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Artificial Placenta

Effects of an artificial placenta on brain development and injury in premature lambs

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ABSTRACT

Purpose: We evaluated whether brain development continues and brain injury is prevented during Artificial Placenta (AP) support utilizing extracorporeal life support (ECLS).
Methods: Lambs at EGA 118 days (term = 145; n = 4) were placed on AP support (venovenous ECLS with jugular drainage and umbilical vein reinfusion) for 7 days and sacrificed. Early (EGA 118; n = 4) and late (EGA 127; n = 4) mechanical ventilation (MV) lambs underwent conventional MV for up to 48 h and were sacrificed, and early (n = 5) and late (n = 5) tissue control (TC) lambs were sacrificed at delivery. Brains were harvested, formalin-fixed, rehydrated, and studied by magnetic resonance imaging (MRI). The gyrification index (GI), a measure of cerebral folding complexity, was calculated for each brain. Diffusion-weighted imaging was used to determine fractional anisotropy (FA) and apparent diffusion coefficient (ADC) in multiple structures to assess white matter (WM) integrity.
Results: No intracranial hemorrhage was observed. GI was similar between AP and TC groups. ADC and FA did not differ between AP and late TC groups in any structure. Compared to late MV brains, AP brains demonstrated significantly higher ADC (0.45 ± 0.08 vs. 0.27 ± 0.11, p = 0.02) and FA (0.61 ± 0.04 vs. 0.44 ± 0.05; p = 0.006) in the cerebral peduncles.
Conclusions: After 7 days of AP support, WM integrity is preserved relative to mechanical ventilation.
Type of study: Research study.

Extremely low gestational age newborns (ELGANs), defined as neonates born at ≤28 weeks estimated gestational age (EGA), face unacceptably high morbidity and mortality, with survival well below 50% for infants born before 24 weeks [1]. Morbidities in these patients arise from the immaturity of multiple organ systems [2–5]. In particular, ELGANs suffer a disproportionate amount of neurologic disability, with nearly 40% of survivors being neurologically impaired [6,7]. Both intracranial hemorrhage (ICH) and white-matter injury (WMI) have been recognized as major problem within this population. The pathophysiology of these injuries is multifactorial, and evidence suggests hypoxia–ischemia, inflammation, mechanical ventilation, fluid shifts, and hemodynamic instability all contribute [8,9].

A radical solution to minimize these factors is to recreate the intrauterine environment with an Artificial Placenta (AP). This consists of venovenous extracorporeal life support (VV-ECLS) with jugular vein drainage and umbilical vein reinfusion, avoidance of mechanical ventilation, maintenance of fluid-filled lungs, and preservation of fetal circulation. We recently reported the ability of the AP to prevent lung injury and allow continued lung development in extremely premature lambs [10]. However, neurologic complications, in addition to being common in ELGANs, are also frequently encountered in patients supported by ECLS [11]. The effects of the AP on the premature brain are unknown.

With AP-supported patients having two major risk factors for neurologic injury, it is imperative to assess the effects of the AP on the premature brain prior to clinical translation. We therefore used MRI to assess cortical folding and gray- and white-matter integrity in extremely preterm lambs supported by the AP, and compared these

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findings to those in gestational age-matched controls, and to age-matched lambs that were mechanically ventilated. Despite the theoretic risk conferred by ECLS, we hypothesized that the AP would not cause ICH or WMI, and would be superior to mechanical ventilation in preserving brain WM integrity.

1. Methods

The sheep in this experiment were treated in compliance with the *Guide for Care and Use of Laboratory Animals* (US National Institutes of Health publication No. 85-23, National Academy Press, Washington D.C., revised 1996) and all methods were approved by the University of Michigan Institutional Animal Care and Use Committee (protocol 00007211).

1.1. AP support

Premature lambs of EGA 118 ± 3 days (n = 4) were delivered via midline laparotomy and transverse hysterotomy. This gestational age was selected, as previous experimentation has determined fetal sheep lung development at this stage is analogous to that of a 24-week human fetus [12]. The right jugular vein was exposed and cannulated with a 10–14Fr drainage cannula (Terumo: Ann Arbor, MI). A 10–12Fr reinfusion cannula was placed in the umbilical vein, and the circuit was completed using ¼" tubing (Tygon: Lima, OH), a roller pump (MC3: Ann Arbor, MI), and oxygenator/heat exchanger (either Capiox Baby Rx, Terumo: Ann Arbor MI, or Medos HiLite, Xenios: Heilbronn, Germany; Fig. 1). Venovenous (VV) ECLS was initiated. A 5Fr triple lumen venous line was placed in the second umbilical vein and used for IV fluid and medication administration, and a 5Fr umbilical arterial line (both lines from Covidien-Medtronic: Minneapolis, MN) was placed in the umbilical artery for hemodynamic monitoring and arterial blood gas (ABG) sampling.

The lambs were then intubated, and the endotracheal tube filled with amniotic fluid and capped. ECLS was managed according to goal ABG parameters: pH 7.30–7.45, pCO_2 35–50 mmHg, pO_2 25–40 mmHg, and SpO_2 65–80%. AP support was continued for 7 days.

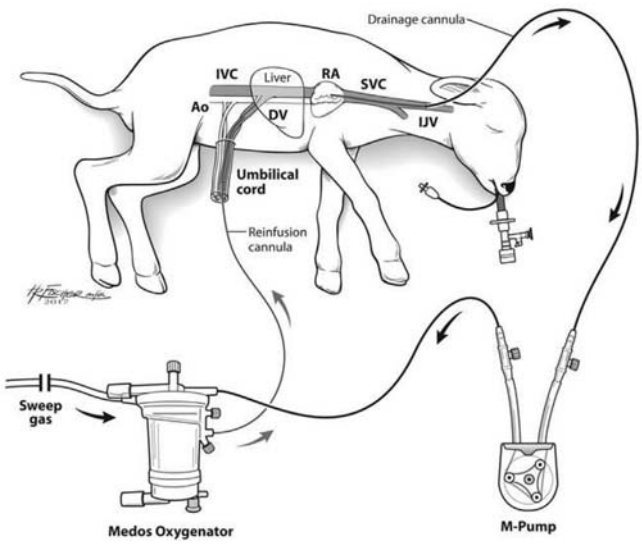

Fig. 1. Schematic of the Artificial Placenta. Blood is drained from the right jugular vein by a collapsible-tubing roller pump (M-pump, MC3: Ann Arbor, MI) and propelled to an oxygenator/heat exchanger (Medos HiLite, Xenios: Heilbronn, Germany), then returned via an umbilical vein. The second umbilical vein is accessed for IV fluid and medication administration, and an umbilical arterial line is placed for hemodynamic monitoring and blood gas sampling. The lamb is intubated and the lungs remain filled with amniotic fluid by clamping the endotracheal tube. Ao, aorta; DV, Ductus venosus; IJV, internal jugular vein; IVC, inferior vena cava; RA, right atrium; SVC, superior vena cava.

Lambs were given total parenteral nutrition (TPN), empiric piperacillin-tazobactam, and solumedrol (0.63 mg/kg every 6 h) at regular intervals. Heparin was infused IV and titrated to an ACT of 200–250.

1.2. Mechanical ventilation (MV) lambs

Early MV (delivered at 118 ± 3 days EGA; n = 4) or late MV (delivered at 128 ± 2 days; n = 4) lambs were delivered in the same method as AP lambs. They were immediately intubated, their lungs were suctioned of fluid, surfactant was administered, and pressure-controlled mechanical ventilation was initiated. Goal ABG values were pH 7.25–7.35, pCO_2 40–60 mmHg, and SpO_2 > 85. If peak airway pressures exceeded 25 cmH_2O, permissive hypercapnia was practiced with administration of IV alkaline therapy. High frequency oscillatory ventilation was employed in the case of refractory hypoxia provided it did not worsen hypercarbia. Support was continued until lung failure, or until 48 h, at which point the animal was sacrificed.

1.3. Tissue control (TC) lambs

Early TC (delivered at 118 ± 3 days EGA; n = 5) and late TC (delivered at 128 ± 3 days; n = 5) lambs were delivered in the same method as AP lambs and immediately sacrificed.

1.4. Brain procurement and preparation

After animal sacrifice, the neck was incised, jugular veins transected, and angiocatheters were placed in the bilateral carotid arteries. The carotid arteries were then each flushed with 60 mL phosphate-buffered saline (PBS) + ethylenediaminetetraacetic acid (EDTA) to clear the cerebral vasculature of blood, followed by 60 mL 10% formalin for fixation. Brains were then harvested intact via craniotomy and placed in 10% formalin.

Prior to imaging, the brains were removed from formalin and soaked in 0.05% sodium azide and 1 equivalent PBS at room temperature. This solution was changed daily for 3–4 weeks prior to MRI [13,14]. For each MRI experiment, the brain was immersed in Fluorinert, a ^1H proton-free, perflurocarbon that improves signal-to-noise ratio in explanted tissue MRI (FC-3283, 3 M Company: Maplewood, MN) [15].

1.5. Magnetic resonance imaging

We used MRI to assess brain development and injury in our experimental lambs. MRI is a validated modality for detecting cerebral abnormalities, including WM injury, in preterm and stillborn neonates, and even in explanted formalin-fixed brains [16,17], making it highly advantageous for our study.

MRI data were collected in an Agilent/Varian 4.7-T small-animal MR scanner equipped with a DirectDrive™ console (Santa Clara, CA), built around an Oxford Instruments (Oxford, United Kingdom) horizontal magnet with a 40-cm clear bore diameter. The system utilizes an actively shielded, 12-cm inner diameter Agilent/Magnex gradient-coil assembly driven by Oy International Electric Company (IEC; Helsinki, Finland) model A-240 amplifiers (300 V and 300 A), producing a maximum magnetic field gradient of 60 G/cm per axis with a rise time of ~300 μs. MRI images were collected with a quadrature transmit/receive coil (outer/inner diameter, 108/63 mm).

Diffusion Tensor Imaging (DTI) measurements were made using a 2D, spin-echo, diffusion-tensor sequence, with [15]. T_1-weighted images were collected using a magnetization-prepared rapid gradient echo (MPRAGE) 3D sequence. The T_1-weighted MPRAGE had the same voxel plan as the DTI measurement (i.e., the data are co-registered), allowing for concomitant analysis of both imaging types [14,18,19].