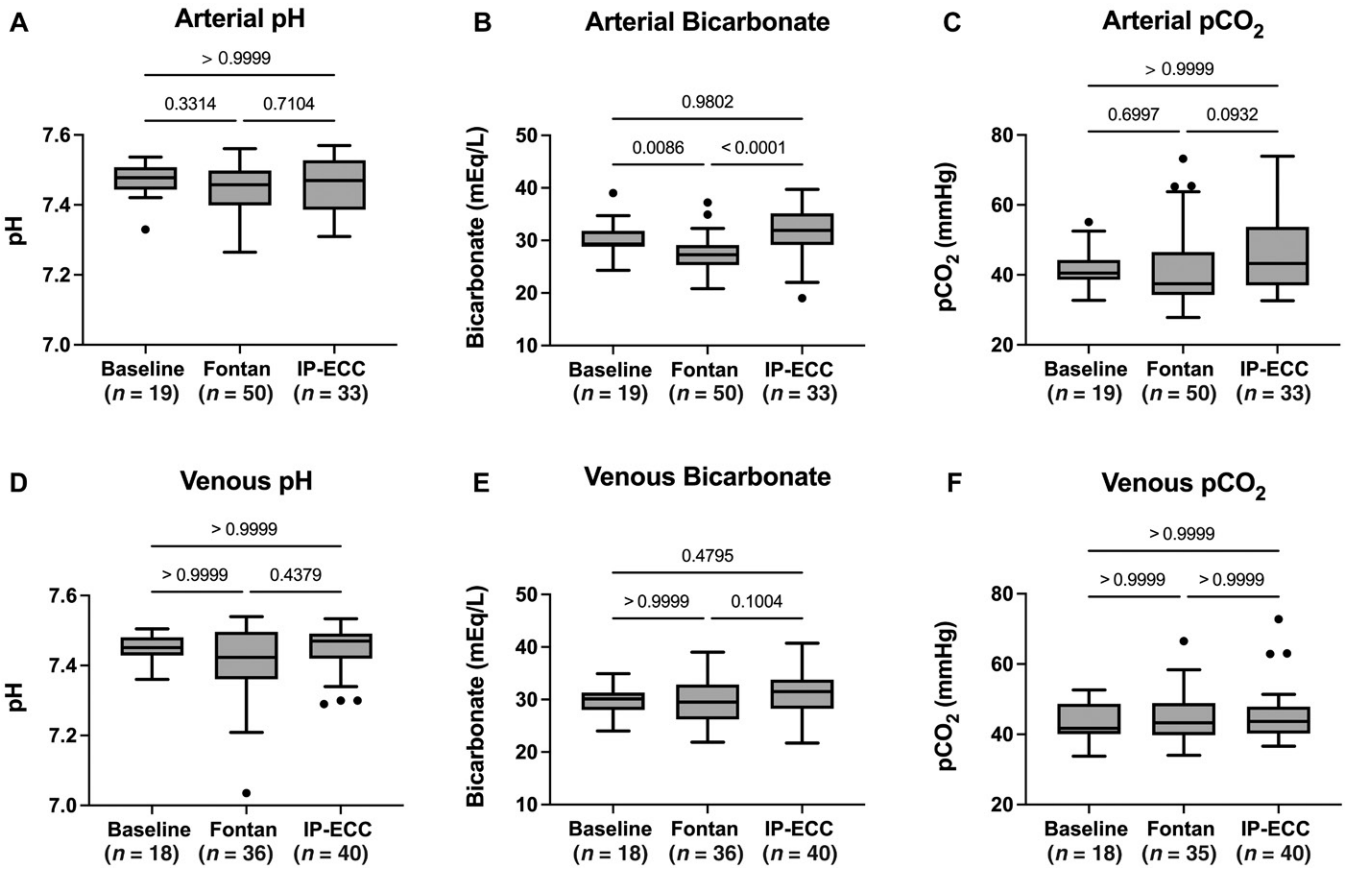

Figure 3. Blood measurements of (A) arterial pH; (B) arterial bicarbonate, (C) arterial pCO_2; (D) venous pH; (E) venous bicarbonate; and (F) venous pCO_2 at baseline and during the 24 h after surgery for the Single-Stage Fontan (Fontan) and inferior vena cava to pulmonary artery extracardiac conduit (IP-ECC) cohorts.

Discussion

Our laboratory has performed staged cardiac reconstructive surgery to establish and repair single ventricle cardiac abnormalities in sheep that were then studied to evaluate the long-term complications of living with Fontan physiology.[35] Cardiovascular, renal, and pulmonary parameters were closely monitored in these animals by using arterial and venous whole blood measurements to ensure optimal peri- and postoperative management. Abnormal postoperative findings included acid-base disturbances, lactic acidosis, and decreased renal function. We performed a retrospective assessment of the arterial and venous whole blood values in these subjects to guide postoperative care of sheep that have undergone major cardiovascular surgery.

As hypothesized, and in accordance with human studies, postoperative parameter deviations from baseline were generally more significant for subjects that underwent an SSF, which is a longer and more complex procedure than an IP-ECC.[2] Specifically, this hypothesis was confirmed for arterial bicarbonate, arterial and venous lactate, and creatinine, markers that are associated with respiratory acidosis and acute kidney injury (AKI) resulting from low cardiac output and reduced respiratory rate after surgery. Postoperative differences in these parameters for the SSF and IP-ECC cohorts could be due to differences in cardiac output, operative stress, or both. The SSF takes longer to perform and results in completely passive venous return to the pulmonary circulation, whereas IP-ECC can be performed in a shorter time and results in only partially passive venous return.

Temporal pH profiles were similar in both cohorts, demonstrating an initial respiratory acidosis as anesthesia was wearing off and subjects had been extubated but were still somewhat sedated. Once the sedation had resolved, respiratory acidosis improved, and metabolic lactic acidosis ensued as the sheep adapted to the new physiology. This recovery pattern is consistent with recovery patterns of humans after cardiac surgery, with a period of low cardiac output for 6 to 18 h after surgery that then resolves. During the period of metabolic acidosis, sheep were sometimes given sodium bicarbonate and respiratory support to buffer the metabolic acidosis and correct deviations in pH. These 2 factors likely combined to obscure the natural course of pH changes. Arterial bicarbonate levels were significantly lower in the SSF cohort as compared with the IP-ECC cohort, corresponding with lactate levels; lactic acidosis is associated with metabolic acidosis that occurs due to tissue hypoxia and anaerobic metabolism.[44] Our nonstandardized administration of exogenous sodium bicarbonate in both postoperative cohorts significantly confounds the interpretation of postoperative pH and bicarbonate findings in this study. Without clinical management, we would expect acidosis and a concomitant drop in bicarbonate in both surgical cohorts, with the change likely greater in the SSF group. Notably, despite the administration of exogenous bicarbonate, pH and bicarbonate levels in the surgical cohorts did not exceed the preoperative baseline. Furthermore, during clinical management, exogenous bicarbonate was administered to SSF animals more often than IP-ECC animals. The finding of significantly lower arterial bicarbonate

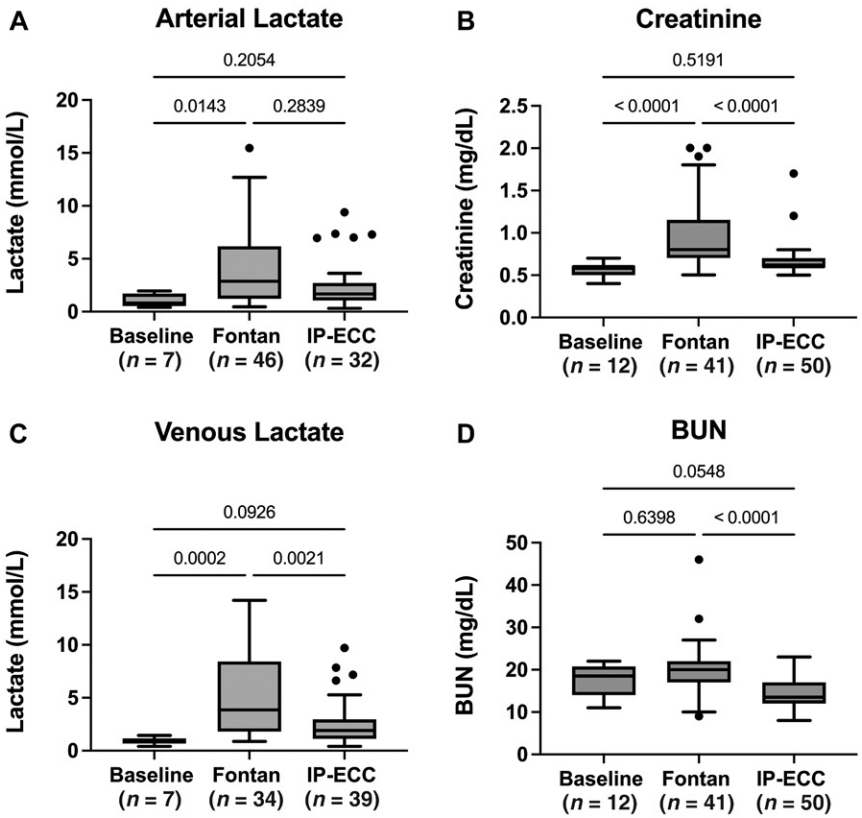

Figure 4. Blood measurements of (A) arterial lactate; (B) creatinine; (C) venous lactate, and (D) blood urea nitrogen (BUN) at baseline and in the 24-h postoperation for the Single-Stage Fontan (Fontan) and inferior vena cava to pulmonary artery extracardiac conduit (IP-ECC) cohorts.

levels in the SSF cohort as compared with baseline and the IP-ECC cohort is even more notable in this context. Taken together, these observations suggest that an underlying acidosis is masked by administration of exogenous bicarbonate. Therefore, although we acknowledge the significant confounding of pH and bicarbonate results due to exogenous sodium bicarbonate administration, our results may still be useful to clinicians who interpret them in this context.

Our temporal patterns in lactic acidosis mirrored those reported in human Fontan studies, with a rapid postoperative

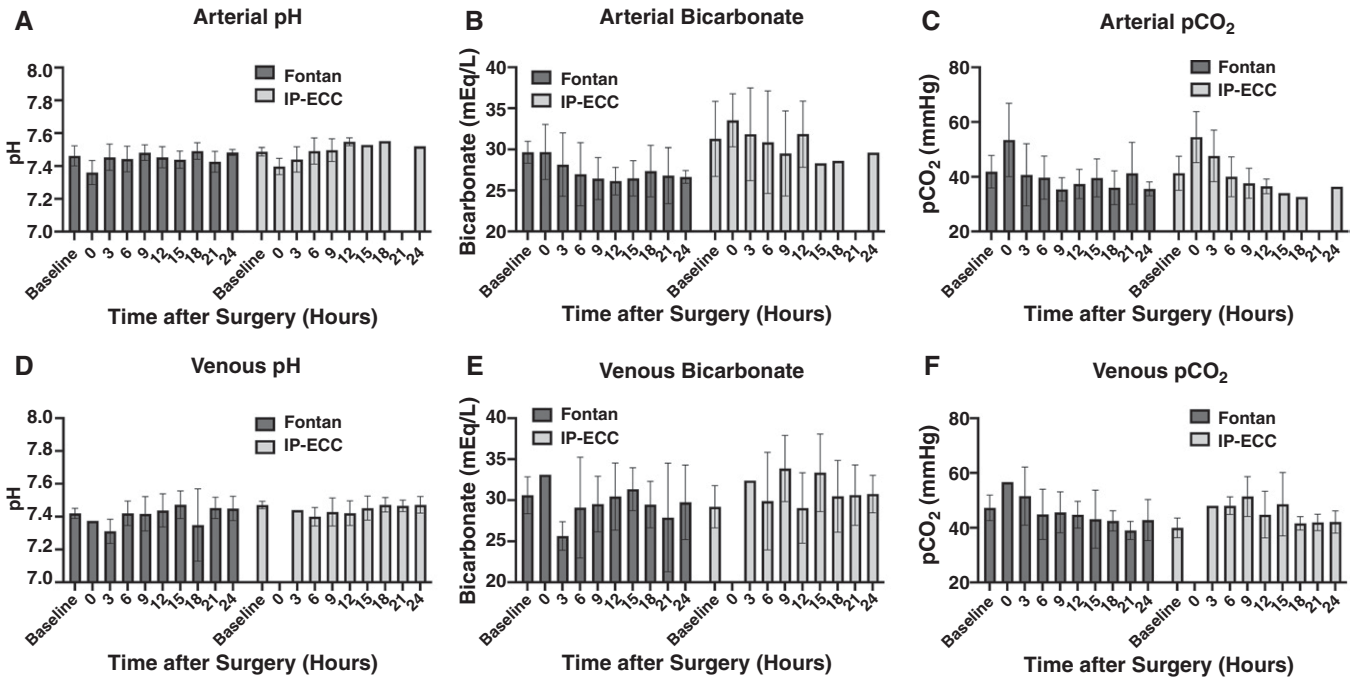

Figure 5. Three-hour interval patterns during the first 24 h after surgery for (A) arterial pH; (B) arterial bicarbonate; (C) arterial pCO_2; (D) venous pH; (E) venous bicarbonate; and (F) venous pCO_2 for the Single-Stage Fontan (Fontan) and inferior vena cava to pulmonary artery extracardiac conduit (IP-ECC) cohorts.

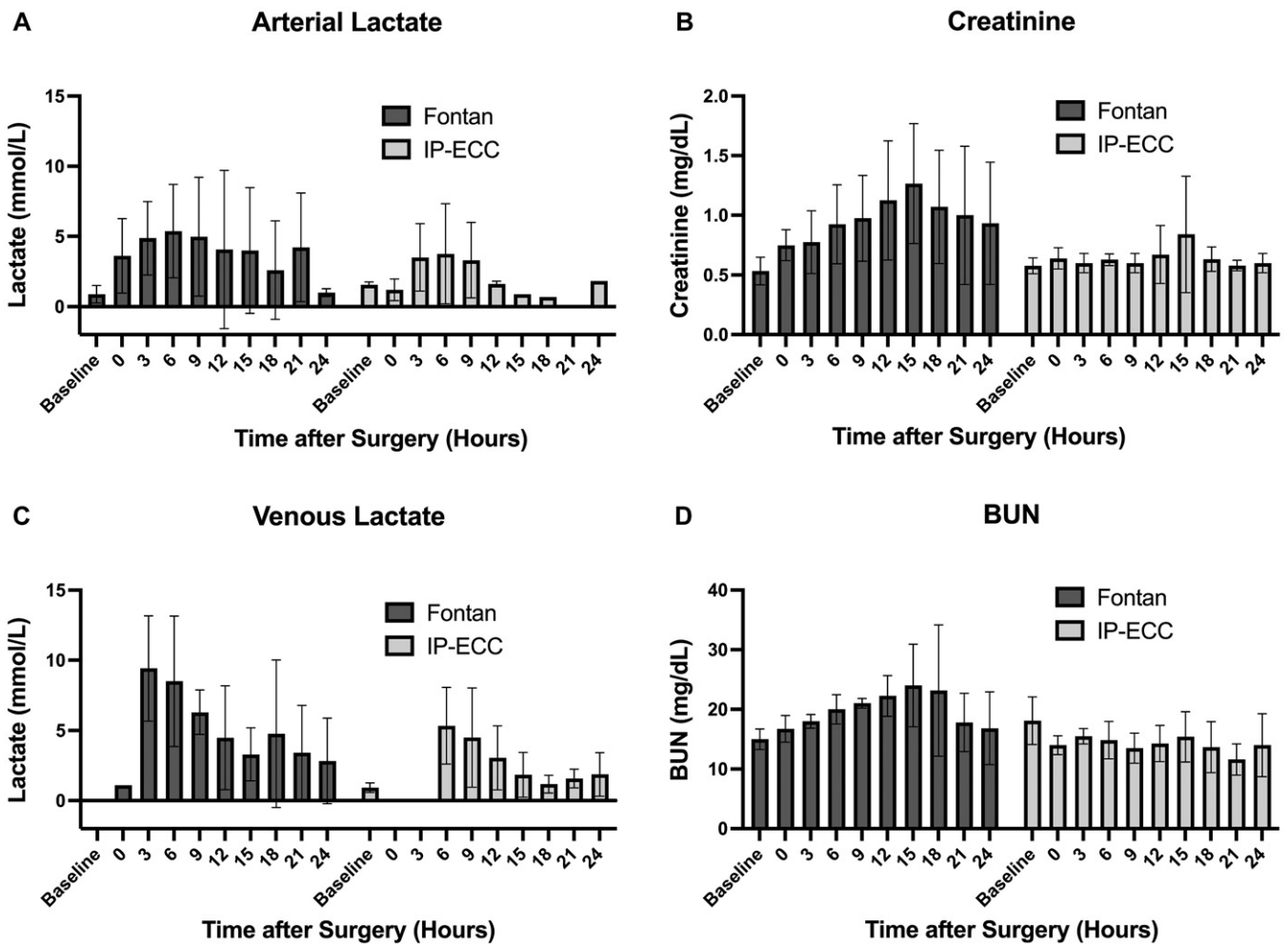

Figure 6. Three-hour interval patterns during the first 24 h after surgery for (A) arterial lactate; (B) creatinine; (C) venous lactate; and (D) blood urea nitrogen (BUN) for the Single-Stage Fontan (Fontan) and inferior vena cava to pulmonary artery extracardiac conduit (IP-ECC) cohorts.

increase in lactate followed by a slow return toward baseline.[17,18] Major cardiac surgeries can drive significant lactate elevation as a consequence of regional or systemic conditions of low perfusion.[13] In fact, some human blood lactate studies actively exclude cardiac surgery patients from analyses of larger cohorts because of skewed lactate levels.[13] Low perfusion leads tissues to convert to anaerobic metabolic processes, generating lactate that subsequently drains into venous circulation.[13,27] Patterns of lactate levels in our study were more apparent in venous samples than arterial samples, consistent with the only ovine study that compared arterial and venous lactate and found a significant 0.44 mmol/L elevation in venous over arterial lactate in a 10-subject ovine cohort at 6 wk after oophorectomy.[27] We could not conduct direct arterial-venous comparisons for each parameter because we never drew arterial and venous samples concurrently. Also of note, the use of lactate levels in cardiac surgery to interpret the presence of an anaerobic state is complicated because lactate can also be elevated by alternate factors, including surgical stress, β-adrenergic drugs, and lung production of lactate.[42] Despite this, lactate was shown to be a reliable predictor of low cardiac output syndrome, which is associated with a decreased cardiac index and signs of organ hypoperfusion.[42]

AKI is a well-documented complication of invasive cardiac surgeries, affecting up to 50% of patients.[11,24] Three main pathophysiological mechanisms contribute to AKI in the setting of cardiac surgery: renal hypoperfusion, inflammation with oxidative stress, and the use of nephrotoxic agents.[11] In our cohort, renal hypoperfusion is likely exacerbated by reduced cardiac output after surgery. Complete or even partially passive venous return reduces cardiac output, affecting end-organ perfusion. Despite extensive investigations into alternative markers for AKI, creatinine and BUN are still the most frequently used.[32] Postoperative increases in both of these biomarkers have been associated with increased mortality.[1,19,24,31,34] In our study, creatinine levels indicated reduced renal function primarily in the SSF cohort, which had an increase in creatinine. The serum creatinine concentrations of most subjects in the SSF cohort did not exceed the normal reference range (0.8 to 1.3 mg/dL).[7] However, in humans, a serum creatinine concentration increase of greater than or equal to 0.3 mg/dL or a percentage increase greater than or equal to 50% is diagnostic for AKI.[29] These guidelines suggest the increase in creatinine that we found in the SSF cohort is clinically significant. The IP-ECC cohort had no significant deviation from baseline in creatinine or BUN.

The primary unexpected finding in our analysis was our BUN data, which showed a significant elevation in the SSF cohort as compared with the IP-ECC cohort, even though neither group deviated significantly from baseline. These results indicate a moderate increase in BUN from baseline for the SSF cohort and a moderate decrease in BUN from baseline for the IP-ECC cohort. However, creatinine, another biomarker of kidney function, showed a significant increase from baseline for the SSF cohort but not in the IP-ECC cohort. The difference in patterns of creatinine and BUN was unexpected and merits further study. A likely

explanation is rooted in the increased fluid loss and decreased fluid intake that occurred in the SSF cohort relative to the IP-ECC cohort. The effect of dilution due to total fluid balance and intravenous fluid intake may affect BUN more than creatinine; clinically, when starting diuretics, we repeatedly noticed a rise in BUN before any change in creatinine. However, we could not adjust for these factors as we did not monitor fluid balance and thus cannot comment definitively on this. The sheep in this cohort were not started on diuretics (furosemide and spironolactone) until postoperative day 3 or later, so these medications had no impact on the 24-h values reported in this study.

This retrospective study was based on data from whole blood testing that was implemented based on clinical need as compared with standardized time points. This approach resulted in gaps in the dataset; all subjects had missing data points at multiple time points, including baseline, for several parameters. Therefore, despite having 21 subjects, we did not have 21 data points for each parameter at all reported time intervals. The specific data points that were missing varied among subjects. We grouped data to mitigate the effect of these gaps on our analysis and conclusions, but due to the small sample size, these gaps may have affected outcomes. The 21 subjects used for this analysis had a wide range of clinical outcomes. Four subjects died within 24 h after surgery, 2 from the SSF cohort and 2 from the IP-ECC cohort, and some of the surviving subjects recovered more quickly than others. As such, this report does not describe the clinical course of serum measurements for a cohort of animals with the same positive (or negative) outcomes. Different values may have occurred in sheep that experienced either normal recovery or rapid decline. Standardization of our included data, such that each subject had one data point per 3-h interval, prevented overrepresentation of subjects that were more actively clinically managed. In the clinic, major cardiovascular procedures can have an array of outcomes, and because our sheep cohort follows this trend, our findings are more representative of typical clinical findings than of a cohort comprised of subjects recovering at a uniform rate (all very quickly or all very slowly). Another limitation is the wide range of ages at surgery (3 to 28 mo) for subjects included in this study. Age and size may have influenced the ability of each subject to recover from the surgery, thus influencing analyte measurements. However, the ages at surgery were statistically equivalent in the SSF and IP-ECC cohorts ($P = 0.9036$) suggesting that comparisons of the 2 cohorts are not skewed by age variability. All subjects were actively managed to support the clinical course; interventions likely affected analyte values. Bicarbonate and pH were the parameters most likely to be affected by clinical management as we routinely administered a dose of bicarbonate (1 mEq/kg) to sheep with a postoperative pH of 7.3 or lower and gave a repeat dose if acidosis did not improve. We did not maintain information on which sheep received bicarbonate, so we cannot compare the treated and nontreated subjects. However, we do know that many more SSF sheep were treated as compared with IP-ECC animals. In the course of managing these animals, we did not notice bicarbonate administration (or lack thereof) was a prognostic indicator. Subjects that received sodium bicarbonate may develop transient and mild metabolic alkalosis. We speculate that the minimal variability from baseline seen in arterial and venous pH and venous bicarbonate in both surgical cohorts indicates a masking of the expected trend of acidosis and concomitant drop in bicarbonate. Despite potential side effects and variables, failure to treat metabolic acidosis with sodium bicarbonate would not be consistent with typical clinical management.

In summary, this retrospective study provides measurements of arterial and venous whole blood analytes in sheep after 2 different major cardiovascular surgeries. When comparing parameters in the 2 cohorts, deviation from baseline findings was greater for the SSF. In contrast to the SSF group, the IP-ECC cohort showed no significant deviation from baseline for any assessed parameters. This finding indicates a higher safety profile for the less complex surgery, as would be expected. However, the marked absence of any deviation from baseline for all parameters in the IP-ECC cohort, despite clinical management, merits future assessment in a larger cohort.

The primary point of comparison that can be made between our results and existing literature is in baseline measurements. Numerous published studies report baseline pH, pCO_2, and bicarbonate data for sheep, but only 2 reported baseline blood lactate, and only one reported baseline blood creatinine and urea.[9,20,27,33,40,43] Because we collected our baseline measurements from anesthetized sheep (always less than 20 min after induction), it is useful that 2 of these studies reported baseline whole blood values in sheep both before and during anesthesia.[33,43] Our baseline measurements agree with those in the existing literature for all 6 parameters that we measured, including studies that used unanesthetized sheep.[9,20,27,33,40,43] We speculate that anesthesia did not cause significant deviations in our baseline measurements due to the short interval between induction and sample collection. A previous study reported that pH, pCO_2, lactate, urea, and creatinine did not change significantly within 20 min after induction of anesthesia in sheep, but bicarbonate was not measured.[43] Beyond baseline comparisons, the aims of our study do not overlap with prior reports of whole blood findings in sheep, so other comparisons with the literature are not possible.

Our study represents the first attempt to characterize postoperative variation in a panel of whole blood analytes in sheep and aims to support real-time diagnostic capabilities and postoperative management. Overall, the data from this study should improve future postoperative treatment of sheep undergoing major cardiovascular procedures to facilitate research on these lifesaving surgeries.

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Conflict of Interest

Dr. Yates has received funding from Alexion Pharmaceuticals related to clinical trial design for studies related to pediatric AKI in postoperative cardiac surgery.

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References

1. **Arnan MK, Hsieh TC, Yeboah J, Bertoni AG, Burke GL, Bahrainwala Z, Grega MA, Baumgartner WA, Gottesman RF.** 2015. Postoperative blood urea nitrogen is associated with stroke in cardiac surgical patients. Ann Thorac Surg **99**:1314–1320. https://doi.org/10.1016/j.athoracsur.2014.11.034.
2. **Attanavanich S, Limsuwan A, Vanichkul S, Lertsithichai P, Ngodngamthaweesuk M.** 2007. Single-stage versus two-stage modified Fontan procedure. Asian Cardiovasc Thorac Ann **15**:327–331. https://doi.org/10.1177/021849230701500413.
3. **Bloom BM, Grundlingh J, Bestwick JP, Harris T.** 2014. The role of venous blood gas in the emergency department: A systematic

review and meta-analysis. Eur J Emerg Med **21**:81–88. https://doi.org/10.1097/MEJ.0b013e32836437cf.
4. **Byrne AL, Bennett M, Chatterji R, Symons R, Pace NL, Thomas PS.** 2014. Peripheral venous and arterial blood gas analysis in adults: Are they comparable? A systematic review and meta-analysis. Respirology **19**:168–175. https://doi.org/10.1111/resp.12225.
5. **Castro D, Patil SM, Zubair M, Keenaghan M.** 2024. Arterial blood gas. In: StatPearls. Treasure Island (FL): StatPearls Publishing.
6. **Chong WH, Saha BK, Medarov BI.** 2021. Comparing central venous blood gas to arterial blood gas and determining its utility in critically ill patients: Narrative review. Anesth Analg **133**:374–378. https://doi.org/10.1213/ANE.0000000000005501.
7. **Cooper TK, Zhong Q, Nabity M, Rosenberg G, Weiss WJ.** 2012. Use of urinary biomarkers of renal ischemia in a lamb preclinical left ventricular assist device model. Artif Organs **36**:820–824. https://doi.org/10.1111/j.1525-1594.2011.01436.x.
8. **Cysyk J, Clark JB, Newswanger R, Jhun C-S, Izer J, Finicle H, Reibson J, Doxtater B, Weiss W, Rosenberg G.** 2019. Chronic in vivo test of a right heart replacement blood pump for failed Fontan circulation. ASAIO J **65**:593–600. https://doi.org/10.1097/MAT.0000000000000888.
9. **Dinev D.** 2003. Peritoneal dialysis in healthy sheep: Changes in acid-base balance, blood electrolytes and some parameters of carbohydrate metabolism. Bulg J Vet Med **6**:159–167.
10. **DiVincenti L, Westcott R, Lee C.** 2014. Sheep (*Ovis aries*) as a model for cardiovascular surgery and management before, during, and after cardiopulmonary bypass. J Am Assoc Lab Anim Sci **53**:439–448.
11. **Djordjević A, Šušak S, Velicki L, Antonič M.** 2021. Acute kidney injury after open-heart surgery procedures. Acta Clin Croat **60**:120–126. https://doi.org/10.20471/acc.2021.60.01.17.
12. **Duchenne J, Claus P, Pagourelias ED, Mada RO, Van Puyvelde J, Vunckx K, Verbeken E, Gheysens O, Rega F, Voigt J-U.** 2019. Sheep can be used as animal model of regional myocardial remodeling and controllable work. Cardiol J **26**:375–384. https://doi.org/10.5603/CJ.a2018.0007.
13. **Evron S, Tress V, Ezri T, Szmuk P, Landau O, Hendel D, Schechter P, Medalion B.** 2007. The importance of blood sampling site for determination of hemoglobin and biochemistry values in major abdominal and orthopedic surgery. J Clin Anesth **19**:92–96. https://doi.org/10.1016/j.jclinane.2006.04.005.
14. **Gallagher EJ, Rodriguez K, Touger M.** 1997. Agreement between peripheral venous and arterial lactate levels. Ann Emerg Med **29**:479–483. https://doi.org/10.1016/S0196-0644(97)70220-8.
15. **Gattinoni L, Pesenti A, Matthay M.** 2018. Understanding blood gas analysis. Intensive Care Med **44**:91–93. https://doi.org/10.1007/s00134-017-4824-y.
16. **Häger C, Biernot S, Buettner M, Glage S, Keubler LM, Held N, Bleich EM, et al.** 2017. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. PLoS One **12**:e0175839. https://doi.org/10.1371/journal.pone.0175839.
17. **Hamamoto M, Imanaka H, Kagisaki K, Yagihara T, Kitamura S, Nishimura M.** 2005. Is an increase in lactate concentration associated with cardiac dysfunction after the Fontan procedure? Ann Thorac Cardiovasc Surg **11**:301–306.
18. **Hamamoto M, Uemura H, Imanaka H, Yagihara T.** 2006. Relevance of the measurement of the concentration of lactate in the serum subsequent to the Fontan procedure in small children. Cardiol Young **16**:275–280. https://doi.org/10.1017/S1047951106000369.
19. **Hartz AJ, Kuhn EM, Kayser KL, Johnson WD.** 1995. BUN as a risk factor for mortality after coronary artery bypass grafting. Ann Thorac Surg **60**:398–404. https://doi.org/10.1016/0003-4975(95)00358-R.
20. **Izer J, Mattern E, Ellwanger J, Wilson R.** 2019. Comparison of arterial and venous blood-gas values in anesthetized Dorset cross-bred lambs (*Ovis aries*) using a point-of-care analyzer. Vet Anaesth Analg **46**:209–213. https://doi.org/10.1016/j.vaa.2018.12.003.
21. **Kirubakaran C, Gnananayagam JEJ, Sundaravalli EK.** 2003. Comparison of blood gas values in arterial and venous blood. Indian J Pediatr **70**:781–785. https://doi.org/10.1007/BF02723794.
22. **Koch CG, Reineks EZ, Tang AS, Hixson ED, Phillips S, Sabik JF, Henderson JM.** 2015. Contemporary bloodletting in cardiac surgical care. Ann Thorac Surg **99**:779–784. https://doi.org/10.1016/j.athoracsur.2014.09.062.
23. **Lee YS, Kim WY, Yoo JW, Jung HD, Min TJ.** 2018. Correlation between regional tissue perfusion saturation and lactate level during cardiopulmonary bypass. Korean J Anesthesiol **71**:361–367. https://doi.org/10.4097/kja.d.17.00002.
24. **Lofgren L, Silverton N, Kuck K.** 2023. Combining machine learning and urine oximetry: Towards an intraoperative aki risk prediction algorithm. J Clin Med **12**:5567. https://doi.org/10.3390/jcm12175567.
25. **Malatesha G, Singh NK, Bharija A, Rehani B, Goel A.** 2007. Comparison of arterial and venous pH, bicarbonate, Pco2 and Po2 in initial emergency department assessment. Emerg Med J **24**:569–571. https://doi.org/10.1136/emj.2007.046979.
26. **Masuda M, Kado H, Shiokawa Y, Fukae K, Suzuki M, Murakami E, Yasui H.** 1998. Clinical results of the staged Fontan procedure in high-risk patients. Ann Thorac Surg **65**:1721–1725. https://doi.org/10.1016/S0003-4975(98)00264-1.
27. **Mathews LA, Barletta M, Almeida DC, Graham LF, Quandt JE.** 2014. Evaluation of serial venous and arterial lactate concentrations in healthy anesthetized sheep undergoing ovariectomy. Vet Anaesth Analg **41**:498–505. https://doi.org/10.1111/vaa.12142.
28. **McBride ME, Berkenbosch JW, Tobias JD.** 2001. Correlation of venous and arterial blood gas values following cardiothoracic surgery in infants and children. J Intensive Care Med **16**:231–235. https://doi.org/10.1046/j.1525-1489.2001.00231.x.
29. **Mehta RL, Kellum JA, Shah SV, Molitoris BA, Ronco C, Warnock DG, Levin A.** 2007. Acute Kidney Injury Network: Report of an initiative to improve outcomes in acute kidney injury. Crit Care **11**:R31. https://doi.org/10.1186/cc5713.
30. **Middleton P, Kelly A, Brown J, Robertson M.** 2006. Agreement between arterial and central venous values for pH, bicarbonate, base excess, and lactate. Emerg Med J **23**:622–624. https://doi.org/10.1136/emj.2006.035915.
31. **Moledina DG, Parikh CR.** 2018. Phenotyping of acute kidney injury: Beyond serum creatinine. Semin Nephrol **38**:3–11. https://doi.org/10.1016/j.semnephrol.2017.09.002.
32. **Nadim MK, Forni LG, Bihorac A, Hobson C, Koyner JL, Shaw A, Arnaoutakis GJ, et al.** 2018. Cardiac and vascular surgery-associated acute kidney injury: The 20th International Consensus Conference of the ADQI (Acute Disease Quality Initiative) Group. J Am Heart Assoc **7**:e008834. https://doi.org/10.1161/JAHA.118.008834.
33. **Onmaz A, Güneş V, Atalan G, Gelfert C, Atalan G.** 2009. Comparison of arterial and venous blood gas values in sheep before and during isoflurane anaesthesia. Rev Med Vet (Toulouse) **160**:356–361.
34. **Parikh CR, Coca SG, Thiessen-Philbrook H, Shlipak MG, Koyner JL, Wang Z, Edelstein CL, et al.** 2011. Postoperative biomarkers predict acute kidney injury and poor outcomes after adult cardiac surgery. J Am Soc Nephrol **22**:1748–1757. https://doi.org/10.1681/ASN.2010121302.
35. **Park H-J, Kelly JM, Hoffman JR, Takaesu F, Schwartzman W, Ulziibayar A, Kitsuka T, et al.** 2022. Computational analysis of serum-derived extracellular vesicle miRNAs in juvenile sheep model of single stage Fontan procedure. Extracellular Vesicle **1**:100013. https://doi.org/10.1016/j.vesic.2022.100013.
36. **Rychik J, Atz AM, Celermajer DS, Deal BJ, Gatzoulis MA, Gewillig MH, Hsia T-Y, et al.** 2019. Evaluation and management of the child and adult with Fontan circulation: A scientific statement from the American Heart Association. Circulation **140**:e234–e284. https://doi.org/10.1161/CIR.0000000000000696.
37. **Sheng F, Chen L, Lin H, Wu H.** 2022. Systematic review and meta-analysis: Value of venous blood gas in the diagnosis of acute exacerbation of chronic obstructive pulmonary disease in emergency department. Ann Palliat Med **11**:1473–1481. https://doi.org/10.21037/apm-22-336.

38. **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. https://doi.org/10.1258/002367704322968821.
39. **Singh V, Khatana S, Gupta P.** 2013. Blood gas analysis for bedside diagnosis. Natl J Maxillofac Surg **4**:136–141. https://doi.org/10.4103/0975-5950.127641.
40. **Sobiech P, Stopyra A, Kuleta Z, Zbanyszek M, Milewski S.** 2005. Acid-base balance parameters of arterial, venous and capillary blood in sheep. Bull Vet Inst Pulawy **49**:125–127.
41. **Sood P, Paul G, Puri S.** 2010. Interpretation of arterial blood gas. Indian J Crit Care Med **14**:57–64. https://doi.org/10.4103/0972-5229.68215.
42. **Taiana M, Tomasella I, Russo A, Lerose A, Ceola Graziadei M, Corubolo L, Rama J, et al.** 2023. Analysis of P(v-a)CO2/C(a-v)O2 ratio and other perfusion markers in a population of 98 pediatric patients undergoing cardiac surgery. J Clin Med **12**:5700. https://doi.org/10.3390/jcm12175700.
43. **Trimmel NE, Hierweger MM, Jucker S, Windhofer L, Weisskopf M.** 2022. Physiologic effects of prolonged terminal anesthesia in sheep (*Ovis gmelini aries*). Comp Med **72**:257–266. https://doi.org/10.30802/AALAS-CM-21-000103.
44. **UpToDate.** [Internet]. 2023. Bicarbonate therapy in lactic acidosis. [Cited 24 February 2023]. Available at: https://www.uptodate.com/contents/bicarbonate-therapy-in-lactic-acidosis.
45. **Van Puyvelde J, Rega F, Minami T, Claus P, Cools B, Gewillig M, Meyns B.** 2019. Creation of the Fontan circulation in sheep: A survival model. Interact Cardiovasc Thorac Surg **29**:15–21. https://doi.org/10.1093/icvts/ivz022.