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Acute fetal cardiovascular adaptation to artificial placenta in sheep model

S. SANCHEZ-MARTINEZ[1,2$] P. C. RANDANNE[1,3$] A. HAWKINS-VILLARREAL[1,4]
K. REZAEI[1,5] R. FUCHO[1] S. BOBILLO-PEREZ[1,6] E. BONET-CARNE[1,2,7] M. ILLA[1,8]
E. EIXARCH[1,9] B. BIJNENS[2,10,11#] F. CRISPI[1,9#] and E. GRATACÓS[1,8,9*#] , Collaborators[†]

[1]*BCNatal Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Universitat de Barcelona, Barcelona, Spain;*
[2]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain;* [3]*Pediatric Cardiology Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain;* [4]*Fetal Medicine Service, Obstetrics Department, Santo Tomás Hospital, University of Panama, Panama City, Panama (on behalf of the Iberoamerican Research Network in Obstetrics, Gynecology and Translational Medicine)* ; [5]*Cardiovascular Surgery Unit, Hospital Universitario Virgen del Rocio, Seville, Spain;* [6]*Paediatric Intensive Care Unit, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain;* [7]*Barcelona Tech, Universitat Politècnica de Catalunya, Barcelona, Spain;* [8]*Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain;* [9]*Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) and Centre for Biomedical Research on Rare Diseases (CIBER-ER), Barcelona, Spain;* [10]*ICREA, Barcelona, Spain;* [11]*Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain*

CONTRIBUTION

What are the novel findings of this work?
We describe the immediate cardiovascular adaptation of the fetus to an artificial placenta (AP) in a sheep model, using invasive and noninvasive hemodynamic monitoring and ultrasonography. Connection to an AP system resulted in a transient fetal hemodynamic response that tended to normalize over hours. In this short-term evaluation, cardiac structure and function were preserved, but a low-resistance, high-volume and high-pressure situation developed, reflected by non-physiologically elevated pressures and pulsatile flow within the output of the AP system.

What are the clinical implications of this work?
The low-resistance, high-volume and high-pressure situation may result eventually in diastolic heart failure and marked cardiac remodeling. Developments in extracorporeal circulation and oxygenation membranes for AP systems should seek to rectify this altered hemodynamic state and reproduce as much as possible the physiological features of placental circulation, with refinement guided by thorough evaluation of fetal cardiac function and morphology in the long term.

ABSTRACT

Objective *To describe the acute cardiovascular adaptation of the fetus after connection to an artificial placenta (AP) in a sheep model, using ultrasound and invasive and noninvasive hemodynamic assessment.*

Methods *This was an experimental study of 12 fetal sheep that were transferred to an AP system, consisting of a pumpless circuit with umbilical cord connection, at 109–117 days' gestation. The study was designed to collect in-utero and postcannulation measurements in all the animals. The first six consecutive fetuses were fitted with intravascular catheters and perivascular probes to obtain invasive physiological data, including arterial and venous intravascular pressures and perivascular blood flows, with measurements taken in utero and at 5 and 30 min after cannulation. These experiments were designed with a survival goal of 1–3 h. The second set of six fetuses were not fitted with catheters, and experiments were aimed at 3–24 h of survival. Echocardiographic assessment of cardiac anatomy and function, as well as measurements of blood flow and pre- and postmembrane pressures recorded by circuit sensors in the AP system, were available for most of the fetuses. These data were acquired in utero and at 30 and 180 min after cannulation.*

Correspondence to: Prof. E. Gratacós, BCNatal (Hospital Sant Joan de Déu and Hospital Clínic), Universitat de Barcelona, Sabino de Arana 1, 08036 Barcelona, Spain (e-mail: egratacos@ub.edu)

$S.S.-M. and P.C.R. contributed equally to this study.

#B.B., F.C. and E.G. contributed equally to this study

†Collaborators are listed at end of article.

ORIGINAL PAPER

Results *Compared with in-utero conditions, the pulsatility index at 30 and 180 min after connection to the AP system was reduced in the umbilical artery (median, 1.36 (interquartile range (IQR), 1.06–1.5) vs 0.38 (IQR, 0.31–0.5) vs 0.36 (IQR, 0.29–0.41); •P < 0.001 for extreme time-points) and the ductus venosus, whereas umbilical venous peak velocity increased (median, 20 cm/s (IQR, 18–22 cm/s) vs 39 cm/s (IQR, 31–43 cm/s) vs 43 cm/s (IQR, 34–54 cm/s); P < 0.001 for extreme time-points) and flow became more pulsatile. Intravascular monitoring showed that arterial and venous pressures increased transiently after connection, with median values for mean arterial pressure at baseline, 5 min and 30 min of 43 mmHg (IQR, 35–54 mmHg), 72 mmHg (IQR, 61–77 mmHg) and 58 mmHg (IQR, 50–64 mmHg), respectively (P = 0.02 for baseline vs 5 min). Echocardiography showed a similar transient elevation of fetal heart rate at 30 and 180 min after connection compared with in utero (median, 145 bpm (IQR, 142–156 bpm) vs 188 bpm (IQR, 171–209 bpm) vs 175 bpm (IQR, 165–190 bpm); P = 0.001 for extreme time-points). Fetal cardiac structure and function were mainly preserved; median values for right fractional area change were 36% (IQR, 34–41%) in utero, 38% (IQR, 30–40%) at 30 min and 37% (IQR, 33–40%) at 180 min (P = 0.807 for extreme time-points).*

Conclusions *Connection to an AP system resulted in a transient fetal hemodynamic response that tended to normalize over hours. In this short-term evaluation, cardiac structure and function were preserved. However, the system resulted in non-physiologically elevated venous pressure and pulsatile flow, which should be corrected to avoid later impairment of cardiac function. © 2023 International Society of Ultrasound in Obstetrics and Gynecology.*

INTRODUCTION

Extreme prematurity is a leading cause of neonatal mortality and morbidity, the rates of which have remained unchanged in infants born at or before 26 weeks' gestation over the last two decades[1–4]. Extreme immaturity represents a biological barrier for current ventilation-based life support systems and explains the high prevalence of chronic morbidity in survivors[5]. In this context, every additional week of gestation results in a significant improvement in neonatal outcome[6]. An artificial placenta (AP) replicates the hemodynamic conditions of a real placenta and is used to support extremely preterm fetuses. Advances over the last 10 years have provided important preclinical evidence in lamb[7,8] and porcine[9] models to support the feasibility of this technology in clinical practice. Pioneering work by Flake *et al.*[7] • demonstrated that connection to an AP system can be achieved reproducibly, with evidence of survival periods of up to 4 weeks, along with good somatic growth and apparently normal organ development.

One of the most critical components of an AP is the extracorporeal circulation system, the aim of which should be to reproduce the physiological hemodynamic conditions of the fetoplacental circulation. Understanding the process of fetal adaptation to an AP over acute and chronic timescales is key to the successful development of this technology. Previous studies have described the hemodynamic adaptation of fetal lambs with regard to various physiological and biochemical variables, including invasive pressure measurements[7,10,11]. In these studies, values were reported on a daily basis, providing important information about fetal hemodynamics under AP support. However, there is little information available on the acute fetal hemodynamic response, that is, within the first hours after initial connection to the AP, in comparison with basal conditions. Understanding this response is essential for ensuring smooth fetal cardiovascular adaptation and long-term survival within the AP system.

The aim of this study was to describe the immediate cardiovascular adaptation of the fetus to an AP in a sheep model, by monitoring fetal intravascular pressures, blood flows and heart rate invasively, and fetal cardiac structure and function on echocardiography, *in utero* and at 5, 30 and 180 min after connection to the AP system.

METHODS

Animals

This was an experimental study in fetal Ripollesa sheep at 109–117 days' gestation (term gestation, 145 days). The study was nested within a larger AP experimental research program, and was started after an initial learning phase during which protocols for umbilical cord cannulation and connection to an extracorporeal circulation system were defined[12]. The study was conducted between September 2021 and March 2022 in 12 consecutive animals that could be cannulated and connected to the AP system without any major complication, such as air or thrombotic embolism or problems with coagulation or cannulation. Basal and postcannulation measurements were taken in all the animals. The first six consecutive fetuses were fitted with intravascular pressure catheters and perivascular flow probes, and were assigned a survival goal of 1–3 h. In this first set of fetuses, invasive hemodynamic assessment was performed *in utero* and at 5 and 30 min after cannulation, while fetal Doppler assessment and echocardiography were performed *in utero* and at 30 and 180 min after cannulation. The second set of six fetuses were not fitted with catheters, and experiments were aimed at 3–24 h of survival. In this subgroup, Doppler and echocardiography were performed in basal conditions *in utero*, and at 30 and 180 min after connection to the AP system. Figure S1 conceptualizes the experimental timeline and illustrates the data that were acquired at each phase of the study.

Animal handling and experimental procedures were performed in accordance with relevant regulation and Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines[13]. The study was approved by the Animal Experimental Ethics Committee of the University of Barcelona (67/20 P1 and 67/20 P2).

Experimental timeline

Maternal surgery and fetal invasive monitoring

Pregnant ewes were anesthetized with intramuscular administration of acepromazine (0.03 mg/kg), ketamine (5 mg/kg), midazolam (0.25 mg/kg) and buprenorphine (0.01 mg/kg), and general anesthesia was maintained with isoflurane (2–3% in oxygen/air mixture at 50% at 1 L/min) and propofol (2–4 mg/kg). Intraoperative hemodynamic monitoring included heart rate, blood pressure and oxygen saturation. A lower midline laparotomy was performed to expose the uterus. Fetal umbilical ultrasound and echocardiography were performed through the uterine wall to obtain *in-utero* imaging data. Subsequently, a small hysterotomy was performed to expose the fetal abdomen and lower extremities. Fetuses received one intramuscular dose of anesthesia (3 µg/kg fentanyl, 0.2 mg/kg midazolam and 1 mg/kg rocuronium). In the first six fetuses only, and in order to monitor continuously intravascular pressures, SPR-320 Mikro-Tip catheters (tip size, 2F; Millar Instruments, Houston, TX, USA) were inserted into the fetal arterial system (via the fetal femoral artery ($n = 3$) or insertion at the umbilical cord free loop and advancement into the intra-abdominal portion of the umbilical artery ($n = 3$)) and the umbilical vein (via insertion at the umbilical cord free loop and advancement into the intra-abdominal portion of the umbilical vein ($n = 6$)) (Figure S2). The catheters were fixed securely using purse-string sutures. In the same fashion, a snapshot of arterial and venous blood flow before connection was obtained using VeriQ™ Medistim ● perivascular transit time-flow measurement probes with a diameter of 5 or 6 mm, depending on vessel thickness. The vessels were held as steadily as possible for 10 s to ensure stable measurements. Lastly, the fetal heart rate was derived as a cyclic measurement over the arterial intravascular pressure.

Connection to AP system

Briefly, arterial/venous cannulas (12/12 Fr, 10/12 Fr or 10/14 Fr, depending on vessel size) were placed in one umbilical vein and two umbilical arteries, secured with silk 2/0 sutures and connected to the extracorporeal circulation system. Our pumpless circuit consisted of a low-resistance hollow fiber oxygenator (Quadrox-ID Neonatal Oxygenator; Maquet, Rastatt, Germany) connected to 1/4-inch inside diameter × 1/16-inch polyvinyl chloride (PVC) tubing (Sorin Group, Milan, Italy). This was an arterial-venous extracorporeal oxygenation circuit, with the two umbilical arteries merged and providing inflow to the oxygenator, whose outflow port was connected to the umbilical vein. The total priming volume of the entire circuit was 92 mL (interquartile range (IQR), 90–98 mL). Before connection, heparinized maternal blood was heated through the oxygenator at 38.3°C, and the sweep gas supplied to the oxygenator was a mixture of medical air, oxygen and nitrogen, to achieve a partial pressure of oxygen of 15–25 mmHg and a partial pressure of carbon dioxide of 35–55 mmHg in the fetal blood.

After connection, circuit postmembrane flow was measured continuously using a clamp-on ultrasound-based ME6PXL flow sensor connected to a TS410 flowmeter module (Transonic Systems Inc., Ithaca, NY, USA). The heart rate of the non-instrumented fetuses was derived from this flow signal. Similarly, circuit premembrane and postmembrane pressures were monitored continuously using physiologic pressure transducers (MLT844; ADInstruments Inc., Dunedin, New Zealand). In addition, fetal arterial and venous intravascular pressures continued to be measured throughout this phase using the Millar Mikro-Tip catheters mentioned above. After ensuring successful connection to the AP system, the fetus was submerged in a protective warm saline bath.

Signal integration

The continuous measurement of intravascular pressures and heart rate from the time of catheterization in the first six animals enabled us to capture the acute response to cannulation and connection and subsequent adaptation to the AP system. These measurements, together with the circuit flow and pre- and postmembrane pressures were integrated, recorded continuously at a sampling rate of 100 Hz, and displayed in real time during the surgical procedure and subsequent monitoring, using LabChart 8 (ADInstruments Inc.) (Figure S1).

Fetal umbilical and cardiovascular ultrasound

Comprehensive fetal umbilical ultrasound and echocardiography were obtained in 10 of the 12 animals. Imaging was performed *in utero* through the uterine wall (before catheterization and instrumentation) and at 30 and 180 min after connection to the AP during a stabilization period, using a Vivid iq (GE Healthcare, Zipf, Austria) machine equipped with a 4.5–11.5-Hz phased-array transducer. Ultrasound examinations were carried out by experts with extensive training in clinical and experimental fetal echocardiography (F.C., A.H.V., P.C.R.), with offline measurements taken by at least two examiners. We have previously reported high inter-observer reproducibility for these measures of cardiac function[14,15]. Transuterine basal echocardiography was performed after Cesarean section; following connection to the AP system, echocardiography was conducted directly in the warm protective bath through a liquid medium. All Doppler measurements were performed in the absence of fetal movements, maintaining the insonation angle as close as possible to 0° and ensuring at least three stable cycles. Frame rates ranged from 50 to 148 frames/s. A standard 6S probe (2.5–8 MHz) was used and neonatal and pediatric cardiology settings for this probe were selected on the Vivid iq machine. The various measurements were performed using EchoPAC software v.204 (GE Healthcare).

Fetoumbilical Doppler ultrasound

Umbilical artery velocities and pulsatility index (PI) were measured at the intra-abdominal (paravesical) portion of the artery. PI was calculated as (peak systolic velocity − end-diastolic velocity) / mean velocity. Measurement of the diameter and flow of the umbilical vein was performed approximately at the middle portion, between its abdominal insertion and the origin of the ductus venosus. The ultrasound beam was focused perpendicularly to the vessel wall to measure the diameter of the umbilical vein. Umbilical vein flow was calculated as (umbilical vein diameter (cm)/2)^2 × π × umbilical • vein velocity–time integral × 60), and indexed to fetal weight in kg. Ductus venosus peak velocities and PI were obtained from a transverse section of the fetal abdomen before its entrance into the inferior vena cava, with the Doppler gate positioned at the isthmic portion.

Fetal echocardiography

A complete two-dimensional (2D) echocardiographic examination was performed initially to assess structural heart integrity, followed by the measurements detailed below.

Cardiac morphometry. This included cardiac, atrial and ventricular areas, ventricular sphericity indices, relative wall thickness and aortic, pulmonary and ductus arteriosus diameters. Left and right atrial areas were delineated on 2D images in an apical four-chamber view at end-ventricular systole (maximum point of atrial distension), and indexed by cardiac area. Cardiac area, left and right ventricular areas, base-to-apex lengths and basal transverse diameters were measured on 2D images in an apical four-chamber view at end-diastole. Left and right ventricular sphericity indices were calculated as base-to-apex length/transverse basal diameter of the left and right ventricles, respectively. Ventricular end-diastolic septal and free-wall thicknesses were measured on M-mode imaging in a transverse four-chamber view. Relative wall thickness was calculated as (free wall thickness + septal wall thickness) / ventricular transverse diameter. Diameters of the aortic and pulmonary valves were measured in frozen real-time images during systole using the leading edge-to-leading edge method. Ductus arteriosus diameter was measured on a 2D image in the three-vessel view.

Systolic function. Evaluation of systolic function included shortening fraction, tricuspid and mitral annulus displacement, stroke volume, cardiac output and ejection time fraction. Left and right shortening fractions were obtained on M-mode imaging in a transverse four-chamber view, using Teicholz's formula. Tricuspid and mitral annulus displacement was measured on M-mode imaging in an apical four-chamber view by placing the cursor at right angles to the atrioventricular junction, marked by the annulus at the mitral or tricuspid valve. The maximum amplitude of motion was taken as the extent of displacement between end-systole and end-diastole, measured in mm. Aortic flow was obtained in an apical or basal five-chamber view of the heart, and pulmonary artery flow was obtained in a right ventricular outflow tract view. Velocity–time integrals were calculated by manual tracing of the spectral Doppler area. Left and right stroke volumes were calculated as π/4 × (aortic or pulmonary valve diameter)^2 × (aortic or pulmonary artery systolic flow velocity–time integral), and indexed to fetal weight in kg. Left and right cardiac outputs were calculated as left or right stroke volume × fetal heart rate. Cardiac index was calculated as the sum of cardiac outputs in both ventricles, indexed by fetal weight. The right-to-left ventricular output ratio was also calculated. Left and right ventricular ejection times were measured from pulmonary and aortic pulsed-wave Doppler systolic flow and calculated as the time interval between the opening and closure of the pulmonary or aortic valve. Cardiac cycle time was defined as the interval between two consecutive valve opening clicks. Ejection time fraction was calculated as (ejection time / cycle time) × 100.

Diastolic function. Evaluation of diastolic function included left and right peak early (E) to late (A) atrioventricular filling velocity ratio (E/A ratio) and inflow time fraction. Atrioventricular flow velocities were obtained in an apical four-chamber view, with the Doppler sample volume placed just below the valve leaflets. The presence of monophasic inflow patterns and tricuspid regurgitation was also recorded. Left and right filling times were obtained from atrioventricular filling flows and were defined as the interval between E-wave onset and A-wave termination. Filling time fraction was calculated as (filling time / cycle time) × 100.

Statistical analysis

The statistics presented in the tables were calculated using SPSS. Categorical • variables are expressed as *n* (%) and were compared using the χ-square test. Continuous variables are reported as median (IQR) and the Mann–Whitney *U*-test was used to compare values at two different time-points; $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Characteristics of animals and circuit hemodynamic data

Of the 12 fetuses included in the study, seven were female. Median gestational age was 110 days (IQR, 109–115 days), median weight was 1.62 kg (IQR, 1.34–1.79 kg) and median survival was 8.3 h (IQR, 1.8–26.5 h). An overview of fetal characteristics and circuit parameters is provided in Table 1.

Data obtained by continuous monitoring of fetal hemodynamics by AP circuit sensors are summarized

in Table 2. The heart rate increased significantly after AP connection, from a median of 143 bpm (IQR, 135–152 bpm) *in utero* to 194 bpm (IQR, 175–219 bpm) at 30 min ($P < 0.001$), and decreased moderately afterwards, although not significantly. This trend was confirmed by echocardiography and extracorporeal sensors. When comparing the situation at 30 *vs* 180 min after connection to the AP system, flow tended to decrease, although not significantly, and both the pre- and postmembrane pressures remained virtually constant.

Invasive hemodynamic monitoring

Fetal heart rate, intravascular arterial and venous pressures (mean value and PI), and perivascular and extracorporeal circuit flow, measured *in utero*, immediately after cannulation and connection to the AP system (5 min) and during early adaptation (30 min) are displayed in Table 3 and Figure 1. A detailed description of the *in-utero* data is provided in the online captions for Figures S3–S5. In the acute phase following connection to the AP system, all the fetuses systematically developed a hypertensive response, as reflected by the significant increase between the measurements taken *in utero* and at 5 min of mean arterial pressure (median, 43 mmHg (IQR, 35–54 mmHg) *vs* 72 mmHg (IQR, 61–77 mmHg); $P = 0.015$) and mean venous pressure (median, 6 mmHg (IQR, 5–7 mmHg) *vs* 10 mmHg (IQR, 8–15 mmHg); $P = 0.004$). Within 30 min after AP connection, these values tended to decrease, although not significantly.

No significant change in arterial pressure pulsatility was observed across the different experimental phases, despite a 24% reduction between the measurements taken *in utero* and at 30 min postconnection. While also non-significant, venous pressure followed an opposite trend, with a 77% increase in pulsatility at 5 min postconnection compared with *in utero*, although this returned to values similar to those registered during the *in-utero* state at 30 min. Similarly, heart rate increased significantly from baseline to 5 min after AP connection (median, 135 bpm (IQR, 131–142 bpm) *vs* 217 bpm

(IQR, 160–250 bpm); $P = 0.004$) and decreased moderately afterwards, although not significantly. Lastly, the total flow through the umbilical cord of the fetuses tended to increase after AP connection and decrease slightly afterwards, although none of these findings were found to be statistically significant (Table 3 ●). [AQ9]

An illustrative example of the changes observed in arterial and venous pressure patterns between the *in-utero* state and at 5 and 30 min after connection to the AP system is shown in Figure 1b. The changes in venous pressure pulsatility across the different phases of the experiment and shape distortion in the arterial pressure signal are evident.

Fetal umbilical and cardiovascular ultrasound

Fetal ultrasonographic and echocardiographic data obtained *in utero* and after connection to the AP system are presented in Table 4 and Figure 2. An acute fetal response to AP connection was observed, including increased peak velocity and reduced PI (diastolic velocities increased more than did systolic velocities) in the umbilical artery and ductus venosus. Median values for umbilical artery PI at baseline, 30 min and 180 min were 1.36 (IQR, 1.06–1.50), 0.38 (IQR, 0.31–0.50) and 0.36 (IQR, 0.29–0.41), respectively ($P < 0.001$ for extreme time-points). An increase in umbilical venous

Table 2 Summary of hemodynamic data obtained from continuous physiological monitoring by circuit sensors, at 30 and 180 min after connection to artificial placenta

Parameter	30 min	180 min	P
n	12	9*	—
Heart rate (bpm)	194 (175–219)†	182 (167–191)†	0.499
Flow (mL/kg/min)	181 (120–200)	154 (132–192)	0.831
Premembrane pressure (mmHg)	27 (25–35)	32 (24–35)	0.921
Postmembrane pressure (mmHg)	18 (16–21)	19 (12–25)	0.926

Data are given as median (interquartile range). *Three fetuses did not survive to 180 min. †$P < 0.001$ *vs in utero*.

Table 1 Overview of experiments

Experiment	Cannula size, French (artery/vein)	Circuit volume (mL)	Sex	GA (days)	Weight (kg)	FiO₂ (%) (range)	Sweep gas (mL/min) (range)	Survival goal (min)	Survival (min)	Invasive data	Ultrasound reported
1	12/12	NA	F	109	1.34	21–21	50–150	60–180	79	Yes	No
2	12/12	NA	F	116	2.30	21–21	200–300	60–180	79	Yes	No
3	10/12	NA	F	115	1.11	21–21	100–250	60–180	68	Yes	Yes
4	10/12	NA	F	109	1.33	14–21	200	60–180	176	Yes	Yes
5	10/12	95	F	110	1.60	21–21	100–200	60–180	140	Yes	Yes
6	10/12	100	M	115	1.71	21–21	100–225	60–180	141	Yes	Yes
7	10/12	90	M	109	1.61	14–16	200–300	180–1440	819	No	Yes
8	10/12	93	M	109	1.84	14–24	200–400	180–1440	1598	No	Yes
9	10/12	90	F	111	1.35	14–18	120–160	180–1440	1719	No	Yes
10	10/14	90	M	110	1.64	14–22	160–400	180–1440	1436	No	Yes
11	10/14	90	F	110	1.78	14–26	160–1000	180–1440	1585	No	Yes
12	10/14	133	M	117	1.80	12–22	180–400	180–1440	3038	No	Yes

F, female; FiO₂, fraction of inspired oxygen; GA, gestational age; M, male; NA, not applicable.

peak velocity was observed (median, 20 cm/s (IQR, 18–22 cm/s) *vs* 39 cm/s (IQR, 31–43 cm/s) *vs* 43 cm/s (IQR, 34–54 cm/s); $P < 0.001$ for extreme time-points), along with an increase in flow, which became more pulsatile after AP connection. Most parameters remained constant over the short-term stabilization phase.

The structure of the fetal heart was mostly preserved, with no significant changes noted in cardiac and ventricular sizes, relative wall thickness or valve diameters, with the exception of a more elongated right ventricle (increased right ventricular sphericity) and reduced left atrial area after AP connection.

Regarding function, fetal heart rate increased significantly after AP connection (median, 145 bpm (IQR, 142–156 bpm) *vs* 188 bpm (IQR, 171–209 bpm) *vs* 175 bpm (IQR, 165–190 bpm); $P = 0.001$ for extreme time-points), which, together with preserved stroke volume, led to increased cardiac output at 30 min. The ratio of right-to-left cardiac output remained stable over time. A significant increase in left and right ejection time fraction was noted after cannulation. Myocardial contractility was mainly preserved, with left ventricular shortening fraction, right ventricular fractional area change and mitral and tricuspid annulus displacement remaining similar between the *in-utero* and postcannulation states. Median values for right fractional area change were 36% (IQR, 34–41%) *in utero*, 38% (IQR, 30–40%) at 30 min and 37% (IQR, 33–40%) at 180 min ($P = 0.807$ for extreme time-points). The increased heart rate resulted in monophasic inflow patterns in 40.0% and 28.6% of animals at 30 and 180 min after AP connection, respectively. While filling time fraction tended to reduce, E/A ratios were preserved in most of the animals after AP connection. The rate of mild tricuspid regurgitation increased significantly from 50% *in utero* to 90–100% after AP connection. There was no evidence of mitral regurgitation or ductal constriction.

DISCUSSION

This study describes the acute fetal adaptive response to an AP system using invasive and non-invasive hemodynamic monitoring and ultrasonography. Connection to the AP caused a transient hemodynamic response that tended to normalize over hours. Cardiac structure and function were preserved in the short term, but the system caused non-physiologically elevated venous pressure and pulsatile flow, which should be corrected to prevent later cardiac dysfunction.

This study complements previous work describing hemodynamics and cardiac function in lamb fetuses connected to a pumpless AP, in which abundant umbilical-artery diastolic flow and fetal tachycardia with mostly preserved cardiac function over a 3-week period were reported[16]. The present study has two key contributions: it provides *in-utero* measurements that allow for intra-animal comparisons and focuses on the short-term acute hemodynamic adaptation of the fetus to AP connection. Attachment to our AP caused an acute tachycardic response that may reflect fetal stress, but is also consistent with adaptation to a low-resistance and low-compliance circuit[16]. This would explain the marked increase in umbilical venous flow and umbilical artery diastolic velocity compared with baseline. Echocardiographic findings of preserved heart structure and contractility, but elongated right ventricle, are also consistent with a fetal heart pumping against a low-resistance AP. The low resistance and compliance of the system are due to the rigidity of cannulas and PVC tubing, in contrast to the vasculature of a normal placenta. This resistance and rigidity cause arterial pulsatility to be transmitted more easily through the circuit, which affects umbilical-vein flow and leads to umbilical-vein pulsatility. Our finding of systemic hypertension is probably a consequence of three factors: the acute increase in heart rate at the time of connection, the dramatically lower compliance of the AP compared with the natural placenta and the wave reflections originating at the interface of two media (e.g. umbilical vessels, cannulas, PVC tubing, oxygenator). Further discussion of this topic can be found in our recently published simplified computational model of an AP[17]. Wave reflections may also explain the shape distortion observed in umbilical-arterial flow

Table 3 Summary of hemodynamic data obtained from invasive fetal monitoring in six fetuses, *in utero* and at 5 and 30 min after connection to artificial placenta

| Parameter	In utero	5 min	30 min	In utero vs 5 min	5 min vs 30 min	In utero vs 30 min
Heart rate (bpm)	135 (131–142)	217 (160–250)	185 (161–217)	0.004	0.454	0.009
Mean arterial pressure (mmHg)	43 (35–54)	72 (61–77)	58 (50–64)	0.015	0.087	0.132
Arterial pulsatility index	0.38 (0.34–0.45)	0.35 (0.33–0.51)	0.29 (0.23–0.37)	0.937	0.240	0.260
Mean venous pressure (mmHg)	6 (5–7)	10 (8–15)	10 (7–12)	0.004	0.615	0.024
Venous pulsatility index	0.13 (0.09–0.17)	0.23 (0.17–0.25)	0.11 (0.05–0.18)	0.180	0.169	0.660
Delta pressure (mmHg)*	37 (29–49)	59 (47–69)	48 (37–57)	0.065	0.240	0.310
Flow (mL/kg/min)†	171 (132–201)	201 (117–235)	120 (99–138)	0.937	0.418	0.240

Data are given as median (interquartile range). Given the pulsatile and rapidly changing nature of the described signals, data reported correspond to values averaged over 1-min timespan. *Mean arterial pressure – mean venous pressure. †Flow *in utero* was measured using perivascular flowmeter, directly on umbilical vein; flow after connection to artificial placenta system was derived non-invasively from extracorporeal circuit and was included for sake of comparison. Flow was indexed by fetal weight.

and pressures (Figure 1b). Additionally, the amount of reflection and the position of measurement along the umbilical vessel affect recorded flows[16], which may explain the difference in flow as measured on echocardiography and by sensors within the AP circuit.

In summary, a low-resistance and low-compliance system may explain the increase in intravascular pressures, heart rate, cardiac output and blood flow. Cardiac contractility was mostly preserved, with increased cardiac output and fusion of inflow waveforms probably
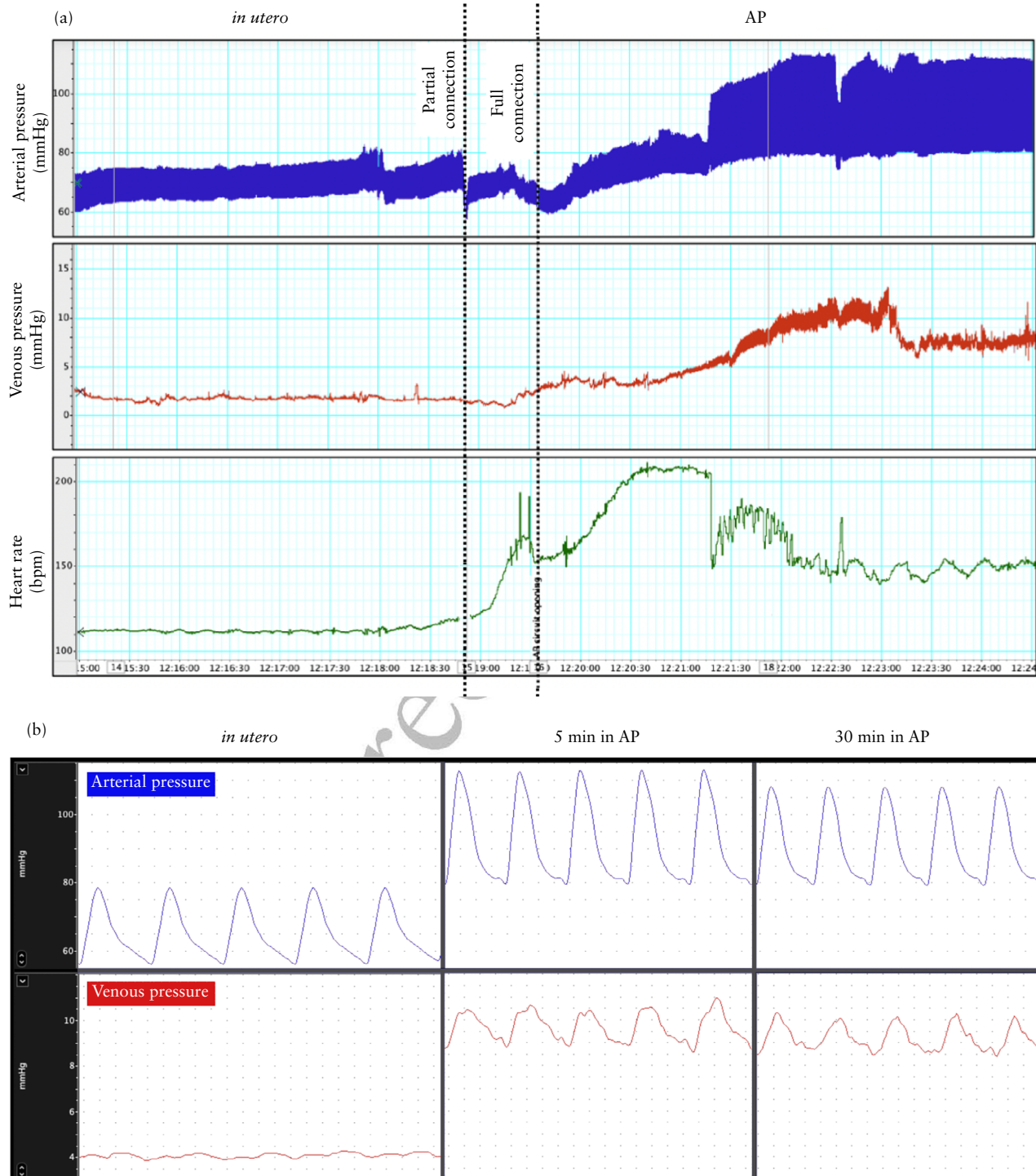
Figure 1 Illustrative examples of traces obtained from continuous invasive monitoring of fetal arterial and venous intravascular pressures (a,b) and heart rate (a) during *in-utero* period, after connection to artificial placenta (AP) and during early adaptation to AP system. Arterial traces are depicted in blue to represent deoxygenated blood and venous traces in red to represent oxygenated blood, in line with previous publications. Changes in venous pressure pulsatility and shape distortion in arterial pressure signal across different phases of experiment are evident.

explained by increases in heart rate. Monophasic inflows suggest mildly impaired diastolic function with reduced filling time fraction and minor tricuspid regurgitation, which could lead to diastolic dysfunction in the long term. Over 180 min, no obvious diastolic dysfunction was observed and the acute response tended to normalize, as reflected by the decrease in heart rate and cardiac output. However, increased umbilical venous flow with a marked increase in pulsatility and pressure remained, which should be controlled to avoid diastolic dysfunction over subsequent days. The measured heart rate, cardiac output, umbilical Doppler parameters and circuit flow were comparable with those reported previously[16]. The tachycardic response, a common finding in our and previous AP models[7,8,10], may be reduced by adapting the model to counteract the low resistance.

Table 4 Fetoumbilical Doppler and fetal echocardiographic measurements obtained *in utero* and at 30 and 180 min after connection to artificial placenta

| | | | | | P | |
| | | | | In utero vs | 30 min vs | In utero vs |
Parameter	In utero *(n = 10)*	30 min (n = 10)	180 min (n = 7)	30 min	180 min	180 min
Fetoumbilical Doppler						
UA pulsatility index	1.36 (1.06–1.50)	0.38 (0.31–0.50)	0.36 (0.29–0.41)	< 0.001	0.493	< 0.001
UA peak systolic velocity (cm/s)	57 (49–60)	64 (51–83)	74 (68–94)	0.21	0.11	0.001
UA peak diastolic velocity (cm/s)	18 (11–20)	44 (32–62)	55 (47–69)	< 0.001	0.13	< 0.001
UV pulsatility index	0.20 (0.14–0.24)	0.45 (0.31–0.54)	0.45 (0.41–0.57)	< 0.001	0.591	< 0.001
UV peak velocity (cm/s)	20 (18–22)	39 (31–43)	43 (34–54)	< 0.001	0.379	< 0.001
UV flow (mL/min/kg)*	229 (198–258)	269 (230–327)	296 (263–316)	0.08	0.770	0.05
DV pulsatility index	0.50 (0.41–0.67)	0.29 (0.22–0.33)	0.36 (0.22–0.41)	< 0.001	0.384	0.011
DV peak systolic velocity (cm/s)	59 (54–70)	85 (73–92)	83 (65–91)	0.001	0.587	0.018
Fetal echocardiography						
Fetal cardiac morphometry						
Cardiac area (mm²/kg)*	880 (767–947)	880 (735–967)	769 (730–880)	0.940	0.204	0.143
RA area/cardiac area (%)	10 (8–13)	9 (7–10)	10 (7–11)	0.131	0.626	0.329
RV area/cardiac area (%)	19 (18–22)	16 (16–21)	17 (16–20)	0.082	0.495	0.067
RV sphericity index	2.0 (1.8–2.3)	2.3 (2.2–2.7)	2.2 (1.9–2.6)	0.038	0.696	0.242
Right relative wall thickness	0.91 (0.80–1.00)	0.99 (0.75–1.15)	0.87 (0.75–1.15)	0.594	0.625	0.796
Pulmonary artery diameter (cm)	0.67 (0.61–0.70)	NA	NA	—	—	—
Ductus arteriosus diameter (cm)	0.49 (0.42–0.50)	0.44 (0.42–0.50)	0.45 (0.39–0.50)	0.207	0.961	0.323
LA area/cardiac area (%)	17 (14–19)	17 (13–17)	15 (12–16)	0.08	0.143	0.032
LV area/cardiac area (%)	25 (21–28)	24 (20–25)	25 (24–27)	0.11	0.464	0.435
LV sphericity index	2.0 (1.8–2.2)	2.1 (1.8–2.2)	2.0 (1.8–2.2)	0.364	0.526	1
Left relative wall thickness	0.91 (0.80–1.00)	0.99 (0.75–1.15)	0.87 (0.75–1.15)	0.594	0.625	0.796
Aortic diameter (cm)	0.59 (0.56–0.60)	NA	NA	—	—	—
Fetal systolic function						
Fetal heart rate (bpm)	145 (142–156)	188 (171–209)	175 (165–190)	< 0.001	0.327	0.001
RV fractional area change (%)	36 (34–41)	38 (30–40)	37 (33–40)	0.596	0.769	0.807
Tricuspid ring displacement (mm)	5.2 (4.9–5.4)	4.9 (4.8–5.1)	4.9 (4.7–5.0)	0.147	0.620	0.077
Right ejection time fraction (%)	44 (39–49)	51 (47–53)	50 (48–50)	0.018	0.696	0.101
Right stroke volume (mL/kg)*	1.8 (1.6–2.0)	1.6 (1.5–2.0)	1.6 (1.4–1.7)	0.142	0.558	0.064
Right cardiac output (mL/kg/min)*	272 (239–296)	336 (272–356)	280 (244–348)	0.05	0.283	0.491
LV shortening fraction (%)	41 (40–44)	38 (36–39)	39 (37–40)	0.08	0.546	0.06
Mitral ring displacement (mm)	5.0 (4.8–5.6)	5.1 (4.9–5.8)	4.8 (4.2–5.0)	0.84	0.069	0.104
Left ejection time fraction (%)	44 (39–47)	50 (46–52)	49 (44–52)	0.007	0.770	0.03
Left stroke volume (mL/kg)*	1.22 (1.16–1.29)	1.19 (1.04–1.48)	1.03 (0.95–1.06)	0.94	0.097	0.04
Left cardiac output (mL/kg/min)*	179 (173–192)	222 (190–273)	172 (165–201)	0.007	0.032	0.558
Right-to-left output ratio	1.49 (1.28–1.57)	1.43 (1.12–1.47)	1.63 (1.21–1.68)	0.41	0.097	0.368
Cardiac index (mL/kg/min)*	446 (425–481)	549 (461–655)	447 (436–547)	0.022	0.079	0.711
Diastolic function						
Tricuspid inflow monophasic	0 (0)	4 (40.0)	2 (28.6)	0.03	0.09	0.037
Tricuspid E/A ratio	0.85 (0.76–1.00)	0.80 (0.72–1.00)	0.67 (0.65–0.80)	0.495	0.059	0.03
Right inflow time fraction (%)	45 (43–48)	42 (38–48)	43 (36–50)	0.568	0.596	0.792
Mild tricuspid regurgitation	5 (50.0)	9 (90.0)	7 (100)	0.012	0.124	0.012
Mitral inflow monophasic	0 (0)	4 (40.0)	2 (28.6)	0.03	0.09	0.037
Mitral E/A ratio	0.65 (0.57–0.73)	0.72 (0.65–0.79)	0.81 (0.77–0.87)	0.193	0.067	0.05
Left inflow time fraction (%)	53 (42–56)	44 (34–46)	42 (37–47)	0.02	0.626	0.05

Data are given as median (interquartile range) or *n* (%). *Indexed by fetal weight. A, atrial contraction; DV, ductus venosus; E, early diastole; LA, left atrium; LV, left ventricle; NA, not applicable; RA, right atrium; RV, right ventricle; UA, umbilical artery; UV, umbilical vein.

Importance of study

This study helps our understanding of the impact of an AP on fetal hemodynamics. After considerable training, a stable connection of the umbilical circulation to an AP system is attainable. The next step is to achieve near-physiological placental circulation, which is extremely challenging using the available circuitry adapted from extracorporeal membrane oxygenation (ECMO) systems. Modeling short- and long-term fetal hemodynamic adaptation is critical to identifying the circuit requirements for maintaining a fetus in near-physiological conditions in an AP. This study demonstrates that a commercial ECMO oxygenator results in a low-resistance, high-volume and high-pressure situation that may result eventually in diastolic heart failure and cardiac remodeling. Improvements in the circuitry and oxygenators used should aim at reducing this high-flow low-resistance situation, with refinement guided by comprehensive fetal cardiac evaluation.

Limitations

One limitation of this study is that the complexity of the surgical procedure resulted in a small sample size. Moreover, catheterization may have affected the accuracy of reported pressures and flows and hindered comparison between catheterized and non-catheterized animals. Maternal and fetal anesthesia may have interacted with heart rate. Echocardiography was only reported for 10 out of 12 fetuses, owing to poor image quality for the catheterized animals. The acute cardiovascular response to AP connection is likely to be influenced by altered loading conditions, but also by other factors related to fetal stress, such as surgery, fetal manipulation,

anesthesia and external stressors of the transition process. Given the small numbers reported, we could not assess the effects of these factors. Although the experiments reported were performed after the investigators had undergone considerable training, we ● cannot rule out the possibility that performance improved over time and influenced some observations. Thus, the immediate adaptation described in the present study might not be of long-standing significance. Further research is needed to fully understand this response mechanism over longer observation periods, and should include assessment of the effects on brain circulation. Furthermore, cannula and circuit sizes were not identical among experiments, which may have impacted on some measurements. Flow was measured in the vessel prior to connection and in the circuit tubing after connection. Both measurement techniques should yield similar results for laminar flow[18], typical in the umbilical vein; however, caution should be taken when comparing flow *in utero* and under AP support. Lastly, median gestational age was 110 days and median fetal weight was 1.62 kg. Ideally, these experiments should be conducted in fetuses of 90 days' gestation and 600 g in weight, which are more similar in size and maturity to clinical conditions ●.

In conclusion, sheep fetuses connected to an AP system display an acute adaptive response to a low-resistance circuit, which tends to normalize over hours, but persists in the form of non-physiologically elevated venous pressure and pulsatile flow. The circuitry and oxygenators in AP systems should be adapted to avoid later cardiac dysfunction. Future studies are warranted to assess cardiac function and remodeling over longer observation periods, after modifying the AP system to mimic more closely the physiological features of the placental circulation.
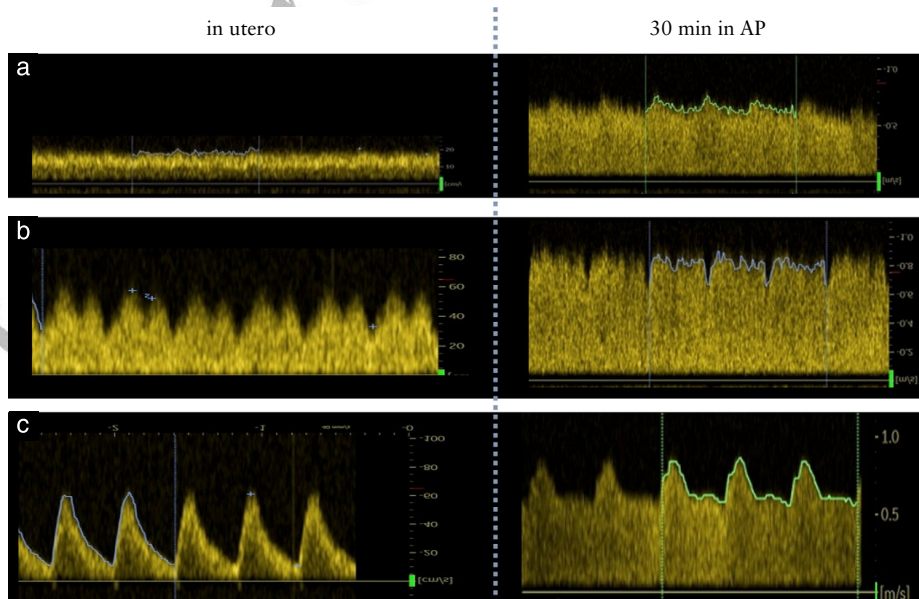
Figure 2 Illustrative examples of umbilical vein (a), ductus venosus (b) and umbilical artery (c) Doppler traces obtained *in utero* and 30 min after connection to artificial placenta (AP).

COLLABORATORS

Aleix Garcia, BCNatal Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Universitat de Barcelona, Barcelona, Spain

Juan Medina, BCNatal Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Universitat de Barcelona, Barcelona, Spain

Miguel Morán, BCNatal Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu), Universitat de Barcelona, Barcelona, Spain

Victor Gómez, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain

Marina Chorda, BCNatal Fetal Medicine Research Center (Hospital Clínic and Hospital Sant Joan de Déu) and Hospital Clinic, Universitat de Barcelona, Barcelona, Spain

Mireia Gispert, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain

Maite Mata, Hospital Clinic, Universitat de Barcelona, Barcelona, Spain

Joan Sanchez de Toledo, Pediatric Cardiology Department, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain

Daniel Pereda, Cardiovascular Surgery Department, Hospital Clínic, Universitat de Barcelona, Barcelona, Spain

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SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

Figure S1 Timeline of physiological monitoring and echocardiography in 12 sheep. Solid colored cells indicate continuous measurement, dots indicate discretely acquired data and blank cells indicate absence of data collection during period of interest.

Figure S2 Photographs of Millar Mikro-Tip catheter insertion into fetal femoral artery (a,b) or free loop of umbilical cord (c).

Figure S3 Summary of *in-utero* umbilical artery and vein intravascular pressures and perivascular flows measured in first six lambs of study over representative three cardiac cycles at baseline. ONLINE VERSION: Summary of *in-utero* umbilical artery and vein intravascular pressures (left panel) and perivascular flows (right panel) measured in first six lambs of study over three representative cardiac cycles at baseline. Solid line indicates sample-wise median and shading represents corresponding interquartile range (IQR). Systolic portion of arterial pressure peaks at median value of 55 (IQR, 42–68) mmHg, and minimum value reached during diastole is 41 (IQR, 25–50) mmHg. Conversely, venous pressure depicts a quasi-continuous waveform centered at 7.1 (IQR, 5.8–7.4) mmHg, with peak-to-peak absolute variation below 0.2 mmHg. Similarly, arterial systolic flow peaks early in the cardiac cycle, with median values around 160 (IQR, 120–195) mL/kg/min, followed by a residual flow rate driven by inertia which steadily decreases until reaching minimum of 40 (IQR, 35–60) mL/kg/min right before start of next cycle. Hosting on average the same amount of flow, umbilical veins portray an almost continuous/laminar flow centered at 78 (IQR, 58–95) mL/kg/min, with much-dampened pulsatility compared with that of the arteries, as a result of compliant properties of placenta. Data were calculated and plotted using MATLAB version 2021a (Mathworks, Natick, MA, USA).

Figure S4 Illustration of three different scenarios that may appear when assessing amount of flow carried by each artery/vein pair. ONLINE VERSION: Illustration of three different scenarios that may appear when assessing amount of flow carried by each artery/vein pair. (a,b,c) All arteries and veins carry very similar amount of blood. (d) Amount of blood carried is symmetric within each artery/vein pair, but one pair carries significantly less blood. (e,f) Amount of blood carried is similar between two arteries and between two veins, but asymmetric within each artery/vein pair.

Figure S5 Averaged profiles of fetal heart rate, intravascular pressures and circuit flow for first six fetuses of study, over a timespan ranging from baseline state at 5 min before cannulation to 30 min after connection to artificial placenta (AP) system. ONLINE VERSION: Averaged profiles of fetal heart rate, intravascular pressures and circuit flow for first six fetuses of study, over a timespan ranging from baseline state at 5 min before cannulation to 30 min after connection to artificial placenta (AP) system. Time = 0 min indicates connection to AP system. Consistently across experiments, arterial pressure and heart rate increase steadily minutes before connection, reaching their maximum value within first 5 min after connection. Venous pressure shows a similar but less pronounced trend. After connection, circuit flow increases steadily, peaking within the 30-min timespan before decreasing to a stable level.