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Gastrointestinal mucosal development and injury in premature lambs supported by the artificial placenta☆,☆☆

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ABSTRACT

Background: An Artificial Placenta (AP) utilizing extracorporeal life support (ECLS) could revolutionize care of extremely premature newborns, but its effects on gastrointestinal morphology and injury need investigation.
Methods: Lambs (116–121 days GA, term = 145; n = 5) were delivered by C-section, cannulated for ECLS, had total parenteral nutrition (TPN) provided, and were supported for 7 days before euthanasia. Early and Late Tissue Controls (ETC, n = 5 and LTC, n = 5) delivered at 115–121 days and 125–131 days, respectively, were immediately sacrificed. Standardized jejunal samples were formalin-fixed for histology. Crypt depth (CD), villus height (VH), and VH:CD ratios were measured. Measurements also included enterocyte proliferation (Ki-67), Paneth cell count (Lysozyme), and injury scores (H&E). ANOVA and Chi Square were used with $p < 0.05$ considered significant.
Results: CD, VH, and VH:CD were similar between groups ($p > 0.05$). AP demonstrated more enterocyte proliferation (95.7 ± 21.8) than ETC (49.4 ± 23.4; $p = 0.003$) and LTC ($66.1 + 11.8$; $p = 0.04$), and more Paneth cells (81.7 ± 17.5) than ETC (41.6 ± 7.0; $p = 0.0005$) and LTC (40.7 ± 8.2, $p = 0.0004$). Presence of epithelial injury and congestion in the bowel of all groups were not statistically different. No villus atrophy or inflammation was present in any group.
Conclusions: This suggests preserved small bowel mucosal architecture, high cellular turnover, and minimal evidence of injury.
Study type: Research paper/therapeutic potential.
Level of evidence: N/A

Prematurity remains a global health problem, with extremely low gestational age newborns (ELGANS, born <28 weeks) suffering the highest morbidity and mortality [1,2]. Of premature infants that survive, morbidities include respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), retinopathy of prematurity (ROP), and necrotizing enterocolitis (NEC) [1–4]. NEC, in particular, is one of the most common and lethal gastrointestinal emergencies of prematurity with a mortality rate as high as 35% and up to 50% needing surgery [5,6]. Most infants who suffer from NEC are supported initially with total parenteral nutrition (TPN). Although this allows nutritional support, the lack of enteral feeds can lead to intestinal atrophy and increased risk of bacterial translocation and sepsis [7–9].

An artificial placenta (AP) could offer a paradigm shift in the management of extremely preterm infants, simulating the intrauterine environment by utilizing extracorporeal life support (ECLS) to allow for the maintenance of fetal circulation, fluid filled lungs, and no mechanical ventilation. While we have previously reported that the AP protects against lung injury and allows normal lung development [10], it is also important to evaluate the effects on other organ systems such as the gastrointestinal tract.

We aimed to evaluate the effects of AP support on bowel morphology and injury. We specifically evaluated mucosal architecture, cellular proliferation, and injury from AP-supported lambs compared to gestational age-matched controls. We hypothesized that architecture would be preserved during AP support and injury would be comparable to tissue controls.

☆ Conflicts of Interest: None

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1. Methods

The experimental procedure was performed in an ovine model following protocol approval by the University of Michigan Institutional Animal Care and Use Committee (IACUC) (protocol 00007211). All sheep used for the experiment were treated in compliance with the Guide for Care and Use of Laboratory Animals, 8th edition [11].

Lambs used for the experiment were divided into three groups: Artificial Placenta (AP) Group, Early Tissue Control (ETC), and Late Tissue Control (LTC). Age and weight of each lamb were recorded.

1.1. AP group

Premature lambs at 116–121 days GA (term = 145; n = 5) were delivered via C-Section. 10–14 Fr cannulas (Terumo: Ann Arbor, MI) were placed in the jugular vein (drainage) and umbilical vein (reinfusion). The circuit was completed with ¼" tubing (Tygon: Lima, OH), a collapsible-tubing roller pump (MC3: Ann Arbor, MI), and oxygenator/heat exchanger (either Medos HiLite, Xenios: Heilbronn, Germany or Capiox Baby Rx, Terumo, Ann Arbor, MI; Fig. 1). VV-ECLS was initiated and the lambs were monitored closely. A 5 Fr arterial line (Covidien-Medtronic: Minneapolis, MN) was placed into the umbilical artery for hemodynamic monitoring and arterial blood gas (ABG) blood draws. The second umbilical vein was cannulated with a 5 Fr triple lumen venous line (Covidien-Medtronic: Minneapolis, MN) for intravenous fluid, TPN, heparin sulfate (SAGENT, Schaumburg, IL) (100 U/h, titrated to a goal activated clotting time (ACT) of 200–250 s), and Prostaglandin E_1 (Pfizer, New York, NY) (0.2 μg/kg/min) infusion to maintain ductal patency. The lambs were intubated and lungs were filled with fluid (amniotic fluid, Ringer's Lactate, or perfluorodecalin [Origen: Austin, TX]).

All lambs were supported on TPN infused via the umbilical vein. The TPN (ExactMix, Baxter Healthcare Corporation, Englewood, CO. Baxter International Inc. Supplied by the University of Michigan HomeMed-Home Infusion Pharmacy) was made with a standard composition including: Amino Acids (15%) (16 GM); Dextrose (45 GM); IntraLipids (80 ML); and electrolytes (Sodium Phosphate (5.4 MM); Potassium Chloride (8.1 MEQ); Magnesium Sulfate (1.6 MEQ); and Calcium Gluconate (1 MEQ)) in 400 mL volume administered at a rate of 5 mL/kg/h. All AP lambs remained nil per os (NPO) during support. Hemodynamics, urine output, and bowel movements were monitored. All lambs were given prophylactic intravenous antibiotics (piperacillin-tazobactam [Hospira Inc., Lake Forest, IL]) and antifungals (fluconazole [SAGENT, Schaumburg, IL]) to prevent infection. Solumedrol (Pfizer, New York, NY) 0.63 mg/kg was given every 12 h to prevent hypocortisolemia. Diazepam (Hospira Inc., Lake Forest, IL) 2.5 mg and Buprenorphine (Parr Inc., Spring Valley, NJ) 0.3 mg were used sparingly for pain or agitation. In cases of volume-resistant hypotension, vasopressors (norepinephrine (Claris LifeSciences Inc., North Brunswick, NJ), epinephrine (Hospira Inc., Lake Forest, IL), or dopamine (Baxter, Deerfield, IL)) was used to maintain a MAP >40 mmHg. AP support was continued for 7–10 days; then the animals were euthanized.

1.2. Tissue control groups

Early and Late Tissue Controls (ETC; n = 5 and LTC; n = 5) were delivered at 115–121 and 125–131 days, respectively, and immediately sacrificed.

1.2.1. Necropsy, tissue preparation, and histological analysis

After sacrifice, the bowel was removed en bloc and formalin-fixed. Standardized 2 cm longitudinal sections of jejunum were taken from 6 cm distal to the duodenojejunal junction to be used for mucosal measurements and staining. Sections from proximal, mid, and distal jejunum, terminal ileum, and cecum were also harvested for injury scoring. All samples were sectioned 3–5 μm thick. Slides were stained

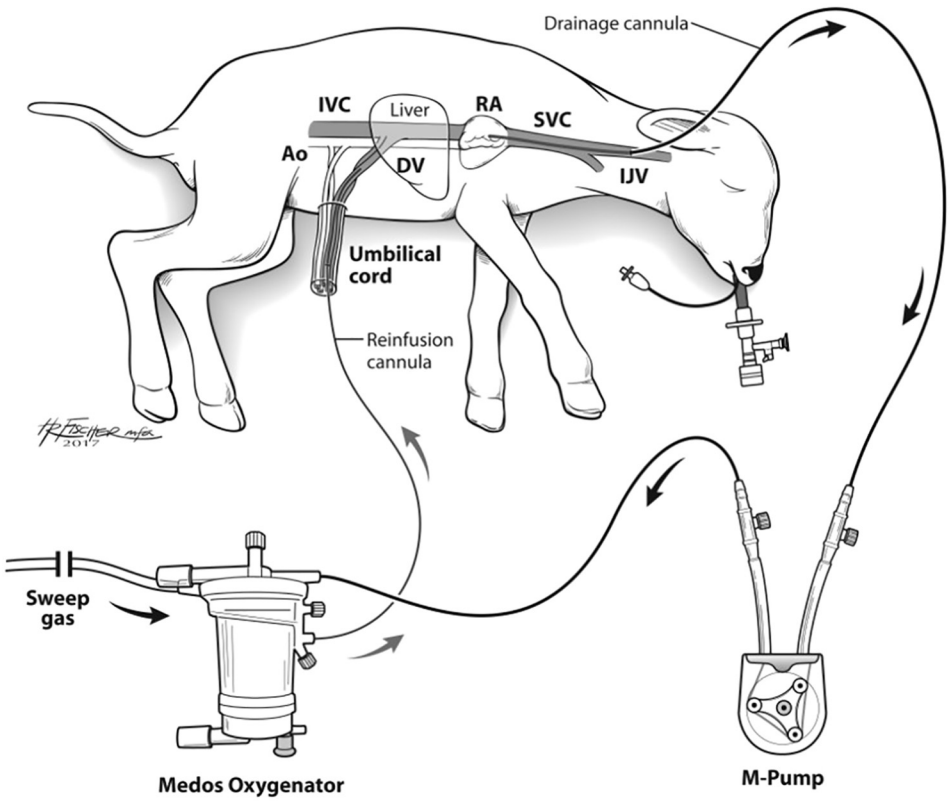

Fig. 1. Schematic of the artificial placenta (AP) circuit in a premature lamb model demonstrating the cannulated lamb for VV ELCS, collapsible-tubing roller pump (M-Pump), and oxygenator/heat exchanger. Ao: Aorta; IVC: Inferior Vena Cava; DV: Ductus Venosus; RA: Right Atrium; SVC: Superior Vena Cava; IJV: Internal Jugular Vein.