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Original Article

Effect of Sildenafil on Pulmonary Circulation and Cardiovascular Function in Near-Term Fetal Sheep During Hypoxemia

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Abstract

Sildenafil is a potential new treatment for placental insufficiency in human pregnancies as it reduces the breakdown of vasodilator nitric oxide. Pulmonary vasodilatation is observed in normoxemic fetuses following sildenafil administration. Placental insufficiency often leads to fetal hypoxemia that can cause pulmonary vasoconstriction and fetal cardiac dysfunction as evidenced by reduced isovolumic myocardial velocities. We tested the hypotheses that sildenafil, when given directly to the hypoxemic fetus, reverses reactive pulmonary vasoconstriction, increases left ventricular cardiac output by increasing pulmonary venous return, and ameliorates hypoxemic myocardial dysfunction. We used an instrumented sheep model. Fetuses were made hypoxemic over a mean (standard deviation) duration of 41.3 (9.5) minutes and then given intravenous sildenafil or saline infusion. Volume blood flow through ductus arteriosus was measured with an ultrasonic transit-time flow probe. Fetal left and right ventricular outputs and lung volume blood flow were calculated, and ventricular function was examined using echocardiography. Lung volume blood flow decreased and the ductus arteriosus volume blood flow increased with hypoxemia. There was a significant reduction in left ventricular and combined cardiac outputs during hypoxemia in both groups. Hypoxemia led to a reduction in myocardial isovolumic velocities, increased ductus venosus pulsatility, and reduced left ventricular myocardial deformation. Direct administration of sildenafil to hypoxemic fetus did not reverse the redistribution of cardiac output. Furthermore, fetal cardiac systolic and diastolic dysfunction was observed during hypoxemia, which was not improved by fetal sildenafil treatment. In conclusion, sildenafil did not improve pulmonary blood flow or cardiac function in hypoxemic sheep fetuses.

Keywords

hypoxemia, fetal, pulmonary circulation, sildenafil

Introduction

The fetal pulmonary vasculature becomes responsive to oxygen with advancing gestational age.[1] The pulmonary circulation responds to a decrease in partial pressure of O_2 (pO_2) by vasoconstriction and reduced lung volume blood flow.[2,3] There is also peripheral vasoconstriction and centralization of the blood flow.[4,5] Fetal hypoxemia is often found in placental insufficiency.[6] Hypoxemia, particularly when associated with acidemia, affects fetal cardiac function.[7-9]

In normal pregnancy, the vasodilator nitric oxide contributes to the vasodilation and reduced vascular resistance seen in the uteroplacental circulation.[10,11] The nitric oxide second messenger cyclic guanosine monophosphate is enzymatically degraded by phosphodiesterases.[12] Sildenafil citrate (sildenafil), an inhibitor of phosphodiesterase-5, is able to enhance the vasodilatory action of nitric oxide.[12] It has been reported to increase placental blood flow and reduce the severity of fetal growth restriction.[13] The pulmonary circulation is responsive

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to sildenafil in normoxemic foetuses.[14] Sildenafil infusion to the fetus led to 60% reduction in pulmonary vascular resistance in normoxemic fetuses in the sheep model.[14] The effect of sildenafil on pulmonary circulation in the presence of hypoxemia or its effect on hypoxemia-induced cardiac dysfunction is not known.

We reasoned that hypoxemia-induced pulmonary vasoconstriction will lead to a reduction in volume blood flow to the lungs and consequently pulmonary venous return to the left atrium. This could potentially be compensated by increased shunting across the foramen ovale. However, fetal ability to increase foramen ovale shunting is limited.[15] Therefore, left ventricular (LV) output could be maintained only if pulmonary volume blood flow could be increased by reversing hypoxemia-induced pulmonary vasoconstriction using sildenafil. Furthermore, experimental studies in the chick embryo have shown that sildenafil prevents the increase in phosphodiesterase expression, protects against oxidative stress, and normalizes nitric oxide bioavailability in the fetal heart in chronic hypoxemic environment.[16]

We hypothesized that administration of sildenafil to the hypoxemic fetus reverses pulmonary vasoconstriction, maintains LV cardiac output, and ameliorates myocardial dysfunction. The first aim of our study was to examine whether sildenafil, when given directly to the fetus, can at least partially relieve reactive pulmonary vasoconstriction in a hypoxemic fetus. This could potentially increase lung volume blood flow and LV output that is crucial to maintain adequate cerebral volume blood flow and perfusion pressure. Second aim was to assess the effects of sildenafil on right ventricular (RV) and LV function in hypoxemic fetus.

Methods

The study protocol was reviewed and approved by the National Animal Experiment Board of Finland (ESAVI/1007/04.10.07/2014). The animal care and experimental procedures were conducted according to the national legislation and the EU Directive 2010/63/EU.[17] A total of 24 pregnant sheep underwent surgery for fetal instrumentation at 128 (standard deviation [SD] = 2.9) days' gestation. The details of the surgical procedure have been previously described.[8] In brief, pregnant sheep were operated under general anesthesia. Induction of anesthesia was achieved with intravenous propofol (4-7 mg/kg) and maintenance with isoflurane (1.5%-2.5%) in an oxygen–air mixture delivered via an endotracheal tube. Intravenous boluses of fentanyl (0.05-0.15 mg) were administered as required. A midline laparotomy was performed, and the fetal head and neck were delivered through a small hysterotomy incision. Catheters were introduced into the internal jugular vein and the carotid artery to access venous and arterial circulations and to collect blood samples. A small left lateral thoracotomy was performed at the level of third intercostal space, and the ductus arteriosus was identified and dissected. A 4.0-mm ultrasonic transit-time flow probe was secured around the vessel for the measurement of ductus arteriosus volume blood flow. The thoracotomy was closed. Electrocardiogram leads were placed under the skin and securely attached on the fetal chest using sutures. A separate catheter was placed in the amniotic cavity to measure intra-amniotic pressure. After the replacement of amniotic fluid by 0.9% warm saline and closure of the surgical wounds, all catheters and cables were tunneled subcutaneously and exteriorized through a small skin incision in the ewe's flank.

After a 5-day recovery period, experiments were performed under general anesthesia as described above. The mean (SD) gestational age was 132 (3.3) days. Fetal systemic venous and carotid artery blood pressure and arterial blood gas values were monitored. Two-dimensional echocardiography was performed using a Vivid 7 ultrasound system (GE Medical Systems, Horten, Norway) with a 10-MHz phased-array transducer. Cine loops of high-resolution cardiac images of the longitudinal and cross-sectional views of the RV and LV were recorded at a high frame rate (more than 150 frames/s) and LV and RV global longitudinal strains were measured off-line by speckle tracking using acoustic-tracking software (EchoPAC; GE Medical Systems). The ductus arteriosus volume blood flow (Q_{DA}) was directly measured by ultrasonic transit-time flow probe. Fetal right ventricular cardiac output (RVCO) and left ventricular cardiac output (LVCO) were calculated using 2-dimensional ultrasound and pulsed-wave Doppler techniques to measure aortic and pulmonary valve diameters and blood flow velocity waveforms as described.[1] Lung volume blood flow (Q_P) was calculated by subtracting Q_{DA} from RVCO. Right pulmonary artery pulsatility index (RPA PI) was calculated from its Doppler blood flow velocity waveform.[1] Tissue Doppler technique was used to record LV and RV lateral wall movement at the atrioventricular valve level during the cardiac cycle.[18] Isovolumic contraction velocity (IVCV) and isovolumic relaxation velocity (IVRV) of the RV- and LV-free wall were measured.[18] The PI of the ductus venosus (DV) was calculated from its blood flow velocity waveform. The analyses of the ultrasound recordings were performed by examiners blinded to the allocation of intervention. Systemic volume blood flow was calculated by subtracting Q_P from combined cardiac output. Systemic vascular resistance was calculated by dividing the mean arterial pressure (MAP, mm Hg) by systemic volume blood flow (mL/min), indexed to fetal weight (kg), and expressed as mm Hg·min/mL/kg.[19]

Fetal cardiac systolic function was assessed by tissue Doppler-derived IVCV and myocardial strain using speckle tracking. Diastolic function was evaluated using tissue Doppler-derived IVRV and DV PIV. The ultrasound examination was repeated during each phase of the experiment to collect data. The still images and video clips of the ultrasound examination were stored digitally. Using off-line analysis, an observer (LA or AB) blinded to group allocation calculated volume flows as well as other echocardiographic measurements, such as myocardial isovolumic velocities and strain.

After baseline data collection, maternal hypoxemia was induced by connecting the ewe to a rebreathing circuit, thereby reducing the maternal fraction of inspired O_2 (FiO_2) to reach

the oxygen saturation level of 80% over a mean (SD) duration of 41.3 (9.5) minutes. This was verified by maternal arterial blood gas analyses. The blood gas values (corrected for 39°C) were analyzed just before ultrasonographic data collection at each study point (Abbott – iSTAT 1, East Windsor, New Jersey). The hypoxemia phase data were collected 30 minutes after desired maternal oxygen saturation level was reached. Thereafter, 12 fetuses were allocated to receive sildenafil infusion into the internal jugular vein (sildenafil citrate 0.8 mg/mL) that was diluted 1:1 in saline and infused at a rate of 2.5 mL/h (1.0 mg/h) and 12 fetuses receiving saline infusion were used as controls, respectively. Data were collected at 30 and 120 minutes following commencement of infusion. After hypoxemia + 120 minutes infusion data collection was completed, maternal hypoxemia was reversed while infusion was continued. Recovery phase data were collected at 30 minutes of maternal normoxemia was achieved. The animals were euthanized at the end of the experiment with an intravenous overdose (1 mg/kg) of pentobarbital sodium to the ewe. Fetal weights were determined postmortem.

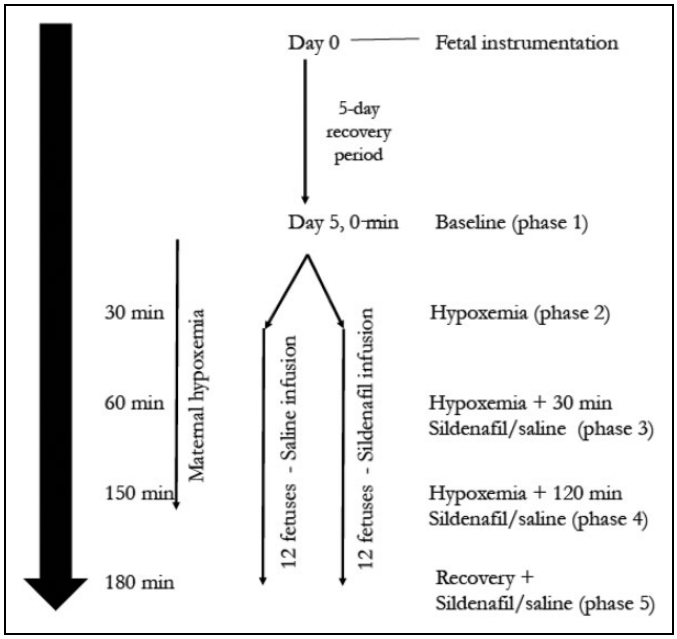

Figure 1. Timeline of the experiment.

Statistics

The data were expressed as mean (SD) unless stated otherwise. Linear mixed model (LMM) was used for repeatedly measured data. The phase of the experiment and sildenafil treatment (vs saline) was included as fixed effects, an interaction term, and individual fetus as random intercept. If LMM showed a significant difference between measurement points (P [time] < .05), then a pairwise comparison between relevant points was performed. The difference between the 2 groups was expressed as P (group). The 2 groups may not show similar changes with time (interaction term). Therefore, this was expressed as P (group × time). The LMM can be applied even when there are missing data. Not all fetuses survived the whole experiment, and there were missing values. Hence, LMM was particularly suitable for this experiment. Statistical analyses were

Table 1. Maternal and Fetal Parameters During the Experiment.

Parameter	Group	Baseline	Hypoxemia	Hypoxemia + Infusion (30 minutes)	Hypoxemia + Infusion (120 minutes)	Recovery + Infusion	*P* (Time)	*P* (Group)	*P* (Time × Group)
Maternal pO_2 (mm Hg)	Control	168 (47.3)	48 (9.0)	45 (8.3)	44 (5.5)	133 (36.0)	**<.005**	.178	.141
	Sildenafil	164 (75.0)	48 (5.6)	47 (4.4)	44 (5.6)	89 (32)			
Maternal pCO_2 (mm Hg)	Control	37 (5.7)	35 (3.2)	35 (3.1)	35 (4.1)	41 (3.1)	**<.005**	**.004**	.169
	Sildenafil	42 (3.8)	39 (3.9)	38 (4.0)	40 (4.3)	41 (4.8)			
FHR (beats/min)	Control	173 (32.1)	170 (27.9)	177 (27.5)	171 (19.4)	158 (23.1)	**.011**	.506	.671
	Sildenafil	171 (30.8)	174 (24.3)	165 (20.5)	157 (20.1)	146 (20.6)			
MAP (mm Hg)	Control	49.1 (10.2)	42.7 (10.7)	47.0 (15.3)	46.3 (10.2)	50 (10.8)	**.001**	.201	**.042**
	Sildenafil	45.4 (9.8)	42.9 (10.7)	40.2 (9.0)	35.3 (3.5)	36.4 (4.4)			
Fetal CVP (mm Hg)	Control	3.5 (5.2)	3.4 (3.7)	3.0 (4.5)	2.2 (5.6)	2.0 (3.4)	.774	.96	.144
	Sildenafil	2.5 (1.7)	2.7 (2.9)	0.67 (1.9)	3.5 (3.0)	3.4 (2.5)			
Fetal arterial pH	Control	7.32 (0.05)	7.30 (0.03)	7.21 (0.06)	7.15 (0.12)	7.18 (0.06)	**<.001**	**.046**	.378
	Sildenafil	7.25 (0.04)	7.25 (0.05)	7.19 (0.08)	7.06 (0.16)	7.09 (0.16)			
Fetal arterial pO_2 (mm Hg)	Control	21 (2.5)	12 (2.9)	12 (2.9)	11 (1.1)	21 (2.9)	**<.001**	.306	**.004**
	Sildenafil	21 (6.2)	13 (1.7)	11 (2.0)	11 (3.5)	15 (5.2)			
Fetal arterial pCO_2 (mm Hg)	Control	49 (7.6)	50 (3.5)	52 (6.1)	52 (7.3)	51 (3.4)	**.004**	**.003**	.136
	Sildenafil	55 (8.6)	55 (7.9)	58 (7.7)	66 (17.7)	57 (8.4)			
Fetal arterial base excess (mM/L)	Control	−1.70 (2.51)	−2.36 (2.92)	−7.00 (5.10)	−10.18 (6.01)	−9.00 (3.57)	**<.001**	.584	.729
	Sildenafil	−3.75 (3.28)	−3.17 (2.89)	−6.08 (4.60)	−11.36 (7.65)	−10.56 (7.89)			
Fetal arterial lactate (mM/L)	Control	4.03 (1.77)	4.03 (1.77)	7.69 (3.52)	9.73 (4.01)	9.59 (4.06)	**<.001**	.968	.262
	Sildenafil	3.62 (1.88)	3.62 (1.88)	6.43 (1.88)	10.33 (4.02)	10.82 (4.56)			

Abbreviations: CVP, central venous pressure; FHR, fetal heart rate; MAP, mean arterial pressure.
Note: Significant results are indicated in bold.

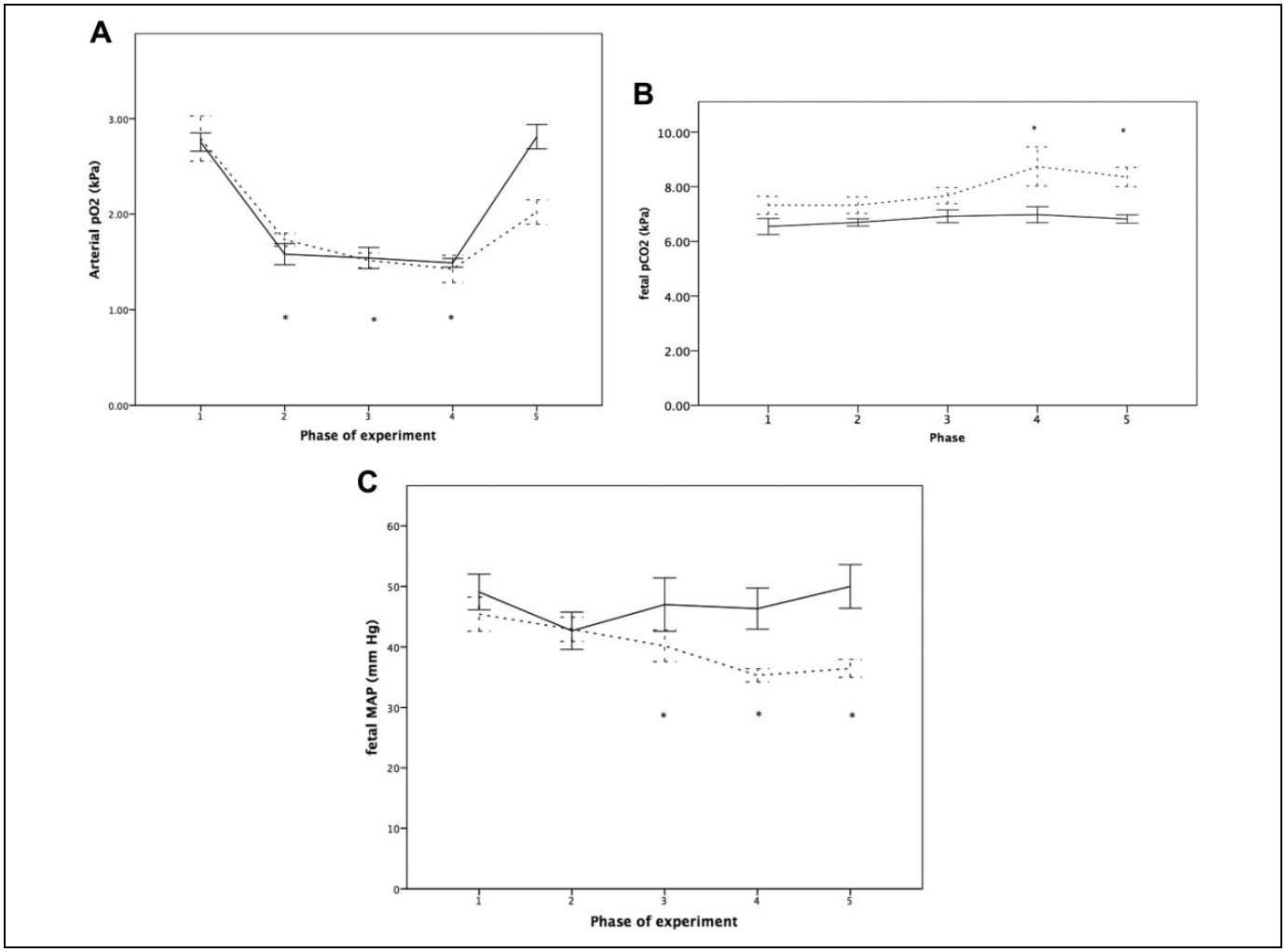

Figure 2. Fetal pO_2 (A), pCO_2 (B), and mean arterial pressure (C) during the experiment. Solid line represents saline and dashed line as sildenafil. Phase 1: baseline; phase 2: hypoxemia; phase 3: hypoxemia + 30 minutes infusion of saline or sildenafil; phase 4: hypoxemia + 120 minutes infusion of saline or sildenafil; phase 5: normoxemia + infusion of saline or sildenafil. A, *denotes significantly difference as compared to baseline and recovery + infusion phase in both groups. Significant difference between sildenafil and saline group ($P < .005$) in the recovery + infusion phase. B, *denotes significantly higher as compared to baseline ($P = .001$) and hypoxemia phase ($P = .003$) only in the sildenafil group. C, *denotes significantly lower as compared to baseline ($P = .012$) only in the sildenafil group.

performed using SPSS (IBM Corp, Released 2011, IBM SPSS Statistics for Windows, version 20.0; IBM Corp, Armonk, New York) and SAS (version 9.3; SAS Institute Inc, Cary, North Carolina). A 2-tailed P value <.05 was considered statistically significant.

Results

Maternal weight, heart rate, and blood pressure values in the sildenafil and control groups were comparable during the entire experiment ($P > .05$ for all; data not shown). Figure 1 shows the timeline of the experiment. Maternal pO_2 at baseline was significantly higher ($P < .005$) than all other experimental phases (Table 1). Maternal partial pressure of CO_2 (pCO_2) at baseline and recovery were significantly different than all other phases.

Mean (SD) fetal weight was 2.44 (0.28) kg and 2.38 (0.34) kg in the control and sildenafil groups, respectively. There was no significant difference between the gestational age of the 2 groups ($P = .345$).

The fetal heart rate in the recovery + infusion phase was significantly lower compared to baseline in both the groups ($P = .002$). As expected, fetal pO_2, pH, and base excess decreased progressively during the experiment (Table 1). In the recovery + infusion phase, fetal pO_2 remained significantly lower in the sildenafil group when compared to the control group (Table 1, Figure 2A). In the sildenafil group, fetal pCO_2 in hypoxemia + 120 minutes infusion phase and recovery + infusion phase was significantly higher as compared to the control group (Table 1, Figure 2B). Fetal lactate progressively increased with no difference between the groups. In the sildenafil group, fetal MAP was significantly lower in hypoxemia +

Table 2. Fetal Cardiac Output, Pulmonary Flow, and Ductal Flow During the Experiment.

Parameter	Group	Baseline	Hypoxemia	Hypoxemia + Infusion (30 minutes)	Hypoxemia + Infusion (120 minutes)	Recovery+ Infusion	*P* (Time)	*P* (Group)	*P* (Time × Group)
RV cardiac output (mL/min/kg)	Control	255.2 (63.8)	258.5 (111.8)	242.1 (89.5)	258.8 (70.0)	248.4 (54.1)	.209	.474	.779
	Sildenafil	288.1 (114.6)	270.1 (106.7)	282.7 (119.8)	260.5 (123.4)	259.5 (109.7)			
LV cardiac output (mL/min/kg)	Control	255.5 (77.7)	208.9 (55.6)	233.9 (42.5)	220.8 (53.3)	222.6 (65.4)	**.001**	.376	.646
	Sildenafil	235.9 (103.7)	209.3 (84.4)	197.1 (54.0)	164.6 (27.3)	166.9 35.5)			
Combined cardiac output (mL/min/kg)	Control	510.7 (115.4)	462.9 (126.2)	474.8 (124.4)	475.5 (99.1)	467.9 (85.6)	**.008**	.956	.979
	Sildenafil	524.0 (139.8)	479.4 (114.1)	479.8 (111.1)	425.1 (112.8)	426.3 (103.5)			
Ductal flow (mL/min/kg)	Control	102.0 (28.7)	165.3 (27)	170.0 (26.6)	155.5 (29.4)	146.8 (40.7)	**.003**	.169	.983
	Sildenafil	90.4 (37.6)	149.8 (42.3)	157.5 (86.9)	124.7 (62.4)	136.4 (61.3)			
Pulmonary blood flow (mL/min/kg)	Control	191.8 (60.6)	139.0 (121.3)	96.5 (103.5)	129.0 (85.3)	125.6 (74.6)	**.001**	.872	.854
	Sildenafil	197.7 (129.0)	120.3 (112.2)	125.2 (117.8)	128.3 (98.3)	114.1 (81.7)			
Right pulmonary artery PI (median [IQR])	Control	12.9 (4.8-40.1	40.2 (26.8-61.7)	52.2 (38.4-94.4)	51.3 (29.6-75.5)	45.7 (30.7-112.7)	**<.005**[a]	.172	.884
	Sildenafil	12.2 (6.8-25.9)	33.1 (30.2-52.6)	60.0 (26.3-72.8)	34.4 (15.5-76.6)	47.8 (24.7-89.6)			
Systemic vascular resistance (mm Hg·min/mL/kg)	Control	0.106 (0.027)	0.095 (0.028)	0.089 (0.015)	0.088 (0.023)	0.109 (0.020)	**.027**	.513	.078
	Sildenafil	0.130 (0.038)	0.104 (0.021)	0.097 (0.030)	0.096 (0.016)	0.095 (0.023)			

Abbreviations: LV, left ventricular; PI, pulsatility index; RV, right ventricular.
[a]Right pulmonary artery PI was log transformed for statistical analysis.

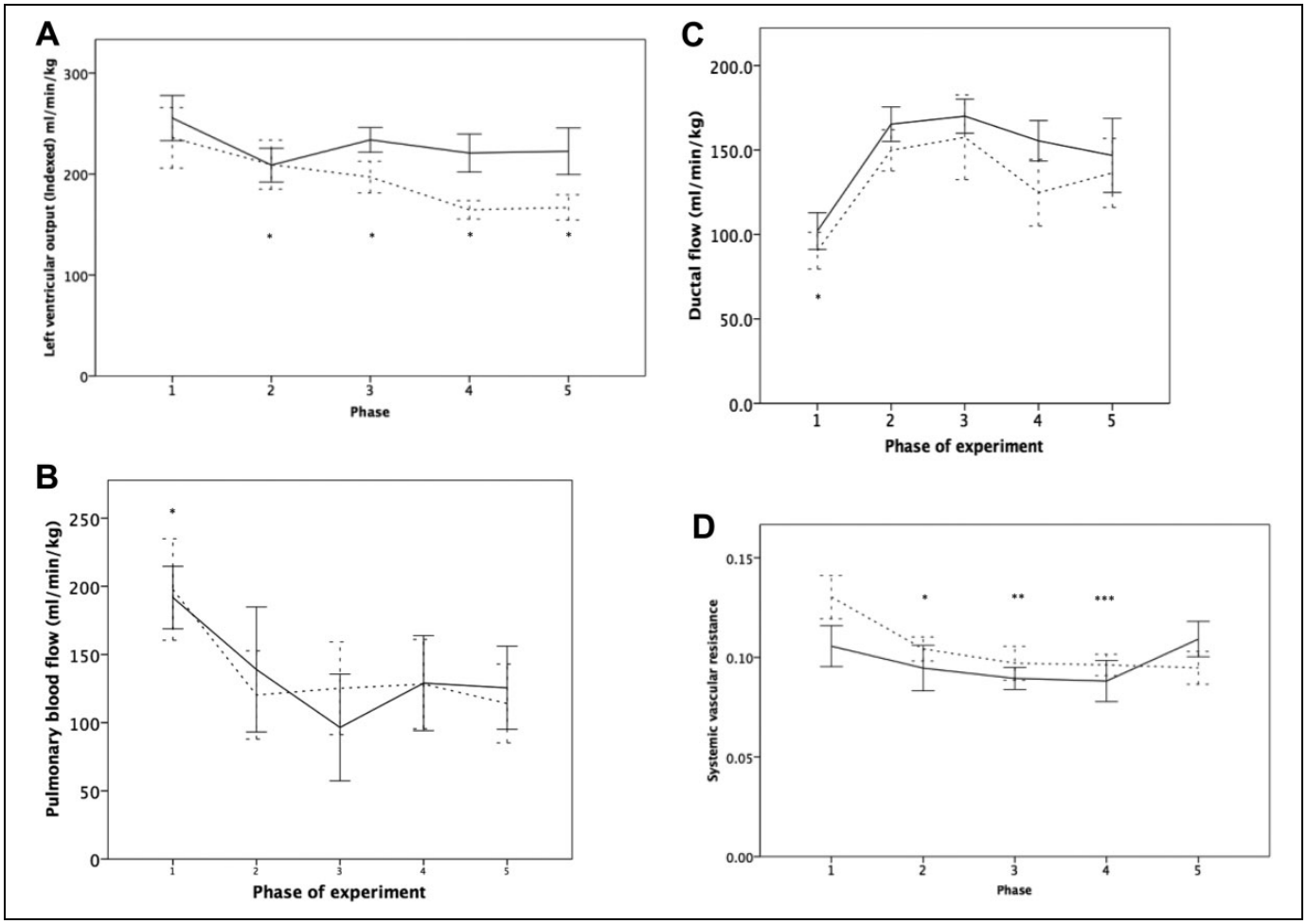

Figure 3. Fetal left ventricular output (A), pulmonary volume blood flow (B), ductus arteriosus volume blood flow (C), and systemic vascular resistance (D) during the experiment. Solid line represents saline and dashed line as sildenafil. Phase 1: baseline; phase 2: hypoxemia; phase 3: hypoxemia + 30 minutes infusion of saline or sildenafil; phase 4: hypoxemia + 120 minutes infusion of saline or sildenafil; phase 5: normoxemia + infusion of saline or sildenafil. A, No group differences ($P = .376$). *Significantly different than baseline ($P < .002$) for each phase. B, no group differences ($P = .872$). *Baseline significantly different than all other phases ($P < .003$). C, No group differences ($P = .169$). *Baseline significantly different than all other phases ($P < .003$). D, No group difference ($P = .513$). Differences marked with *$P = .025$, **$P = .007$, and ***$P = .004$ significantly different compared to baseline. No difference in the recovery + infusion phase ($P = .079$).

30 minutes infusion, hypoxemia + 120 minutes infusion, and recovery + infusion phases than at baseline and hypoxemia phase (Table 1, Figure 2C). Fetal MAP remained stable in the saline group. Fetal systemic venous pressure remained stable in both groups during the entire experiment.

Fetal hypoxemia did not affect RVCO that remained unchanged in both groups. However, LVCO and combined cardiac output fell significantly in both groups during hypoxemia and remained significantly lower in the recovery + infusion phase than at baseline (Table 2, Figure 3A). In both groups, Q_P decreased and both Q_{DA} and RPA PI increased significantly during hypoxemia with no difference between the sildenafil and control groups (Table 2, Figure 3B and C). During hypoxemia, systemic vascular resistance fell significantly in both groups, and it returned to baseline level in the recovery + infusion phase (Table 2, Figure 3D). Again, there was no significant difference between the sildenafil and control groups.

At baseline, LV global longitudinal strain showed more deformation ($P = .001$) than the RV global longitudinal strain with no difference between the groups (Table 3). During hypoxemia, LV global longitudinal strain deformation was reduced ($P = .003$) in both groups (Figure 4A). On the other hand, RV global longitudinal strain remained stable in both groups during hypoxemia. Both RV and LV IVRV (Figure 4B and C) decreased significantly during hypoxemia. On the other hand, only RV IVCV was affected by hypoxemia (Figure 4D), while LV IVCV remained stable (Table 3). In fetal cardiac functional parameters, we found no significant differences between the sildenafil and control groups during the experiment. The DV PIV increased significantly in both groups during recovery + infusion phase compared to baseline (Table 3, Figure 4E).

Response of the fetal heart rate and MAP to acute hypoxemia in the study is shown in Figure 5A and B, respectively.

Table 3. Fetal Left and Right Ventricular Global Longitudinal Strain, Ductus Venosus PI, IVCV, and IVRV at MV as well as TV Measured During the Experiment in the Study Group Compared to the Control Group.

Parameter	Group	Baseline	Hypoxemia	Hypoxemia + Infusion (30 minutes)	Hypoxemia + Infusion (120 minutes)	Recovery + Infusion	*P* (Time)	*P* (Group)	*P* (Time × Group)
DV PI	Control	0.83 (0.39)	0.75 (0.33)	0.77 (0.29)	0.80 (0.23)	1.01 (0.41)	**.001**	.353	.295
	Sildenafil	0.86 (0.32)	0.65 (0.22)	0.92 (0.51)	0.98 (0.45)	1.18 (0.49)			
LV global longitudinal strain (%)	Control	−18.3 (3.04)	−16.3 (2.41)	−16.4 (3.70)	−14.3 (4.13)	−14.9 (2.87)	**.0031**	.72	.34
	Sildenafil	−18.7 (3.82)	−14.8 (2.69)	−15.3 (3.38)	−15.0 (4.27)	−17.8 (2.86)			
RV global longitudinal strain (%)	Control	−13.8 (4.60)	−14.7 (4.86)	−12.2 (6.13)	−12.3 (6.66)	−13.1 (6.58)	.39	.44	.93
	Sildenafil	−14.3 (5.35)	−14.8 (2.66)	−12.5 (3.50)	−13.5 (5.14)	−15.3 (2.19)			
MV IVCV (cm/s)	Control	7.04 (3.67)	6.12 (2.82)	6.12 (2.26)	6.34 (3.51)	7.01 (2.63)	.222	.318	.952
	Sildenafil	6.76 (3.36)	5.44 (2.91)	4.98 (2.70)	4.81 (2.83)	4.95 (2.07)			
TV IVCV (cm/s)	Control	5.69 (2.10)	5.11 (1.94)	4.72 (2.05)	4.03 (1.57)	5.11 (1.60)	**.008**	.584	.909
	Sildenafil	5.29 (2.42)	4.99 (1.85)	4.79 (2.33)	2.79 (1.38)	4.55 (1.81)			
MV IVRV (cm/s)	Control	2.76 (1.20)	2.70 (0.95)	2.29 (0.93)	2.90 (1.46)	2.79 (1.31)	**.001**	.168	.144
	Sildenafil	2.84 (0.93)	1.97 (1.03)	1.63 (0.85)	1.64 (0.91)	1.85 (0.60)			
TV IVRV (cm/s)	Control	2.98 (1.18)	2.71 (1.12)	2.42 (0.98)	2.94 (1.45)	2.90 (1.11)	**.029**	.558	.987
	Sildenafil	2.78 (1.06)	2.56 (0.78)	2.25 (0.91)	2.56 (0.96)	2.44 (0.99)			

Abbreviations: DV PI, ductus venosus pulsatility index; IVCV, isovolumic contraction velocity; IVRV, isovolumic relaxation velocity; LV, left ventricular; RV, right ventricular; MV, Mitral valve; TV, Tricuspid valve.

A reduction of fetal heart rate is seen with acute hypoxemia, but this lasts over 5 to 10 minutes of acute hypoxemia.

Discussion

Our study, using an instrumented sheep at near-term gestation model, demonstrates that sildenafil infusion given directly to the fetal circulation did not reverse hypoxemia-induced vasoconstriction in the pulmonary arterial bed. Furthermore, sildenafil did not ameliorate fetal cardiac systolic or diastolic dysfunction during hypoxemia.

Fetal hypoxemia altered the fetal central circulation. During fetal hypoxemia, we found a decrease in lung volume blood flow and a concomitant increase in the volume blood flow across the ductus arteriosus. Our results confirm previous observations.[4] Consequent to a drop in lung volume blood flow, venous return to the left atrium decreases, thus leading to reduced LV and combined cardiac outputs. Fetal LVCO is a sum of volume blood flow across the foramen ovale and lung volume blood flow. We have previously shown that ductus arteriosus occlusion increases only lung volume blood flow while the volume blood flow across the foramen ovale remains unchanged.[15] This suggests that foramen ovale volume blood flow is at its maximum capacity, and fetus is unable to increase it. Therefore, lung volume blood flow becomes critical in maintaining adequate LVCO and cerebral blood flow and perfusion.[19] In the present study, sildenafil infusion given directly to the fetal circulation did not reverse increased pulmonary vascular impedance induced by fetal hypoxemia and did not increase lung volume blood flow. These findings in the sheep fetus are potentially even more clinically important for the human fetus because in the sheep fetus at near-term gestation, the lungs receive 7% of combined cardiac output,[20] which makes it 20% of the LV output. In the human fetus, the proportion of LV output contributed by pulmonary venous return is even greater. Pulmonary venous return constitutes 50% to 60% of LV output in the third trimester of pregnancy. Sildenafil infusion was given via a central vein (superior vena cava) and therefore should have reached the lungs without passing through the metabolically active organs, such as the placenta or the liver.

Fetal pulmonary circulation is known to be responsive to sildenafil at this gestation. Significant vasodilatation is produced by sildenafil infusion in normoxemic and hyperoxic sheep fetuses.[14] It is possible that other vascular regions of the circulation are more sensitive to sildenafil, and its effect on pulmonary circulation is masked by vasodilatation in the systemic circulation. Endothelial nitric oxide synthase (eNOS) requires O_2 to produce NO,[21] and thus it is possible that the pO_2 levels in the hypoxemic fetus are low enough to limit eNOS activity. Therefore, another possible explanation is that in the presence of hypoxemia, pulmonary vasculature is no longer responsive to sildenafil treatment. Reduced lung blood flow may limit the amount of sildenafil reaching the lung circulation, thereby masking sildenafil-induced vasodilatation. Fetal MAP responded differently to hypoxemia in sildenafil group and this finding could support our theory. The finding of a reduction in the MAP in response to hypoxemia and sildenafil agrees with a previous report.[22] The pO_2 levels were lower, pCO_2 higher, and MAP lower in the recovery phase compared to baseline, only in the sildenafil group. This may be due to problems with placental gas exchange associated with sildenafil exposure. We are not aware of any studies reporting altered gas exchange with sildenafil. The observed differences could also be secondary to altered distribution of the cardiac output. We observed that systemic vascular resistance was reduced following hypoxemia. It has previously been shown that hypoxemia leads to a redistribution of blood flow and that

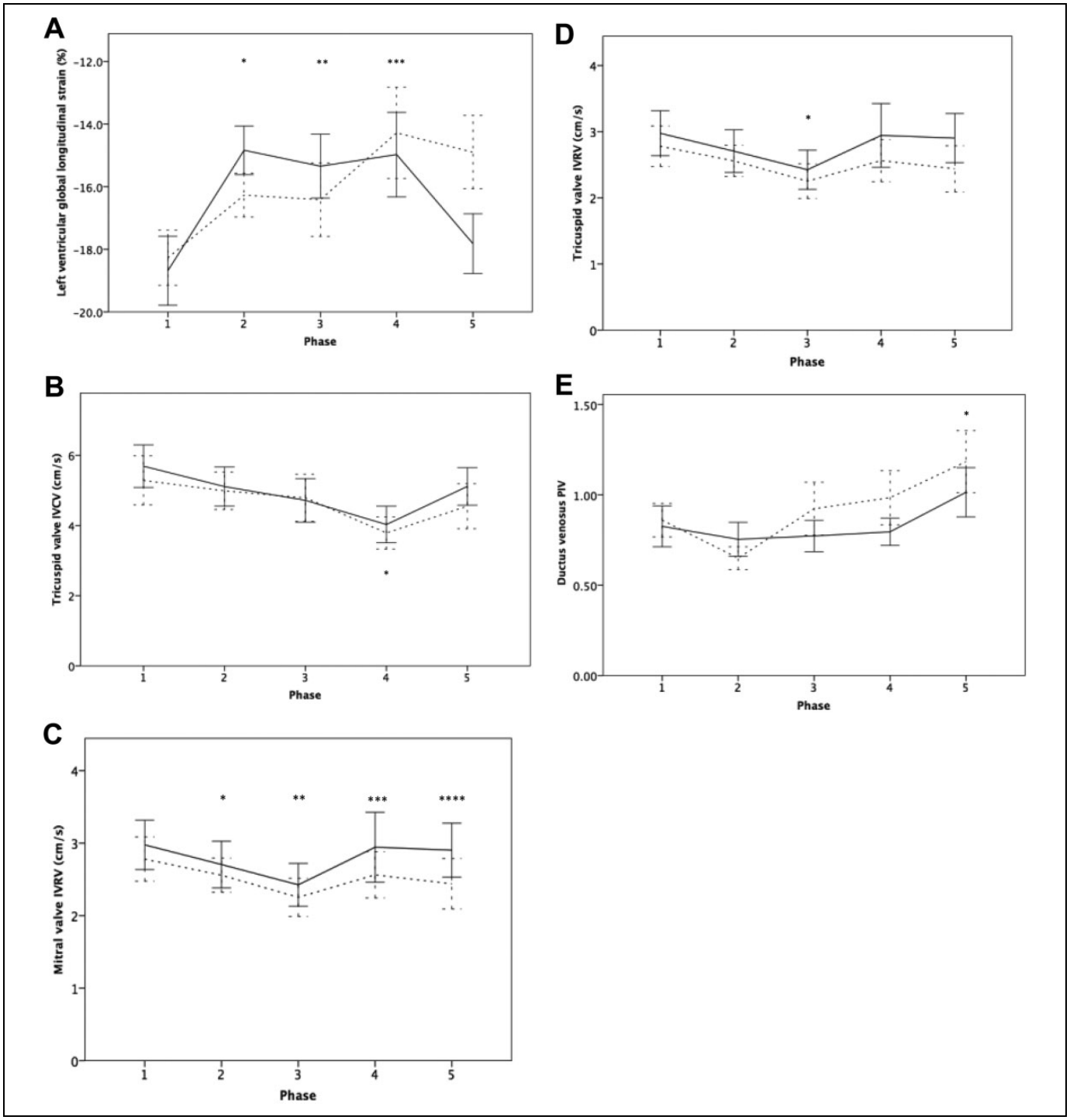

Figure 4. left ventricular (LV) global longitudinal strain (A), tricuspid valve isovolumetric contraction velocity (B), isovolumetric relaxation velocity (IVRV) of mitral valve (C), tricuspid valve (D), and ductus venosus PIV (E) during the experiment. Solid line represents saline and dashed line as sildenafil. Phase 1: baseline; phase 2: hypoxemia; phase 3: hypoxemia + 30 minutes infusion of saline or sildenafil; phase 4: hypoxemia + 120 minutes infusion of saline or sildenafil; phase 5: normoxemia + infusion of saline or sildenafil. A, No group differences ($P = .723$). Differences marked with *$P = .002$, **$P = .008$, and ***$P < .001$ significantly different compared to baseline. No difference in the recovery + infusion ($P = .055$). B, No group differences ($P = .584$). *Hypoxemia + 120 minutes infusion significantly different compared to baseline ($P < .001$). C, No group differences ($P = .168$). Differences marked with *$P = .017$, **$P < .001$, ***$P = .023$, and ****$P = .045$ significantly different compared to baseline. D, No group differences ($P = .558$). *Significantly different from baseline ($P = .007$) in both groups. In E, no difference with phase in the saline group. *Significantly different from baseline ($P = .008$) in the sildenafil group.

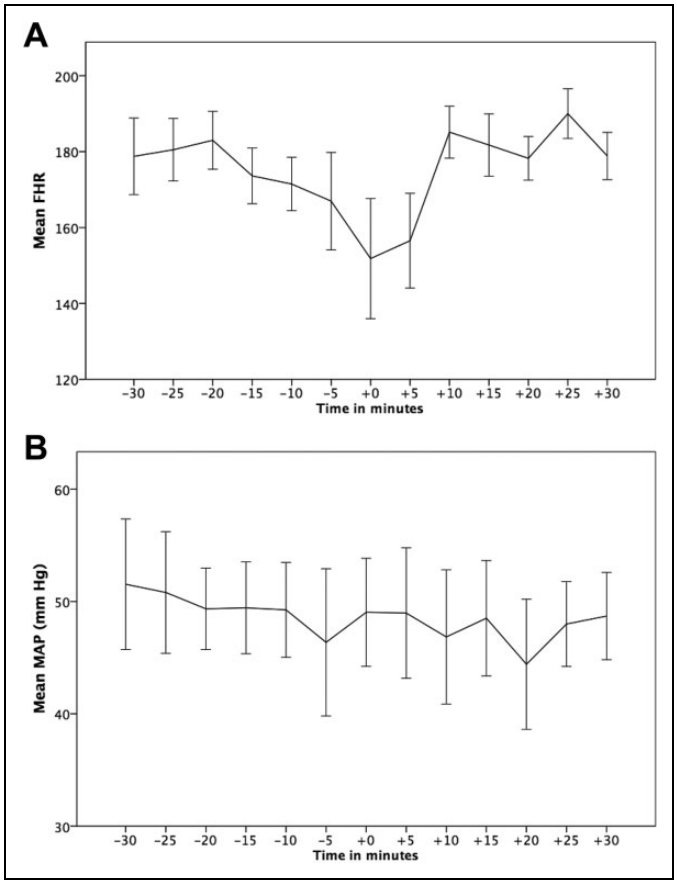

Figure 5. Response of fetal heart rate (A) and mean arterial pressure (B) to hypoxemia. Measurements are taken every 5 minutes. Acute hypoxia was commenced at $t = 0$. Error bars denote 1 SEM. SEM indicates standard error of the mean.

resistance in the carcass and splanchnic circulation increases.[4] Giussani et al[23] demonstrated that hypoxia was associated with significant reduction in femoral artery blood flow and increase in carotid artery blood flow. At a glance, our results may seem to contradict these reports. However, the placental circulation receives 40% of the cardiac output in sheep fetuses[24] and is a major contributor to systemic vascular resistance. Our calculation of systemic vascular resistance included resistance in all vascular beds, except the pulmonary vascular bed. Therefore, it is not surprising that the total systemic vascular resistance (which included placental and cerebral circulations) showed a small reduction during hypoxemia with a reduction in both combined cardiac output and MAP. Response of the fetal MAP to hypoxia is variable, depending on where the blood pressure was measured. Studies where this measurement is performed in the cerebral arteries report that carotid vascular resistance did not change and remained low.[23] On the other hand, studies where the MAP was measured in brachial[25] or femoral artery[23] report that MAP increased significantly in response to hypoxia. This discrepancy explains why there was no statistically significant change in the total peripheral vascular resistance despite a reduction in LV and total cardiac output. It has previously been shown that neuroendocrine system is involved in fetal defense against hypoxemia.[26] However, this should be involved to a similar extent in either group since the 2 groups were otherwise comparable. A reduction in fetal heart rate was seen with hypoxemia, similar to previous observations.[23] However, this response did not last for >10 minutes. Therefore, the fetal heart rate in the hypoxemia phase was no different to that seen at baseline.

Fetal hypoxemia led to diastolic dysfunction in both ventricles as indicated by decreased IVRV. In addition, we found diminished RV IVCV and LV global longitudinal strain, suggesting systolic dysfunction during hypoxemia. These results confirm previous observations.[8,18] In the present study, we hypothesized that sildenafil would ameliorate the myocardial dysfunction caused by hypoxemia. Experimental studies in the chick embryo have shown that in chronic hypoxemic environment, sildenafil prevents the increase in phosphodiesterase expression, protects against oxidative stress, and normalizes nitric oxide bioavailability in the fetal heart.[16] All these findings support the hypothesis that sildenafil protects the fetal heart in hypoxemia. However, in the present study, we found no significant differences in fetal cardiac functional parameters between fetuses who received sildenafil or saline during hypoxemia. Our results suggest that these myocardial protective measures activated by sildenafil treatment are not reflected in improved cardiac function in hypoxemic sheep fetuses. Furthermore, species differences and length of hypoxic exposure may play a role in these findings.

We found an increase in the DV PI during recovery phase in both groups. Fetal systemic venous pressure did not change during the entire experiment, thus excluding it as a cause of increased DV pulsatility. Therefore, the most likely explanation for increased DV pulsatility is that the fetal heart rate was significantly lower in the recovery phase, as a negative correlation is known to exist between heart rate and PI of vessels.[27]

Our study has some limitations. The surgical procedures may constitute a significant stress on the sheep fetuses, and it may be argued that the conditions are quite different from human fetuses exposed to hypoxemia. The postoperative recovery period should be long enough for the recovery of fetal myocardial function.[28]

Normal arterial blood gas values at the baseline phase suggest conditions close to physiologic circulatory state. Availability of invasive data and fetal oxygenation status lend important validation to the results, which is usually impossible to obtain in clinical settings. The study was carried out in a narrow gestational age window of 120 to 134 days and may limit the validity and significance outside this time period. The sheep model has been extensively used for research in fetal cardiac function and hemodynamics. We used sildenafil as an intravenous infusion to the fetus. Studies on the use of sildenafil in placental insufficiency administer the drug to the mother over a period of days to weeks[13,29] as opposed to hours in the present study. Therefore, extrapolation of these results to human pregnancy should be done cautiously. The use of anesthesia may have affected the results of the experiment. However, indexed cardiac output, baseline arterial pO_2, and

arterial pO_2 following hypoxemia are remarkably similar to those reported by Cohn et al[5] in unanesthetized animals. Isoflurane can modify fetal cardiovascular regulation. Invasive and Doppler echocardiographic volume blood flow calculations correlate well (Schmidt et al, 1991). Moreover, the intraobserver variabilities of Doppler ultrasound parameters in fetal sheep are comparable to those found in human fetuses.[1,30] Invasive measurements could have been obtained in unanesthetised animals. However, obtaining reliable ultrasound measurements without the use of anesthesia is technically not possible and is in the postoperative period unethical. Furthermore, newborn lambs under isoflurane anesthesia can increase cardiovascular performance during stress.[31]

In summary, fetal hypoxemia led to pulmonary arterial vasoconstriction, decreased lung volume blood flow, increased shunting through the ductus arteriosus, and a reduction in LVCO. Sildenafil, when given directly to a hypoxemic fetus, could not reverse this redistribution of cardiac output. Furthermore, fetal cardiac systolic and diastolic dysfunction observed during hypoxemia could not be ameliorated by sildenafil treatment.

Authors' Note

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Authors' Contribution

AB contributed to acquisition, analysis or interpretation of data for the work, and drafting the manuscript; LA contributed to acquisition, analysis or interpretation of data for the work, and revising it critically for important intellectual content; JR and GA contributed to conception and design of the work, acquisition, analysis and interpretation of data for the work, and revising it critically for important intellectual content; HH, JJ, MK, TE, PO, and MH revised the manuscript critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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