Ultrasound in Med. & Biol., Vol. 42, No. 11, pp. 2589–2598, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2016.07.006

● *Original Contribution*

EFFECT OF HYPOXEMIA WITH OR WITHOUT INCREASED PLACENTAL VASCULAR RESISTANCE ON FETAL LEFT AND RIGHT VENTRICULAR MYOCARDIAL PERFORMANCE INDEX IN CHRONICALLY INSTRUMENTED SHEEP

AMAR BHIDE,[*†] OLLI VUOLTEENAHO,[‡] MERVI HAAPSAMO,[§] TIINA ERKINARO,[¶] JUHA RASANEN,[‖#] and GANESH ACHARYA[*†**]

[*] Women's Health & Perinatal Research Group, UiT—The Arctic University of Norway, Tromsø, Norway; [†] Department of Obstetrics and Gynecology, University Hospital of Northern Norway, Tromsø, Norway; [‡] Biomedicine Unit, Department of Physiology, University Hospital of Oulu, Oulu, Finland; [§] Department of Obstetrics and Gynecology, University Hospital of Oulu, Oulu, Finland; [¶] Department of Anesthesiology, University Hospital of Oulu, Oulu, Finland; [‖] Department of Obstetrics and Gynecology, University of Eastern Finland, Kuopio, Finland; [#] Oregon Health and Sciences University, Portland, Oregon, USA; and [**] Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institute, Stockholm, Sweden

(*Received* 15 *July* 2015; *revised* 9 *June* 2016; *in final form* 6 *July* 2016)

Abstract—Myocardial performance index (MPI) is increased in growth-restricted fetuses with placental insufficiency, but it is unknown if this is due to fetal hypoxemia or increased placental vascular resistance (R_{plac}). We used chronically instrumented sheep fetuses (n = 24). In 12 fetuses, placental embolization was performed 24 h before experiments. On the day of the experiment, left (LV) and right (RV) ventricular MPIs were obtained by pulsed Doppler at baseline and in the hypoxemia and recovery phases. At baseline, R_{plac} was greater and fetal pO_2 lower in the placental embolization group, but RV and LV MPIs were comparable to those of the control group. During hypoxemia, mean LV MPI increased significantly only in fetuses with an intact placenta (0.34 vs. 0.46), returning to baseline during the recovery phase. Right ventricular MPI was unaffected. We conclude that fetal LV function is sensitive to acute hypoxemia. Exposure to chronic hypoxemia could pre-condition the fetal heart and protect its function with worsening hypoxemia. (E-mail: abhide@sgul.ac.uk)

Key Words: **Cardiovascular function, Hypoxemia, Sheep model.**

INTRODUCTION

The myocardial performance index (MPI) was originally described in the evaluation of dilated cardiomyopathy (Tei et al. 1995). It reflects combined systolic and diastolic cardiac function in both adults and children and is independent of age, ventricular geometric assumptions, heart rate (HR) and blood pressure (Tei et al. 1995, 1996). This observation has been extended to fetuses, and MPI has been studied as a possible marker of fetal cardiac dysfunction. However, the fetal circulation is quite different from the adult circulation. Fetal systemic and pulmonary circulations work in parallel rather than in series, as in adults. The dominant ventricle in adult life is the left ventricle. In the fetus, it is the right ventricle, which supplies blood to most of the body and the placenta. The fetal left ventricle preferentially perfuses the brain. In the setting of increased placental vascular resistance, the afterload on the right ventricle can be elevated. Vascular resistance in the cerebral circulation, however, remains relatively low. Fetuses that are growth restricted because of placental insufficiency appear to have increased left ventricular MPI (Crispi et al. 2008). However, it is uncertain whether the change in MPI is a reflection of hypoxemia, changes in cardiac loading conditions or a direct effect of myocardial cell damage. There have been a few previous attempts to study the relationship between hypoxemia and MPI. One such study (Guorong et al. 2007) reported

Address correspondence to: Amar Bhide, Fetal Medicine Unit, 4th Floor, Lanesborough Wing, St. George's Hospital, Blackshaw Road, SW17 0 QT, United Kingdom. E-mail: abhide@sgul.ac.uk

Conflict of interest disclosure: None of the authors report any conflict of interest.

elevated left as well as right ventricular MPIs in hypoxemia caused by acute cord occlusion. To our knowledge, the effect of acute hypoxemia (without changes in preload or afterload) and the effects of chronic hypoxemia with elevation of placental vascular resistance (R_{plac}) on fetal right and left ventricular MPIs have not been studied.

We hypothesized that increased R_{plac} and chronic fetal hypoxemia caused by placental embolization lead to global myocardial dysfunction and increased fetal left and right ventricular MPI. Furthermore, we wanted to investigate whether fetuses with increased R_{plac} and chronic hypoxemia respond differently to an acute reduction in fetal pO_2 compared with fetuses with intact placenta.

METHODS

All experiments were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe 1986) and European Union Directive ETS 123 (1997). The Animal Care and Use Committee of the University of Oulu approved the study protocol.

Surgical preparation and instrumentation

Data from 24 chronically instrumented pregnant sheep at 115–129/145 d of gestation were used for this report. The details of the instrumentation have been described previously (Erkinaro et al. 2004, 2009). In brief, a laparotomy was performed under general anesthesia and endotracheal intubation. The fetal lower body was exteriorized through a hysterotomy, and 18G polyurethane catheters were introduced into the descending aorta and inferior vena cava *via* the femoral artery and vein. A 4-mm-transit-time ultrasonic flow probe (Transonic Systems) was placed around the umbilical arteries to measure placental volume blood flow (Q_{Plac}). After replacement of amniotic fluid with 0.9% warm saline and closure of the surgical wounds, all catheters and probes were tunneled subcutaneously and exteriorized through a small skin incision in the ewe's flank. Post-operative analgesia was provided with a fentanyl patch (50 mcg/h) attached to the ewe's tail, with additional intramuscular injections of fentanyl 1.5 to 2 mcg/kg twice daily. After 4 d of recovery and 24 h before the experiment, placental embolization was performed in 12 sheep using 45- to 150-mm microspheres (Contour Emboli, Target Therapeutics, Fremont, CA, USA) to simulate placental pathophysiology in pregnancies complicated by placental insufficiency. A dry volume of 0.25 mL of microspheres was suspended in 0.5 mL of 20% albumin and diluted with 10 mL of 0.9% saline. This solution was injected into the fetal descending aorta in 1-mL increments every 15 min until fetal arterial oxygen saturation decreased by 30% from pre-embolization values. The control group included 12 sheep with intact placental circulation.

Throughout the recovery period of 4–5 d, the ewes received daily intravenous infusions of 1 L of Ringer's lactate solution with ampicillin 1 g, and the fetuses were given intravenous injections of benzyl penicillin 1×10^6 IU.

Experimental protocol

On the fifth postoperative day, general anesthesia was induced with propofol 4–7 mg/kg and maintained with isoflurane 1–1.5% in an oxygen/air mixture via an endotracheal tube and mechanical ventilation. Muscle relaxation was induced with rocuronium 20 mg and monitored with a neurostimulator, with additional boluses given as needed. A 16G polyurethane catheter was inserted into the maternal descending aorta through a femoral artery.

When all hemodynamic parameters were stabilized, both invasive and Doppler ultrasonographic baseline measurements were obtained (baseline). After this, maternal and fetal hypoxemia, defined as maternal oxyhemoglobin saturation of 80%, was induced by replacing oxygen with medical air in the rebreathing circuit, and a set of measurements identical to those at baseline were obtained after 15 min of maternal hypoxemia (hypoxemia). Thereafter, the maternal inhaled oxygen concentration was returned to baseline, and the ewe and her fetus were allowed to recover from hypoxemia for 15 min before obtaining the recovery phase measurements (recovery).

Invasive measurements

Maternal arterial pressures and heart rate were measured with disposable pressure transducers (DT-XX, Ohmeda, Hatfield, UK). The transducers used for fetal arterial and venous blood pressure measurements were reusable (Biopac Systems, Santa Barbara, CA, USA). Maternal and fetal mean arterial pressures (MAPs) were computed arithmetically (MAP = diastolic pressure + [systolic pressure–diastolic pressure]/3), and HRs were computed from the arterial waveforms. Placental (R_{Plac}) vascular resistance was computed by dividing fetal MAP by Q_{Plac}. All variables were recorded continuously at a sampling rate of 100 Hz using a polygraph (UIM100 A, Biopac Systems, Santa Barbara, CA, USA) and computerized data acquisition software (Acqknowledge, Version 3.5.7 for Windows, Biopac Systems, Santa Barbara, CA, USA). The recordings were later analyzed at 1-min periods, and the median value of the 6,000 measurements per variable was chosen to represent

a particular minute. Maternal and fetal arterial blood samples drawn at the end of each phase were immediately analyzed for acid–base and lactate values (39°C).

Ultrasonographic data acquisition

During each phase, Doppler ultrasonographic recordings (Acuson Sequoia 512, Mountain View, CA, USA) from the fetal umbilical artery were obtained. Mean values for pulsatility index (PI = [peak systolic velocity−end diastolic velocity]/time-averaged mean velocity over the cardiac cycle) of the umbilical artery (UA PI) were derived from three consecutive blood flow velocity waveforms. From aortic and pulmonary valve blood flow velocity waveforms, the time-velocity integral was obtained by planimetry of the area underneath the Doppler spectrum (Erkinaro et al. 2007). The angle of insonation was kept at $<15°$. Pulmonary and aortic valve diameters were measured during systole using the leading edge method to calculate their cross-sectional areas (CSAs). Volumetric blood flows (Q) across the pulmonary and aortic valves were calculated (Q = CSA × time−velocity integral × HR). Right ventricular output equals the volume blood flow across the pulmonary valve, and left ventricular output equals the volume blood flow across the aortic valve, and their sum is the combined cardiac output (Erkinaro et al. 2007). Fetal cardiac outputs were weight indexed. Left ventricular MPI was calculated using the method described previously (Friedman et al. 2003; Hernandez-Andrade et al. 2005). Briefly, pulsed Doppler ultrasound was used to insonate LV inflow (mitral valve) and outflow (aortic valve). A relatively wide gate size (3–5 mm) was used to obtain waveforms from the two valves simultaneously. A fast sweep speed (5–10 cm/s) was used to record the Doppler waveforms from successive cardiac cycles, and the image was frozen. The mitral and aortic valve movements (clicks) seen on the Doppler velocity waveform patterns were used as the reference points while measuring the cardiac cycle time intervals that are the components of MPI. Measurements of a and b components of MPI were made from the same cardiac cycle. The component a was measured as the time interval from the closure click to the subsequent opening click of the mitral valve, the b component was measured from the opening to the closure of the aortic valve and MPI was calculated with the formula MPI = $(a-b)/b$, where a is the sum of isovolumic contraction time (ICT), isovolumic relaxation time (IRT) and ejection time (ET), and b is the ET. Left ventricular IRT was measured from the closure of the aortic valve to the opening of the mitral valve, and ICT, from closure of the mitral valve to the opening of the aortic valve. Time interval measurements were obtained from three consecutive cardiac cycles, and the average values were used for analyses. Left ventricular IRT and ICT were corrected for the duration of the cardiac cycle and expressed as a percentage of the total duration of the cardiac cycle. All Doppler recordings were made during a stable heart rate in the absence of fetal movements or breathing. Right ventricular MPI was calculated from separate cardiac cycles, because it is not possible to image the tricuspid and pulmonary valves simultaneously. Component a was measured as the time interval from the closure click to the subsequent opening click of the tricuspid valve, and the b component was measured from the opening to the closure of the pulmonary valve. Right ventricular MPI was calculated as above.

Quantitative real-time reverse transcription polymerase chain reaction

The expression of 11 genes in the fetal left and right ventricular myocardium was studied using quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). Total RNA was extracted from myocardial tissue samples obtained from the left and right ventricles and purified using Qiagen Rneasy reagents with DNAse treatment. cDNA first strand was synthesized from RNA using Moloney murine leukemia virus reverse transcriptase. The quantitative PCR reactions were performed with an ABI 7300 Real Time PCR System using TaqMan chemistry. The primers and probes were designed with Primer Express software (Applied Biosystems); 18 S housekeeping gene expression was used to normalize the gene expression data, as described previously (Majalahti-Palviainen et al. 2000). The primers and bifunctional fluorogenic probes (5′-FAM and 3′-TAMRA) employed are listed in Supplementary Table 1 (online only, available at http://dx.doi.org/10.1016/j.ultrasmedbio.2016.07.006).

Statistical analysis

Differences between groups were tested using the independent sample t-test. The general linear model for repeated measurements ANOVA (analysis of variance) was used to test within-subject and between-subject variances, as well as interactions. Bonferroni *post hoc* test was used for pairwise comparisons. Distribution of gene expression data was tested for normality using the Shapiro–Wilk test. Skewed data were log-transformed to achieve normal distribution. Gene expression between fetuses with intact placentas and placental embolization was compared using an unpaired t-test. Expression in the left and right ventricles of the same fetus was compared using a paired t-test. A p value < 0.05 was considered to indicate statistical significance. SPSS software, Version 20 (IBM, Armonk, NY, USA), was used for the statistical analysis.

RESULTS

On the day of the experiment, the mean (standard deviation) gestational age was 126 (4.9) d in the control group and 123 (7.4) d in the placental embolization group. In the control group, maternal mean weight was 73 (7.5) kg, and fetal mean weight, 2,597 (704) g; the corresponding values were 69.5 (19.9) kg and 1,827 (532) g in the placental embolization group, respectively.

During the experiment, maternal pO_2 decreased significantly during the hypoxemia phase in both groups without any difference in the degree of maternal hypoxemia between the groups. Maternal mean arterial blood pressure did not change significantly during the experiment (Table 1).

At baseline, fetal pO_2 was significantly lower, and pCO_2 and lactate were significantly higher, in the placental embolization group than in the controls (Table 2). However, pH and base excess values, as well as mean arterial pressures in the descending aorta, were comparable in the two groups. During the hypoxemia phase, fetal pO_2 decreased significantly in the placental embolization and control groups, and during the recovery phase, it returned to baseline levels in both groups. Fetal pH, pCO_2 and base excess values did not change significantly during the whole experiment.

At baseline, weight-indexed Q_{Plac} was significantly lower, and weight-indexed R_{Plac} and UA PI values were significantly greater, in the placental embolization group than in the control group (Table 2). Fetal weight-indexed right and left ventricular and combined cardiac outputs did not differ between the groups. Furthermore, left and right ventricular MPIs were comparable in the two groups. During the hypoxemia phase, weight-indexed R_{Plac} further increased in the placental embolization group. Left ventricular MPI increased significantly during the hypoxemia phase in the control group (Fig. 1d), whereas right ventricular MPI was not affected by hypoxemia (Fig. 2). A significant increase in both left ventricular IRT and ICT contributed to the elevated MPI (Fig. 1a–c). In the placental embolization group, further reduction in fetal pO_2 (hypoxemia phase) did not affect left or right ventricular MPIs. During the recovery phase, left ventricular MPI returned to baseline level in the control group. Fetal HR and weight-indexed left, right and combined cardiac outputs remained comparable to baseline values during the experiment (Table 2).

Placental embolization causes a chronic and major change in the physiologic environment of the fetus. Therefore, to be able to better interpret the functional effects of the acute hypoxic challenge, we measured the mRNA levels in the fetal heart by qRT-PCR of 11 genes, representing various aspects of cardiac physiology: direct responses to hypoxia (*HIF1a* [hypoxia-inducible factor 1α], *ANGPT1* [angiopoietin 1]), contractile function (*TNNC1* [troponin C], *ATP2A2* [sarco/endoplasmic reticulum Ca^{2+}-ATPase, SERCA2], phospholamban), metabolic function (*CPT1A* [carnitine palmitoyltransferase]), endocrine function (*ANP* [atrial natriuretic peptide] and *BNP* [brain natriuretic peptide]), neural regulation (*ADRBI* [β_1-adrenoreceptor], *TAC1* [tachykinin]) and cytokine regulation (*STAT3* [signal transducer and activator of transcription 3]). Statistically significant effects were found with *ATP2A2* in the left ventricle, and *TNNC1*, *ADRB1* and *STAT3* in the right ventricle, all of which were lower after placental embolization (Table 3 and Fig. 3).

DISCUSSION

In this study, we found both left and right ventricular global function of sheep fetuses with increased R_{plac} and chronic hypoxemia to be comparable to that of fetuses with intact placental circulation and normoxemia. Interestingly, when fetal oxygenation was acutely reduced, left ventricular dysfunction developed only in normoxic fetuses with intact placental circulation. In these fetuses,

Table 1. Maternal parameters during the experiment

Characteristic	Embolized or not	Baseline	Hypoxemia	Recovery	*p* value		
					Within subject	Between subject	Interaction
Heart rate	No	128 (8)	129 (14)	129 (15)	0.45	0.75	0.42
	Yes	132 (18)	129 (28)	120 (15)			
Mean arterial pressure (mm Hg)	No	94 (9)	95 (8)	95 (7)	0.3	0.91	0.43
	Yes	94 (10)	98 (14)	92 (12)			
pO_2 (kPa)	No	15.24 (5.1)	8.12 (1.5)	15.06 (4.7)	<0.005	0.30	0.43
	Yes	14.9 (4.7)	6.43 (1.1)	13.12 (2.6)			
pCO_2 (kPa)	No	5.18 (0.5)	4.84 (0.4)	4.99 (0.4)	0.003	0.75	0.82
	Yes	5.12 (0.4)	4.85 (0.6)	4.89 (0.5)			
pH	No	7.32 (0.06)	7.34 (0.04)	7.32 (0.04)	0.006	0.011	0.32
	Yes	7.38 (0.05)	7.39 (0.04)	7.36 (0.04)			
Base excess (mmol/L)	No	−4.57 (3.2)	−5.07 (2.8)	−5.43 (2.6)	0.12	0.049	0.41
	Yes	−2.03 (3.5)	−2.63 (2.2)	−4.00 (2.4)			

Table 2. Fetal parameters during the experiment

Characteristic	Embolized or not	Baseline	Hypoxemia	Recovery	*p* value		
					Within subject	Between subject	Interaction
Umbilical artery PI	No	0.78 (0.15)	0.72 (0.17)	0.76 (0.17)	0.27	<0.0005	0.07
	Yes	1.22 (0.28)	1.39 (0.53)	1.18 (0.32)			
Placental volume flow (mL/kg/min)	No	154.3 (39.8)	160.9 (55.2)	138.5 (44.1)	0.23	0.009	0.007
	Yes	105.7 (36.4)	90.1 (39.5)	102.7 (54.2)			
Placental vascular resistance (mmHg/kg/mL/min)	No	0.36 (0.090)	0.36 (0.122)	0.42 (0.143)	0.023	<0.0005	0.07
	Yes	0.50 (0.118)	0.63 (0.252)	0.56 (0.193)			
Fetal heart rate (bpm)	No	171 (18)	180 (20)	155 (15)	0.09	0.52	0.20
	Yes	159 (23)	172 (40)	163 (25)			
Right ventricular output (mL/kg/min)	No	413.7 (111.8)	491.5 (188.2)	407.6 (122.7)	0.09	0.61	0.06
	Yes	483.1 (234.0)	491.5 (188.2)	457.8 (149.5)			
Left ventricular output (mL/kg/min)	No	261.2 (56.1)	285.3 (86.9)	241.7 (70.9)	0.12	0.98	0.48
	Yes	278.0 (151.1)	265.5 (88.3)	247.2 (98.7)			
Combined cardiac output (mL/kg/min)	No	667.0 (156.5)	772.3 (269.6)	641.3 (172.3)	0.16	0.336	0.63
	Yes	765.7 (382.4)	750.2 (276.8)	715.0 (223.9)			
Mean arterial pressure (mm/Hg)	No	52 (6)	55 (6)	53 (6)	0.043	0.50	0.38
	Yes	49 (6)	53 (10)	53 (9)			
pH	No	7.32 (0.03)	7.34 (0.03)	78.33 (0.04)	0.27	0.35	0.06
	Yes	7.31 (0.05)	7.31 (0.07)	7.30 (0.05)			
pO_2 (kPa)	No	3.2 (0.52)	2.3 (0.43)	3.06 (0.48)	<0.005	<0.005	0.30
	Yes	1.99 (0.64)	1.20 (0.49)	2.14 (0.65)			
pCO_2 (kPa)	No	6.6 (0.73)	6.5 (0.68)	6.3 (0.89)	0.83	0.026	0.87
	Yes	7.40 (0.58)	7.12 (0.64)	6.9 (0.82)			
Base excess (mEq/L)	No	−0.19 (2.1)	0.27 (1.9)	−0.84 (2.5)	0.008	0.92	0.10
	Yes	1.2 (2.6)	−0.07 (3.7)	−1.53 (3.7)			
Left ventricular MPI	No	0.34 (0.063)	0.46 (0.102)	0.37 (0.072)	<0.0005	0.82	0.029
	Yes	0.36 (0.069)	0.41 (0.065)	0.42 (0.825)			
Left ventricular isovolumetric contraction time (% of cardiac cycle)	No	4.74 (1.3)	6.56 (1.6)	5.03 (1.5)	0.007	0.81	0.09
	Yes	5.06 (1.4)	5.75 (1.0)	5.85 (2.1)			
Left ventricular isovolumetric relaxation time (% of cardiac cycle)	No	9.86 (1.4)	12.81 (3.0)	10.02 (1.6)	0.002	0.10	0.95
	Yes	10.12 (1.9)	11.49 (1.9)	11.20 (2.4)			
Left ventricular ejection time (% of cardiac cycle)	No	43.01 (3.5)	42.44 (3.1)	41.07 (3.5)	0.21	0.95	0.89
	Yes	42.39 (4.2)	42.75 (6.0)	41.14 (5.1)			
Right ventricular MPI	No	0.24 (0.096)	0.24 (0.076)	0.23 (0.095)	0.68	0.77	0.65
	Yes	0.25 (0.11)	0.19 (0.218)	0.23 (0.086)			

PI = placental insufficiency; MPI = myocardial performance index.

the right ventricle maintained its global function. In fetuses with increased R_{plac} and chronic hypoxemia, further reduction in fetal oxygenation had no effect on left or right ventricular global function.

In the placental embolization group, we observed reduced Q_{plac}, increased R_{plac} and fetal hypoxemia at baseline compared with fetuses with intact placental circulation. These circulatory changes were associated with abnormal umbilical artery blood flow velocity waveforms with increased umbilical artery impedance. All these findings are also seen in human pregnancies with placental insufficiency. Together, our baseline results indicate that the embolization procedure was sufficient to mimic placental insufficiency.

The most striking finding in the present study is that during acute reduction in fetal oxygenation, left ventricular global dysfunction was seen only in previously normoxemic fetuses with intact placental circulation, but not in fetuses with chronic hypoxemia and increased R_{plac}. Furthermore, right ventricular global function was maintained during acute hypoxemia in fetuses with intact placental circulation. The importance of this observation further strengthens when we look at the fetal pO_2 levels during the experiment. In fetuses with intact placental circulation, the fetal mean pO_2 level during the acute hypoxemia phase was still higher than that in the fetuses with placental embolization at baseline. In addition, in the placental embolization group, there was a further reduction in fetal pO_2 during the hypoxemia phase. One explanation for our findings could be that increased R_{plac} and chronic fetal hypoxemia after placental embolization lead to pre-conditioning of the fetal left ventricle that protects it from further hypoxemia. There is support for such ischemic pre-conditioning and cardioprotection in previous work. Myocardial pre-conditioning is a powerful endogenous adaptive phenomenon first reported by Murry et al. (1986). They reported that episodes of sublethal ischemia enhance the resistance of the myocardium to subsequent ischemic insult. In mice studies, Mohammed Abdul and co-workers (2014) reported that even small changes in oxygen tension are associated with cardioprotection and increased exercise endurance

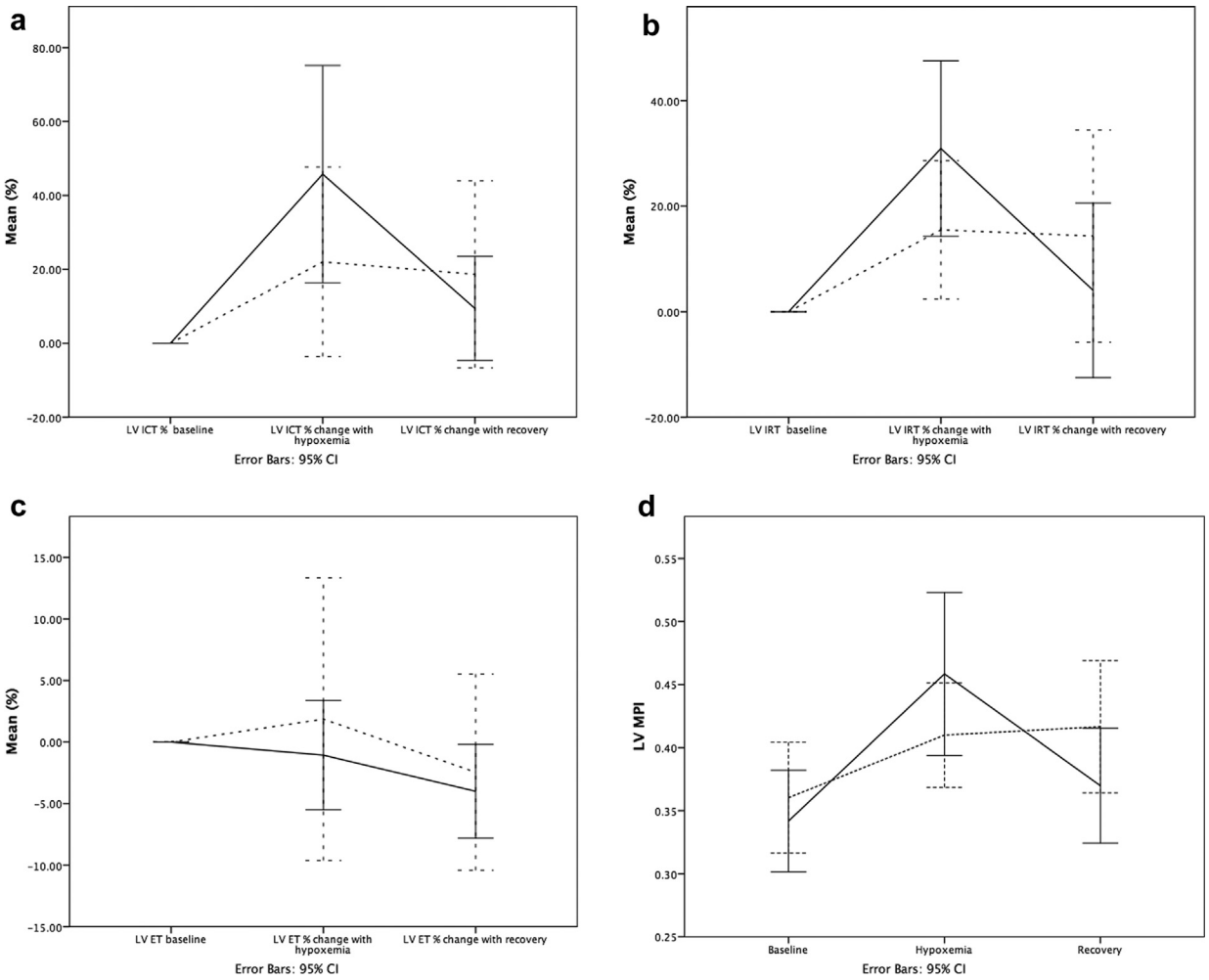

Fig. 1. Changes in left ventricular contractility at baseline, hypoxemia and recovery in fetuses with intact placentas (*solid line*) and placental embolization (*dotted line*). Percentage change in left ventricular isovolumic contraction time (ICT) (a), ejection time (ET) (b) and isovolumic relaxation time (IRT) (c). (d) Left ventricular myocardial performance index (LV MPI). CI = confidence interval.

through upregulation of SUR2A, a regulatory subunit of sarcolemmal ATP-sensitive K^+ channels. Hypoxia is known to upregulate hypoxia-inducible factor (HIF) in adults, which controls a large variety of genes related to angiogenesis, reduction in mitochondrial oxidative metabolism and protection from reactive oxygen species (Semenza 2014). Previous work with cardiac myocyte-specific deletion of HIF-1α found that deleted mice had abnormal cardiac function because of abnormal sarcoplasmic reticulum calcium pump and reduced calcium re-uptake (Huang et al. 2004). Under hypoxic conditions of embryonic/fetal life, mitochondrial number and metabolic activity are lower and are under the influence of HIF. Its upregulation can lead to lower reactive oxygen species (ROS) production, hence protecting the heart from further hypoxemic challenge (Neary et al. 2014). A previous study reported that chronic anemia leading to reduced oxygen delivery increases the myocardial expression of glycolytic enzymes (Mascio et al. 2005) in chronically instrumented fetal sheep, suggesting adaptation or pre-conditioning of the myocardium as a result of hypoxemia. Similarly, placental embolization was associated with myocardial expression of angiogenic factors leading to hypertrophy and hypertension, which is a component of fetal adaptation (Murotsuki et al. 1997).

Our gene expression data did not indicate any differential activation of directly hypoxia-sensitive genes (*HIF1a* and *ANGPT1*) with placental embolization. Instead, the data indicated significantly lower expression of genes involved in cardiac contractile function (*TNNC1* and *ATP2A2*), as well as its regulation (*ADRB1*). Mutations in *TNNC1*, a Ca^{2+} sensor and key regulator of contraction, are known to affect both the contractile function and structure of the ventricles, and can cause both dilated and hypertrophic cardiomyopathy (Kalyva et al. 2014; Robinson et al. 2007). On the other hand, we

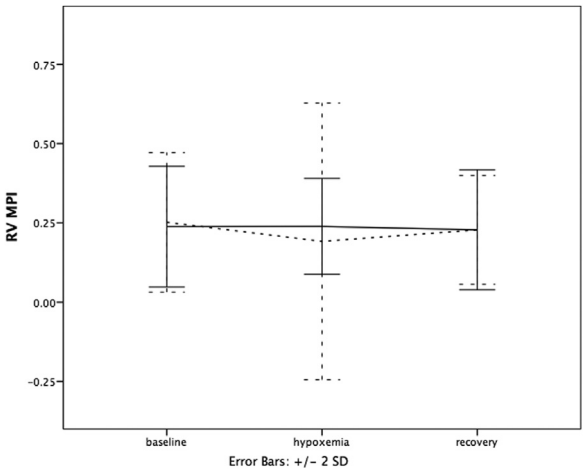

Fig. 2. Changes in right ventricular myocardial performance index (RV MPI) at baseline, hypoxemia and recovery in fetuses with intact placentas (*solid line*) and placental embolization (*dotted line*).

found decreased SERCA2 mRNA levels after placental embolization. Both groups were exposed to hypoxemia, and there was no un-exposed control group. A group not exposed to hypoxemia is needed to assess if the gene expression in the embolized group represents down-regulation or reduced upregulation.

An explanation of the findings is that hypoxemia leads to upregulation of cardiac genes in both groups, but less so with prior placental embolization. Herrera et al. (2016) previously reported that the cardiovascular responses to acute hypoxia are blunted in the chronically hypoxic fetus. They suggest that the blunting of the cardiovascular responses to hypoxia may indicate a change in control strategy triggered by chronic hypoxia, switching toward compensatory mechanisms that are more cost effective in terms of oxygen uptake. Admittedly, this experiment was performed in animals at high altitude, and compensatory responses such as increase in the hematocrit take several days to weeks. In comparison, the hypoxemia caused by placental embolization was relatively short-lived (24 h before the experiments). However, hypoxia-induced changes in gene expression are seen within hours of exposure (Maxwell et al. 2007). It is possible that the blunting of the cardiovascular responses to hypoxemia in fetuses with prior hypoxemia exposure seen in this study may at least partly be mediated through changes in gene expression.

Several techniques are available to study cardiac function (Crispi and Gratacos 2012). Traditionally, fetal cardiac function was assessed by measuring blood flow using conventional Doppler or cardiac morphometry in 2-D or M-mode. This is considered to be a reliable technique to study systolic function. Diastolic function can be studied with the *E/A* ratio using conventional ultrasound or velocity of annular valve motion measured by tissue Doppler. The MPI is a measure of global cardiac function and was used in the present study. Ventricular MPI calculation takes into account both isovolumic relaxation and contraction times, as well as the ejection time. In the present study, both isovolumic relaxation and contraction times increased, whereas ejection time was not affected by acute hypoxemia in fetuses with intact placental circulation. It is known that isovolumic time intervals of the cardiac cycle are much less affected by cardiac loading conditions than ventricular filling and ejection times (Lavine 2005). We have reported that in fetal sheep, the earliest abnormal finding in cardiac function during worsening hypoxemia and acidosis is diastolic dysfunction (Mäkikallio et al. 2006). In the present study, there were signs of both systolic and diastolic dysfunction in the left ventricle during the hypoxemia phase in fetuses with intact placental circulation. We did not find any significant change in the right ventricular MPI in response to acute hypoxemia. It is known that the left ventricular response to a β-adrenergic stimulus during hypoxemia is less than that of the right ventricle (Rasanen et al.

Table 3. Gene expression data for the fetuses with intact placentas compared with those with placental embolization*

	Left ventricle			Right ventricle		
Gene	Placental embolization	Intact placenta	Significance	Placental embolization	Intact placenta	*p* value
STAT3	1.98 (0.74)	2.54 (0.52)	0.080	1.73 (0.64)	2.40 (0.59)	0.034
HIF1A	2.2 (0.87)	2.58 (0.52)	0.297	2.46 (0.74)	2.23 (0.65)	0.48
*ADRB*1	2.46 (0.74)	2.67 (0.16)	0.401	1.89 (0.49)	2.36 (0.31)	0.032
ANGPT	1.91 (0.47)	2.15 (0.88)	0.443	1.54 (0.49)	1.68 (0.51)	0.56
NPPB	1.60 (1.20)	1.82 (0.87)	0.66	1.59 (1.07)	2.19 (0.74)	0.19
CPT1A	2.71 (0.70)	2.54 (0.82)	0.63	1.58 (0.47)	2.11 (1.37)	0.133
TAC1	2.77 (0.95)	2.04 (1.37)	0.188	1.97 (0.77)	1.87 (1.60)	0.86
NPPA	−0.34 (0.94)	−0.45 (0.58)	0.79	−0.32 (0.76)	−0.62 (0.80)	0.42
PLN	−0.13 (0.56)	0.38 (0.50)	0.057	−0.42 (0.46)	−0.38 (0.53)	0.87
ATP2a2	0.87 (0.55)	1.35 (0.25)	0.033	0.56 (0.26)	0.75 (0.27)	0.133
TNNC1	11.4 (3.87)	14.9 (4.58)	0.092	8.75 (1.36)	11.9 (3.36)	0.036

* Data for *STAT3*/18 S, *HIF1a*/18 S, *ADRB*1/18 S, *ANGPT*/18 S, *NPPB*/18 S, *CPT1a*/18 S, *bTAC1*/18 S, *NPPA*/18 S and *PLN*/18 S was log-transformed.

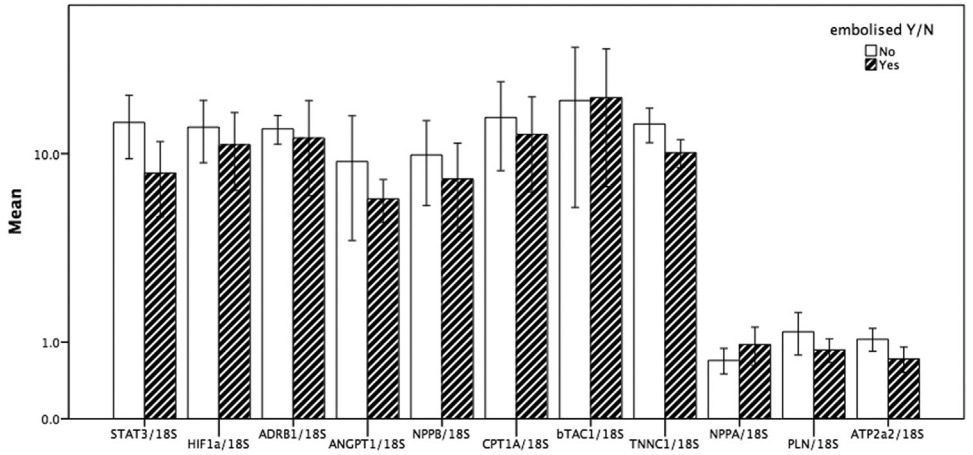

Fig. 3. Myocardial expression data, as measured by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), for 11 genes representing the direct responses to hypoxia (*HIF1a*, *ANGPT1*), contractile function (*ATP2A2*, *TNNC1*), metabolic function (*CPT1*), endocrine function (*NPPA*, *NPPB*), neural regulation (*ADRB1*, *TAC1*) and cytokine regulation (*STAT3*). The data are log transformed for all genes except *TNNC1* and *ATP2a2*. Significant differences between the intact placenta and placental embolization groups were found for the expression of *STAT3* ($p = 0.005$), *ADRB1* ($p = 0.049$), *TNNC1* ($p = 0.009$) and *ATP2a2* ($p = 0.022$).

1991). Our results are in partial agreement with a previous study in fetal sheep that reported significantly increased left and right ventricular MPIs when hypoxemia and acidosis were induced by umbilical cord occlusion (Guorong et al. 2007). The most important difference between the present study and that by Guorong et al. is that apart from leading to hypoxemia and acidosis, umbilical cord occlusion acutely leads to significant reduction in the preload and increase in the afterload. In the present study, we wanted to observe the effects of both acute hypoxemia and chronic hypoxemia with increased placental vascular resistance on left and right ventricular global function.

Fetal weight-indexed left, right and combined cardiac outputs were comparable in the two groups and did not change significantly during the experiment. Previous sheep studies have generally found either increased or unchanged fetal cardiac outputs during acute hypoxemia (Junno et al. 2013; Kamitomo et al. 1993). However, one study reported a reduction in fetal cardiac output in response to acute hypoxemia (Tchirikov et al. 2010). In that study, fetal pO_2 at baseline was 6.2 kPa, and it was reduced to 1.0 kPa during hypoxemia. One explanation of the conflicting findings could be that in the study by Tchirikov et al. (2010), fetuses were hyperoxemic at baseline. Our results are in agreement with human fetal studies of placental insufficiency indicating comparable weight-indexed cardiac outputs for fetuses with normal placental hemodynamic findings (Kiserud et al. 2006). A previous study (Junno et al. 2013) reported that the fetal left ventricle is more sensitive to progressively worsening hypoxemia and acidemia. The findings of the present study are in agreement. In the study by Junno et al. (2013), left or right ventricular MPI did not change in response to hypoxemia. However, this was in the setting of increased R_{plac}, which was achieved with angiotensin II infusion, which also increases fetal arterial blood pressure and has a positive inotropic effect on the heart.

Left ventricular MPI has also been studied in human fetuses. It was reported that left ventricular MPI in early-onset fetal growth restriction is one of the first indices to become abnormal (Cruz-Martinez et al. 2011a). It was unclear whether an abnormal MPI was caused by an increase in the resistance in the placental circulation or hypoxemia. Furthermore, it has been reported that abnormal left ventricular MPI was found in 28% of small-for-gestational-age fetuses at term with a normal umbilical artery blood velocity waveform suggesting normal placental circulatory physiology (Cruz-Martinez et al. 2011b). The authors speculated that the left ventricular MPI was more sensitive to hypoxemia than increased placental vascular resistance. Our findings support this speculation, and in addition, our results suggest that mechanisms leading to abnormal MPIs in fetuses with abnormal placental function are different from those in fetuses with normal placental hemodynamics.

Obvious strengths of our study are that the experiments were performed in chronically instrumented sheep under strictly controlled conditions, and we obtained data from invasive measurements to validate non-invasive measurements. Furthermore, hypoxemia was created under controlled conditions, and this also was validated by direct measurements of pO_2 in the maternal and fetal circulations.

One of the limitations is that the right ventricular MPI cannot be calculated from one cardiac cycle, as is the case for the left ventricular MPI. This is because it is impossible to record right ventricular inflow and outflow velocities simultaneously using pulsed-wave Doppler. However, under controlled conditions, where there is little variation in heart rate and loading conditions, separate inflow and outflow Dopplers can be obtained, and the data should be just as accurate. The experimental conditions differ from normal physiology, because the sheep and the fetus are under a general anesthetic. Isoflurane can modify fetal cardiovascular regulation. However, newborn lambs under isoflurane anesthesia are able to increase cardiovascular performance during stress (Brett et al. 1989). Previous work has indicated that uterine and placental volume blood flows are comparable before and after general anesthetic (Acharya et al. 2004), suggesting unaltered cardiovascular response. Therefore, the results are likely to be close to physiologic conditions.

CONCLUSIONS

Cardiac responses to acute reduction in oxygenation were explored in normoxemic fetuses with intact placental circulation and in fetuses with chronic hypoxemia and increased placental vascular resistance. Both right and left ventricular global function was maintained in fetuses with chronic hypoxemia and increased placental vascular resistance. Further reduction in fetal oxygenation had no impact on global ventricular function. Interestingly, in normoxemic fetuses, acute reduction in oxygenation deteriorated only left ventricular global function. Right ventricular global function was unaffected. We propose that the fetal left ventricle is more sensitive to acute hypoxemia than the right ventricle. However, in chronic hypoxemia, the fetal heart could undergo pre-conditioning that would help the ventricles to maintain function by protecting them from further deterioration with worsening of hypoxemia.

Acknowledgments—This study was partially funded by a grant from the North Norway Regional Health Authority, project no. 12050.—We thank Seija Seljanpera and Veikko Lahteenmaki for their technical assistance in the Animal Laboratory.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ultrasmedbio.2016.07.006.

REFERENCES

Acharya G, Erkinaro T, Makikallio K, Lappalainen T, Rasanen J. Relationships between Doppler-derived umbilical artery absolute velocities, cardiac function, and placental volume blood flow and resistance in fetal sheep. Am J Physiol Heart Circ Physiol 2004; 286:H1266–H1272.

Brett CM, Teitel DF, Heymann MA, Rudolph AM. The young lamb can increase cardiovascular performance during isoflurane anesthesia. Anesthesiology 1989;71:751–756.

Crispi F, Gratacós E. Fetal cardiac function: technical considerations and potential research and clinical applications. Fetal Diagn Ther 2012;32:47–64.

Crispi F, Hernandez-Andrade E, Pelsers MM, Plasencia W, Benavides-Serralde JA, Eixarch E, Le Noble F, Ahmed A, Glatz JF, Nicolaides KH, Gratacos E. Cardiac dysfunction and cell damage across clinical stages of severity in growth-restricted fetuses. Am J Obstet Gynecol 2008;199:254.e1–254.e8.

Cruz-Martinez R, Figueras F, Benavides-Serralde A, Crispi F, Hernandez-Andrade E, Gratacos E. Sequence of changes in myocardial performance index in relation to aortic isthmus and ductus venosus Doppler in fetuses with early-onset intrauterine growth restriction. Ultrasound Obstet Gynecol 2011a;38:179–184.

Cruz-Martinez R, Figueras F, Hernandez-Andrade E, Oros D, Gratacos E. Changes in myocardial performance index and aortic isthmus and ductus venosus Doppler in term, small-for gestational age fetuses with normal umbilical artery pulsatility index. Ultrasound Obstet Gynecol 2011b;38:400–405.

Erkinaro T, Kavasmaa T, Ylikauma L, Mäkikallio K, Haapsamo M, Acharya G, Ohtonen P, Alahuhta S, Räsänen J. Placental and fetal hemodynamics after labetalol or pindolol in a sheep model of increased placental vascular resistance and maternal hypertension. Reprod Sci 2009;16:749–757.

Erkinaro T, Mäkikallio K, Acharya G, Päkkilä M, Kavasmaa T, Huhta JC, Alahuhta S, Räsänen J. Divergent effects of ephedrine and phenylephrine on cardiovascular hemodynamics of near-term fetal sheep exposed to hypoxemia and maternal hypotension. Acta Anaesthesiol Scand 2007;51:922–928.

Erkinaro T, Mäkikallio K, Kavasmaa T, Alahuhta S, Räsänen J. Effects of ephedrine and phenylephrine on uterine and placental circulations and fetal outcome following fetal hypoxaemia and epidural-induced hypotension in a sheep model. Br J Anaesth 2004;93:825–832.

Friedman D, Buyon J, Kim M, Glickstein JS. Fetal cardiac function assessed by Doppler myocardial performance index (Tei index). Ultrasound Obstet Gynecol 2003;21:33–36.

Guorong L, Shaozheng H, Zhenghua W, Boyi L, Qiuyue C, Peng J, Ruiyuan S. Tei index for prenatal diagnosis of acute fetal hypoxia due to intermittent umbilical cord occlusion in an animal model. Prenat Diagn 2007;27:817–823.

Hernandez-Andrade E, Lopez-Tenorio J, Figueroa-Diesel H, Sanin-Blair J, Carreras E, Cabero L, Gratacos E. A modified myocardial performance (Tei) index based on the use of valve clicks improves reproducibility of fetal left cardiac function assessment. Ultrasound Obstet Gynecol 2005;26:227–232.

Herrera EA, Rojas RT, Krause BJ, Ebensperger G, Reyes RV, Giussani DA, Parer JT, Llanos AJ. Cardiovascular function in term fetal sheep conceived, gestated and studied in the hypobaric hypoxia of the Andean altiplano. J Physiol 2016;594:1231–1245.

Huang Y, Hickey RP, Yeh JL, Liu D, Dadak A, Young LH, Johnson RS, Giordano FJ. Cardiac myocyte-specific HIF-1 alpha deletion alters vascularization, energy availability, calcium flux, and contractility in the normoxic heart. FASEB J 2004;18:1138–1140.

Junno J, Bruun E, Gutierrez JH, Erkinaro T, Haapsamo M, Acharya G, Räsänen J. Fetal sheep left ventricle is more sensitive than right ventricle to progressively worsening hypoxemia and acidemia. Eur J Obstet Gynecol Reprod Biol 2013;167:137–141.

Kalyva A, Parthenakis FI, Marketou ME, Kontaraki JE, Vardas PE. Biochemical characterisation of troponin C mutations causing hypertrophic and dilated cardiomyopathies. J Muscle Res Cell Motility 2014;35:161–178.

Kamitomo M, Alonso JG, Okai T, Longo LD, Gilbert RD. Effects of long-term, high-altitude hypoxemia on ovine fetal cardiac output

and blood flow distribution. Am J Obstet Gynecol 1993;169: 701–707.
Kiserud T, Ebbing C, Kessler J, Rasmussen S. Fetal cardiac output, distribution to the placenta and impact of placental compromise. Ultrasound Obstet Gynecol 2006;28:126–136.
Lavine SJ. Effect of heart rate and preload on index of myocardial performance in the normal and abnormal left ventricle. J Am Soc Echocardiogr 2005;18:133–141.
Majalahti-Palviainen T, Hirvinen M, Tervonen V, Ilves M, Ruskoaho H, Vuolteenaho O. Gene structure of a new cardiac peptide hormone: A model for heart-specific gene expression. Endocrinology 2000;141: 731–740.
Mäkikallio K, Erkinaro T, Niemi N, Kavasmaa T, Acharya G, Päkkilä M, Räsänen J. Fetal oxygenation and Doppler ultrasonography of cardiovascular hemodynamics in a chronic near-term sheep model. Am J Obstet Gynecol 2006;194:542–550.
Mascio CE, Olison AK, Ralphe JC, Tomanek RJ, Scholz TD, Segar JL. Myocardial vascular and metabolic adaptations in chronically anemic fetal sheep. Am J Physiol Regul Integr Comp Physiol 2005;289:R1736–R1745.
Maxwell PJ, Gallagher R, Seaton A, Wilson C, Scullin P, Pettigrew J, Stratford IJ, Williams KJ, Johnston PG, Waugh DJ. HIF-1 and NF-kappaB-mediated upregulation of CXCR1 and CXCR2 expression promotes cell survival in hypoxic prostate cancer cells. Oncogene 2007;26:7333–7345.
Mohammed Abdul KS, Jovanović S, Sukhodub A, Du Q, Jovanović A. Upregulation of cardioprotective SUR2 A by sub-hypoxic drop in oxygen. Biochim Biophys Acta 2014;1843:2424–2431.
Murotsuki J, Challis JR, Han VK, Fraher LJ, Gagnon R. Chronic fetal placental embolization and hypoxemia cause hypertension and myocardial hypertrophy in fetal sheep. Am J Physiol 1997;272: R201–R207.
Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–1136.
Neary MT, Ng KE, Ludtmann MH, Hall AR, Piotrowska I, Ong SB, Hausenloy DJ, Mohun TJ, Abramov AY, Breckenridge RA. Hypoxia signaling controls postnatal changes in cardiac mitochondrial morphology and function. J Mol Cell Cardiol 2014;74:340–352.
Räsänen J, Alahuhta S, Kangas-Saarela T, Jouppila R, Jouppila P. The effects of ephedrine and etilefrine on uterine and fetal blood flow and on fetal myocardial function during spinal anaesthesia for caesarean section. Int J Obstet Anesth 1991;1:3–8.
Robinson P, Griffiths PJ, Watkins H, Redwood CS. Dilated and hypertrophic cardiomyopathy mutations in troponin and alpha-tropomyosin have opposing effects on the calcium affinity of cardiac thin filaments. Circ Res 2007;101:1266–1273.
Semenza GL. Hypoxia-inducible factor 1 and cardiovascular disease. Annu Rev Physiol 2014;76:39–56.
Tchirikov M, Strohner M, Scholz A. Cardiac output and blood flow volume redistribution during acute maternal hypoxia in fetal sheep. J Perinat Med 2010;38:387–392.
Tei C, Dujardin KS, Hodge DO, Bailey KR, McGoon MD, Tajik AJ, Seward SB. Doppler echocardiographic index for assessment of global right ventricular function. J Am Soc Echocardiogr 1996;9:838–847.
Tei C, Ling LH, Hodge DO, Bailey KR, Oh JK, Rodeheffer RJ, Tajik AJ, Seward JB. New index of combined systolic and diastolic myocardial performance: A simple and reproducible measure of cardiac function—A study in normals and dilated cardiomyopathy. J Cardiol 1995;26:357–366.