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Condensation: Nifedipine deteriorates fetal sheep right ventricular function and reduces right ventricular cardiac output under hypoxemia at near term gestation.

Short Title: Nifedipine and fetal cardiac function

AJOG at a Glance*:*

A. Why was the study conducted?

- Nifedipine is commonly used drug in pregnancies complicated by maternal hypertensive disorders.
- Despite its widespread use during pregnancy, the possible detrimental effects of nifedipine on fetal cardiac function, especially during hypoxemia, are not fully investigated.
- In a chronically instrumented sheep model, we studied whether nifedipine impacts on fetal cardiac function under hypoxemia.

B. What are the key findings?

- Under hypoxemia, nifedipine decreased global longitudinal strain, was related to signs of diastolic dysfunction, and ultimately reduced cardiac output in the fetal right ventricle.

C. What does this study add to what is already known?

- In hypoxemic environment, nifedipine can have detrimental effects on fetal right ventricular function that is the dominant ventricle during the second half of pregnancy.

Structured Abstract

Background: Nifedipine is widely used drug in pregnancies complicated by maternal hypertensive disorders that can be associated with placental insufficiency and fetal hypoxemia. The evidence regarding fetal myocardial responses to nifedipine in hypoxemia is limited.

Objective: We hypothesized that nifedipine would not impair fetal sheep cardiac function under hypoxemic environment. Especially, we investigated the effects of nifedipine on fetal ventricular functional parameters and cardiac output.

Study Design: A total of 21 chronically instrumented fetal sheep at 122-134 gestational days (term 145 days) were included in this study. Fetal cardiac function was evaluated by measuring global longitudinal strain, indices describing ventricular systolic and diastolic function, and cardiac outputs using 2D speckle tracking, and tissue and spectral pulsed-wave Doppler echocardiography. Fetal carotid artery blood pressure and blood gas values were invasively monitored. After baseline data collection, fetal hypoxemia was induced by maternal hypoxygenation. Following hypoxemia phase data collection, 9 fetuses received nifedipine infusion, and 12 fetuses received saline infusion. Data were collected 30 and 120 minutes after the infusion was started. After 120 minutes data collection, maternal and fetal oxygenation were normalized, and normoxemia phase data were collected, while infusion was continued.

Results: Hypoxemia decreased fetal carotid artery mean arterial pressure from 40 (8) mmHg to 35 (8) mmHg (p <0.007), and left ventricular global longitudinal strain showed less deformation than at baseline (p=0.001). Under hypoxemia, nifedipine caused a reduction in right ventricular global longitudinal strain (p <0.05), a decrease in right ventricular isovolumic relaxation velocity and its deceleration (p<0.01) indicating diastolic dysfunction and a drop in right ventricular cardiac output (p<0.05). Nifedipine did not alter fetal left ventricular functional parameters or cardiac output.

When normoxemia was restored, fetal right ventricular functional parameters and cardiac output returned to baseline level.

Conclusions: In hypoxemic fetus, nifedipine impaired right ventricular function and reduced its cardiac output. The detrimental effects of nifedipine on fetal right ventricular function were abolished, when normoxemia was restored. Our findings suggest that in hypoxemic environment nifedipine triggers detrimental effects on fetal right ventricular function.

Keywords: ultrasound, physiology, antihypertensive medication, heart, pregnancy, blood flow, hemodynamics, hypertension

Introduction

Nifedipine, a dihydropyridine calcium channel blocker, is commonly used drug in pregnancies complicated by maternal hypertensive disorders. Especially in preeclampsia, placental impairment is a common finding that can hamper fetal oxygenation and other gas exchange. Placental transfer of nifedipine to fetal circulation is substantial because umbilical cord serum concentration of nifedipine is about 93% of corresponding maternal serum concentration [1]. Studies on sheep have shown that maternally administered nifedipine does not affect fetal blood pressure. However, it can impair uterine blood flow, potentially resulting in fetal hypoxemia and acidemia. [2] In human fetuses, maternally administered nifedipine has no detectable effect on uterine or umbilical artery blood flow velocity waveforms obtained by Doppler ultrasonography [3]. Furthermore, we have shown that nifedipine when given directly to fetal circulation under hypoxemia does not alter placental hemodynamics or umbilical artery blood flow velocity waveform [4].

Myocardial contractility depends on multiple factors that include the amount of contractile proteins, Ca^{2+}, the sensitivity of the troponin-tropomyosin system to changes in Ca^{2+} concentration within the cell and the interaction between actin and myosin [5]. Nifedipine reduces developed pressure in immature rabbit heart at substantially lower concentrations than required in the adult heart [6]. The age-related differences in mechanical function to calcium antagonists suggests that contractile force in the neonatal heart is more dependent on the slow inward calcium current than in the adult heart [5]. In addition, electrophysiological studies using a mouse heart have shown a higher sensitivity of ventricular L-type Ca^{2+} channel current to verapamil in neonatal and infant stages than in child and adult stages [7].

These results suggest that myocardial responsiveness to calcium channel blockers changes with maturation and age. In addition, the evidence concerning fetal myocardial responses to calcium channel blockers in hypoxemic environment is limited. Therefore, we designed a chronically instrumented fetal sheep model to investigate the effect of nifedipine on fetal cardiac function when

the fetus is hypoxemic. We hypothesized that nifedipine when given directly to the fetus would not have detrimental effect on fetal cardiac function during hypoxemia. Specifically, we investigated the effect of nifedipine on fetal left (LV) and right (RV) ventricular 1) global longitudinal strain, 2) indices describing ventricular systolic and diastolic function, and 3) cardiac outputs.

Materials and Methods

In this study, we included 21 sheep of Finnish breed with time-dated pregnancies. The study protocol was 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 [8,9] and the EU Directive 2010/63/EU [10].

Fetal instrumentation

Fetal instrumentation was performed at 117-130 gestational days (term 145 days) under general anesthesia induced with intravenous propofol (4–7 mg/kg) and maintained with isoflurane (1.5–2.5%) in an oxygen-in–air mixture delivered via an endotracheal tube. For pain relief i.v. boluses of fentanyl (0.05–0.15 mg) were administered when required.

Following a midline laparotomy and a small hysterotomy, fetal head and upper body were delivered. Polyvinyl catheters were introduced into the external jugular vein and the carotid artery placing the catheter tips in the superior vena cava and carotid artery. A 3- lead 28-gauge silver-coated copper electrocardiogram wire (New England Wire Tech., Lisbon, NH, USA) was attached subcutaneously on the fetal chest. A separate polyvinyl catheter was placed in the amniotic cavity to monitor intra-amniotic pressure. The lost amniotic fluid was replaced with warm 0.9% saline solution. All incisions were closed, and the fetus received an intra-amniotic injection of penicillin G (1 million Units). All catheters and wires were tunnelled to a pouch on the ewe's flank. Post-operative pain was controlled with oxycodone given via an epidural catheter that was placed to the ewe before the surgery.

Experimental protocol

After a 4–5-day recovery, experiments were performed under general anesthesia induced with a single bolus of propofol and maintained by isoflurane in an oxygen-in-air mixture. The depth of anesthesia was titrated to minimize its effect on maternal heart rate and blood pressure and allow for ultrasound examination without discomfort. A 16-gauge polyurethane catheter was inserted into the maternal femoral artery. Thereafter, the ewe was placed supine with a right lateral tilt and allowed to stabilize for 30 minutes before obtaining the baseline measurements. After baseline measurements were collected, the ewe was connected to a re-breathing circuit to induce maternal and fetal hypoxemia. Maternal FiO_2 was reduced to reach the peripheral oxygen saturation level of 80%. This was confirmed by maternal arterial blood gas values. Hypoxemia phase data (hypoxemia) were collected 30 minutes after the desired maternal oxygen saturation level was reached. After hypoxemia phase data collection was completed, 9 fetuses were allocated to receive nifedipine infusion at a rate of 1.0 mL/h (700 µg/mL) (5 µg/kg/min) into the superior vena cava. The nifedipine dose was based on the studies by Blea et al. [11] and Nugent et al. [12] and is approximately equivalent to maternal oral intake of 10 mg nifedipine[1]. In the control group, 12 fetuses received saline infusion. Data were collected at 30 (hypoxemia + 30 min infusion) and 120 (hypoxemia + 120 min infusion) minutes following commencement of infusion. After hypoxemia+120 min infusion phase data collection was completed, maternal oxygenation was returned to baseline level while infusion was continued. Maternal normoxemia was achieved within 3 minutes. Recovery phase data collection (normoxemia + infusion) was started 30 minutes after maternal normoxemia was achieved (Figure 1). The mean infusion time was about 150 minutes in each group, and the calculated total mean dose of nifedipine was 1.75 mg. The steady state of the nifedipine concentration in the fetal circulation is achieved in 30-40 min after maternal infusion [1,11]. The total dose of nifedipine in this study is equivalent to oral administration of 10 mg nifedipine given 5 times during a two-hour period. The animals were euthanised at the end of the experiment

with an intravenous overdose (100 mg/kg) of pentobarbital sodium to the fetus and ewe. Fetal weights were determined postmortem.

Monitoring protocol

Maternal and fetal arterial blood pressures were continuously monitored with disposable pressure transducers (DT-XX, Ohmeda, Hatfield, UK). Fetal blood pressure values were referenced to intra-amniotic pressure. Maternal heart rate was determined from the arterial pressure waveforms. Fetal electrocardiogram leads were connected to the ultrasound equipment to obtain fetal heart rate. Maternal and fetal blood gas values were corrected to 39°C and analyzed at each study point using an Abbot i-Stat 1 arterial blood gas analyzer (i-Stat, East Windsor, NJ, USA).

Ultrasonographic data acquisition

Doppler ultrasonography was performed at the end of each phase by a single investigator (J.R.) using a Vivid 7 Dimension ultrasound system (GE Vingmed Ultrasound, Horten, Norway) with a 10 MHz-phased array transducer. The high-pass filter was set at minimum, and the angle of insonation was kept below 15 degrees. Ultrasonographic images were analysed offline by a single observer (L.A.) blinded to allocation of animals to nifedipine or control groups.

Cine-loop images of four-chamber views were obtained. Myocardial deformation (strain) was obtained by 2D speckle tracking to assess ventricular systolic function [13]. Cine-loop clips of the ultrasound examination were stored digitally. Further processing and measurements were performed using an image data acquisition software (EchoPAC, GE Medical Systems, Chicago, United States). Myocardial global longitudinal strain was measured for the LV and RV in a four-chamber view. An automated tracking algorithm outlined the myocardium in consecutive frames in the cardiac cycle. If needed the manual adjustment of the region of interest was performed. The mean value of the two measurements was used for analysis.

Tissue Doppler technique was applied to measure LV and RV lateral wall movements at the atrioventricular valve level during the cardiac cycle. The sample volume (1–1.5 mm) was placed at the level of the atrioventricular valve annuli and aligned as parallel as possible to the myocardial wall (<15° angle of insonation). Myocardial velocities were recorded during three to six cardiac cycles at a sweep speed of 100 mm/s. Isovolumic relaxation (IVRV) and contraction (IVCV) velocities of the LV and RV free wall were measured. Fetal cardiac diastolic function was evaluated using the deceleration of IVRV ($IVRV_{dec}$). Systolic function was assessed by the acceleration of IVCV ($IVCV_{acc}$). [14,15] To calculate fetal cardiac outputs, the diameters of the aortic (AoV) and pulmonary (PV) valves were measured in frozen real-time images during systole with the leading-edge to leading-edge method. The mean value of three separate valve diameter measurements was used to calculate the cross-sectional area (CSA) of the valve. From the blood flow velocity waveforms of the AoV and PV, time-velocity integrals (TVI) were measured and volumetric blood flows (Q) across the AoV and PV were calculated (Q = CSA × TVI × fetal heart rate) [16]. Left ventricular cardiac output (LVCO) equals the AoV volumetric blood flow and right ventricular cardiac output (RVCO) equals the PV volumetric blood flow, and their sum is the combined cardiac output (CCO). Fetal cardiac outputs were weight indexed.

Statistical analysis

Linear Mixed Model (LMM) was used for repeatedly measured data. Phase of the experiment and infusion of nifedipine versus saline were included as fixed effects, an interaction term, and individual fetus as the random intercept. If LMM showed a significant difference between measurement points ($p(time) < 0.05$), then a pairwise comparison between relevant points was performed. Difference between the groups was expressed as p(group). The groups may not show similar changes with time (interaction term). Therefore, this was expressed as p(group*time). Statistical analyses were performed using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics

for Windows, Version 25. Armonk, NY, USA). Data are presented as mean and standard deviation (SD) unless stated otherwise. Two-tailed p value < 0.05 was considered statistically significant.

Results

Maternal arterial blood pressure and heart rate remained within normal physiologic range in both groups during the experiment. Reduction in maternal pO_2 during hypoxemia phases was comparable between the groups (data not shown). Mean fetal weight was 2.44 (2.00- 2.99) kg and 2.51 (2.06 -2.80) kg (p=0.51) in the control and nifedipine groups. The experiments were done at the mean (SD) gestational age of 128 (2) and 126 (5) days in the control and nifedipine groups (p=0.37), respectively.

At baseline, LV global longitudinal strain showed more deformation (p= 0.001) than RV global longitudinal strain. Other ultrasonographic parameters describing ventricular systolic and diastolic function or cardiac outputs did not differ between LV and RV.

During hypoxemia phase, fetal pO_2 decreased (p<0.001) compared to baseline (Table 1). Other fetal blood gas values remained comparable to baseline level. Fetal mean arterial pressure (MAP) decreased (p <0.007) compared to baseline (Table 1). Left ventricular global longitudinal strain demonstrated less deformation (p = 0.01) than at baseline (Table 2). Fetal tissue-Doppler derived indices of LV and RV systolic and diastolic function did not change significantly during hypoxemia phase when compared to baseline. Furthermore, fetal cardiac outputs did not differ from the baseline values.

In hypoxemia + 30 min infusion phase, fetal pO_2 (p<0.001) and base excess (p<0.001) were lower and lactate (p<0.001) concentrations higher than at baseline (Table 1). Both LV and RV global longitudinal strains did not change significantly from the hypoxemia phase values (Table 2). In the nifedipine group, LV IVCV (p=0.02), RV IVRV (p<0.001), and its deceleration (p<0.001) were lower than at baseline. In the control group, RV $IVCV_{acc}$ (p=0.003) was significantly lower when

compared to the baseline with no difference between the groups (Table 3). In both groups, LV and RV cardiac outputs did not differ from the baseline values (Table 2).

In hypoxemia + 120 min infusion phase, fetal blood gas values were comparable to hypoxemia + 30 min infusion phase (Table 1). In the nifedipine group, RV global longitudinal strain showed less deformation than at baseline (p=0.03) or when compared to the control group (p=0.02) (Table 2). In addition, LV IVCV (p=0.003), and RV IVRV (p<0.001) and its deceleration (p=0.001) were less than at baseline in fetuses receiving nifedipine (Table 3). Furthermore, in the nifedipine group, RVCO was lower compared to hypoxemia (p<0.05) and hypoxemia + 30 min infusion (p<0.05) phases, as well as to the control group fetuses (p<0.05) (Table 2, Figure 2). In the control group, fetal cardiac parameters remained comparable to previous phase.

In normoxemia + infusion phase, fetal pO_2 was lower (p<0.05) in the nifedipine group than in the control group. In the nifedipine group, fetal LV and RV global longitudinal strains, tissue Doppler derived indices of fetal cardiac function, as well as cardiac outputs returned to baseline level.

Comment

Principal Findings

Our focus was to investigate the effect of nifedipine on fetal cardiac function during hypoxemia. We gave nifedipine to fetal circulation, to study its direct effect on fetal cardiac function rather than those that are secondary to changes in maternal cardiovascular and uteroplacental hemodynamics. Nifedipine infusion was associated with impaired fetal RV function that manifested as reduced global longitudinal strain, decreased $IVRV_{dec}$ suggesting diastolic dysfunction, and finally as a drop in the RV output. These alterations cannot be explained by an increase in the afterload because fetal MAP decreased during hypoxemia. After fetal hypoxemia was reversed, fetal cardiac functional parameters in the nifedipine group were comparable to baseline values suggesting that hypoxemic environment triggers the detrimental effects of nifedipine on fetal RV function.

Results in the Context of What is Known

At baseline with fetal normoxemia, LV global longitudinal strain showed more deformation than the corresponding RV strain that is in agreement with previous observations [17,18]. Under hypoxemia, LV global longitudinal strain showed less deformation than during normoxemia, while hypoxemia had no effect on RV global longitudinal strain. Reduced LV global longitudinal strain during hypoxemia could be a consequence of a drop in LV preload, direct myocardial dysfunction caused by hypoxemia or a combination of the two [18]. During nifedipine infusion, RV global longitudinal strain showed a reduction in deformation, while the corresponding LV global longitudinal strain was not affected. However, during normoxemia phase when nifedipine infusion was continued, RV global longitudinal strain returned to baseline level. Our results suggest that nifedipine can have a detrimental effect on fetal RV function, especially when the fetus is hypoxemic. Hypoxemia itself, however, did not seem to alter RV global longitudinal strain, as in the control group it remained unchanged. Ventricular longitudinal strain is affected by ventricular loading conditions. In human fetuses, RV global longitudinal strain shows less deformation with advancing gestational age [19]. This is proposed to reflect increased RV afterload, i.e., fetal blood pressure that increases with advancing gestation. In the present study, fetal MAP decreased during hypoxemia, thus the effect of reduced afterload on RV global longitudinal strain should have been the opposite. Therefore, we believe that the reduction in RV global longitudinal strain was a direct effect of nifedipine itself and reflected deteriorated RV systolic function.

We found that in fetuses who received nifedipine infusion, RV IVRV and its deceleration decreased. These parameters assess the movement of the ventricular lateral wall during early diastole, when the intraventricular pressure decreases from the systemic to atrial level. The $IVRV_{dec}$ describes the ventricular diastolic function. The myocardial relaxation is an active process requiring energy to transport Ca^{2+} from cytosol into the sarcoplasmic reticulum. The release of Ca^{2+} from the sarcoplasmic reticulum is more a passive process requiring less energy [20]. Therefore, diastolic

function is impaired earlier and to a greater extent than systolic function if the energy supply is limited.

Under hypoxemia, fetal RVCO decreased significantly in fetuses who received nifedipine infusion. Ventricular output depends on diastolic and systolic function, as well as ventricular loading conditions. Fetal sheep studies have shown that RVCO can increase during hypoxemia [21]. In addition, it seems that fetal RV can maintain its function better than the LV during worsening hypoxemia and acidemia [22]. In sheep fetuses with increased placental vascular resistance and acute metabolic acidosis, RV and LV global cardiac function is preserved [23]. Furthermore, in human fetuses with severe placental insufficiency and signs of increased systemic venous pressure, weight-indexed combined fetal cardiac output is comparable to the fetuses with uncomplicated pregnancies [24]. Based on these findings, we propose that reduced RV cardiac output in the nifedipine group during hypoxemia is a result of nifedipine itself. Interestingly, these detrimental effects of nifedipine on fetal RV function disappeared when fetal normoxemia was restored.

Clinical and Research Implications

Nifedipine is widely used in pregnancies complicated by maternal hypertensive disorders. From clinical perspective, it is important to know the possible unfavorable effects of nifedipine on the fetus, especially on fetal cardiac function. This experimental model was developed to investigate the drug effects on the hypoxemic fetus, because placental insufficiency and fetal hypoxemia are commonly seen in pregnancies complicated by maternal hypertensive disorders. The main finding that nifedipine disturbs fetal RV function and ultimately RV cardiac output is clinically important, because in fetal circulation the RV is responsible for the blood flow in the fetal lower body and placenta. Furthermore, RV is the dominant ventricle in the fetal circulation during the second half of pregnancy carrying more than 50% of fetal combined cardiac output [26]. Our results suggest that hypoxemia is needed to trigger the detrimental effects of nifedipine on fetal RV, because during normoxemia and nifedipine infusion RV functional parameters, as well as RV cardiac output were

restored. We propose that human fetuses who suffer from hypoxemia and a significant placental insufficiency, i.e. abnormal umbilical artery blood flow pattern could be more vulnerable to the detrimental effects of nifedipine on fetal cardiac function. Previously, we found that beta-blockers can differently affect fetal RV and LV, i.e. maternal administration of pindolol decreased fetal left ventricular cardiac output and induced vasoconstriction in the pulmonary vasculature [27]. The next step in experimental research would be to mimic placental insufficiency by embolizing placental vasculature in order to increase placental vascular resistance. Then we could explore, whether nifedipine had more detrimental effect on cardiac function when hypoxemia is associated with significant placental insufficiency. In addition, we have to determine the mechanistic pathways that lead to fetal cardiac dysfunction following nifedipine administration. Finally, it is important to investigate human fetal cardiac function in order to understand whether the response to nifedipine will be similar to sheep fetuses.

Strengths and limitations

The main strengths of our study include that we gave the nifedipine infusion directly into the fetal circulation in relevant concentration to investigate its effects on cardiac function to avoid those alterations that could be secondary to changes in maternal hemodynamics. In addition, we used multiple different and independent ultrasonographic modalities that are validated in previous studies to examine cardiac function [28].

Our study has certain limitations. Fetal surgical intervention could constitute a major stress. However, the recovery period following surgery should be long enough for full recovery of fetal cardiovascular physiology as evidenced by normal blood gas values at baseline [29]. The experiments were performed under general anesthesia that could modify fetal cardiovascular responses to hypoxemia. However, the cardiovascular system of the newborn lamb can increase oxygen delivery in response to hypoxemia during isoflurane anesthesia. At reasonable anesthetic depth, and without

myocardial or peripheral cardiovascular disease, the newborn lamb can coordinate neural, endocrine, and local tissue responses to increase cardiovascular performance in hypoxemia [30]. Finally, validation studies in sheep fetuses have proven that invasive and Doppler echocardiographic volume blood flow calculations correlate well [28]. The intraobserver variabilities of Doppler ultrasonographic parameters of fetal sheep cardiovascular hemodynamics are comparable to those found in human fetuses during the second half of pregnancy [31,32].

Conclusion

We conclude that under hypoxemic conditions, nifedipine impaired fetal RV function that manifested as reduced global longitudinal strain, diastolic dysfunction, and as a drop in the RV output. After recovery from hypoxemia, cardiac functional parameters in fetuses receiving nifedipine were comparable to baseline values. This suggests that hypoxemic environment triggers the detrimental effects of nifedipine on fetal RV function.

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Author contributions

LA - Acquisition, analysis, or interpretation of data for the work, drafting the manuscript.

AB - Acquisition, analysis, or interpretation of data for the work, revising the manuscript critically for important intellectual content.

JL - Acquisition, analysis, or interpretation of data for the work, revising the manuscript critically for important intellectual content.

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	Baseline	**Hypoxemia**	**Group**	**Hypoxemia + 30 min infusion**	**Hypoxemia + 120 min infusion**	**Normoxemia + infusion**	***p*-group**	***p*-time**	***p*-time*group**
pH	7.30 (0.05)	7.30 (0.04)	**Control**	7.21 (0.11)	7.15 (0.12)	7.18 (0.06)	0.87	**0.001**	0.95
			Nifedipine	7.21 (0.11)	7.15 (0.14)	7.19 (0.10)			
pO_2 (kPa) ***(mmHg)***	2.76 (0.36) *20.70 (2.7)*	1.63 (0.46) *12.22 (3.45)*	**Control**	1.54 (0.38) *11.55 (2.85)*	1.49 (0.15) *11.18 (1.13)*	2.81 (0.38) *21.08 (2.85)*	0.85	**0.001**	0.09
			Nifedipine	1.64 (0.44) *12.30 (3.30)*	1.66 (0.51) *12.45 (3.83)*	2.32 (0.29)***** *17.40 (2.18)**			
pCO_2 (kPa) ***(mmHg)***	6.81 (1.03) *51.08 (7.73)*	6.84 (0.61) *51.30 (4.57)*	**Control**	6.92 (0.81) *51.90 (6.08)*	6.98 (0.97) *52.35 (7.28)*	6.82 (0.45) *51.15 (3.38)*	0.07	0.12	0.68
			Nifedipine	7.13 (0.67) *53.48 (5.03)*	7.79 (1.51) *58.43 (11.33)*	7.30 (1.02) *54.75 (7.65)*			
Base excess (mmol/l)	-0.11(2.73)	-1.17 (3.21)	**Control**	-7.00 (5.09)	-10.18 (6.01)	-9.00 (3.57)	0.37	**0.001**	0.85
			Nifedipine	-6.00 (6.06)	-8.88 (5.49)	-7.33 (4.76)			
Lactate (mmol/l)	2.28 (1.33)	3.89 (1.73)	**Control**	7.69 (3.52)	9.73 (4.01)	9.59 (4.05)	0.84	**0.001**	0.72
			Nifedipine	7.50 (2.83)	10.41 (3.40)	10.40 (3.46)			
MAP (mmHg)	40 (8)	35 (8)	**Control**	38 (13)	35 (9)	39 (7)	0.53	**0.018**	0.35
			Nifedipine	36 (4)	32 (7)	32 (5)			

Table 1. Fetal arterial blood gas values and carotid artery mean arterial blood pressure.

Values are means with SD in parentheses. *P-group* indicates the level of difference between the control and nifedipine groups, *p*-time indicates the change in measurements over time and *p*-time*group indicates the group x time interaction. * $p<0.05$ between groups in pairwise comparisons.

Table 2. Fetal heart rate and left and right ventricular functional parameters and cardiac outputs during the experiment.

	Baseline	**Hypoxemia**	**Group**	**Hypoxemia + 30 min infusion**	**Hypoxemia + 120 min infusion**	**Normoxemia + infusion**	***p*-group**	***p*-time**	***p*-time*group**
FHR (bpm)	171 (31)	167 (21)	**Control**	162 (27)	176 (16)	144 (25)	0.54	**0.046**	0.10
			Nifedipine	156 (22)	158 (32)	159 (28)			
Global longitudinal strain (%)									
Left ventricle	-17.1(3.6)#	-14.4 (2.8)	**Control**	-14.4 (3.0)	-12.7 (4.1)	-16.7 (2.6)	0.62	**0.017**	0.21
			Nifedipine	-14.6 (3.0)	-14.3 (4.1)	-15.3 (3.1)			
Right ventricle	-13.9 (3.6)	-12.4 (3.1)	**Control**	-12.6 (3.4)	-13.7 (3.8)	-14.8 (2.2)	**0.046**	0.18	0.52
			Nifedipine	-11.8 (3.4)	-9.7 (3.1)*****	-11.9 (2.7)			
Diastolic function									
Left ventricle									
IVRV (cm/s)	2.63 (1.04)	2.36 (0.91)	**Control**	2.39 (0.94)	2.90 (1.46)	2.79 (1.31)	0.18	0.30	0.32
			Nifedipine	2.03 (0.95)	1.95 (0.69)	2.70 (1.13)			
$IVRV_{dec}$ (cm/s^2)	3.40 (2.52)	2.39 (0.85)	**Control**	2.39 (0.94)	3.09 (1.83)	2.23 (0.76)	0.74	0.07	0.22
			Nifedipine	2.03 (0.95)	2.08 (0.77)	3.37 (0.94)			
Right ventricle									
IVRV (cm/s)	3.33(1.23)	2.65 (1.13)	**Control**	2.42 (0.98)	2.94 (1.45)	2.90 (1.11)	0.068	**0.001**	**0.009**

			Nifedipine	2.59 (1.13)	2.23 (0.50)	2.97 (0.48)			
$IVRV_{dec}$ (cm/s^2)	3.05 (2.03)	2.17 (0.84)	**Control**	2.19 (0.88)	2.51 (1.64)	1.97 (1.23)	0.46	**0.008**	**0.007**
			Nifedipine	2.47 (1.05)	2.75 (1.04)	3.02 (1.28)			
Systolic function									
Left ventricle									
IVCV (cm/s)	7.34 (3.67)	6.15 (3.21)	**Control**	6.12 (2.26)	6.34 (3.51)	7.01 (2.63)	0.60	**0.021**	0.40
			Nifedipine	5.14 (1.38)	4.47 (0.93)	7.85 (3.76)			
$IVCV_{acc}$ (cm/s^2)	5.40 (2.04)	4.25 (1.57)	**Control**	4.50 (1.65)	5.40 (1.54)	4.89 (2.30)	0.62	0.20	0.28
			Nifedipine	4.55 (2.07)	3.59 (1.11)	6.41 (4.69)			
Right ventricle									
IVCV (cm/s)	5.60 (2.28)	4.77 (1.79)	**Control**	4.72 (2.05)	4.03 (1.57)	5.11 (1.60)	0.44	0.12	0.76
			Nifedipine	4.39 (1.35)	4.52 (2.58)	4.27 (1.70)			
$IVCV_{acc}$ (cm/s^2)	5.84 (2.70)	4.57 (1.95)	**Control**	3.53 (1.10)	3.92 (1.34)	5.25 (1.93)	0.65	**0.001**	0.98
			Nifedipine	3.92 (1.73)	4.06 (1.93)	6.02 (2.97)			

Cardiac output (ml/min/kg)									
Left ventricle	246 (76)	210 (65)	**Control**	234 (42)	234 (64)	236 (62)	0.64	0.056	0.74
			Nifedipine	212 (93)	215 (39)	236 (89)			
Right ventricle	231 (58)	242 (91)	**Control**	242 (89)	259 (70)	248 (54)	0.085	0.096	0.74
			Nifedipine	205 (53)	168 (55)*	192 (50)			
Combined	476 (107)	455 (116)	**Control**	475 (124)	493 (107)	484 (93)	0.16	0.13	0.70
			Nifedipine	417 (119)	383 (71)	428 (72)			

Values are means with SD in parentheses. *P-group* indicates the level of difference between the control and nifedipine groups, *p*-time indicates the change in measurements over time and *p*-time*group indicates the group x time interaction. # $p<0.001$ between right and left ventricles, * $p<0.05$ between groups in pairwise comparisons.

Figure legends:

Figure 1. Timeline of the experiment.

Figure 2. Fetal right ventricular cardiac output (RVCO) in the control and nifedipine groups during the experiment.

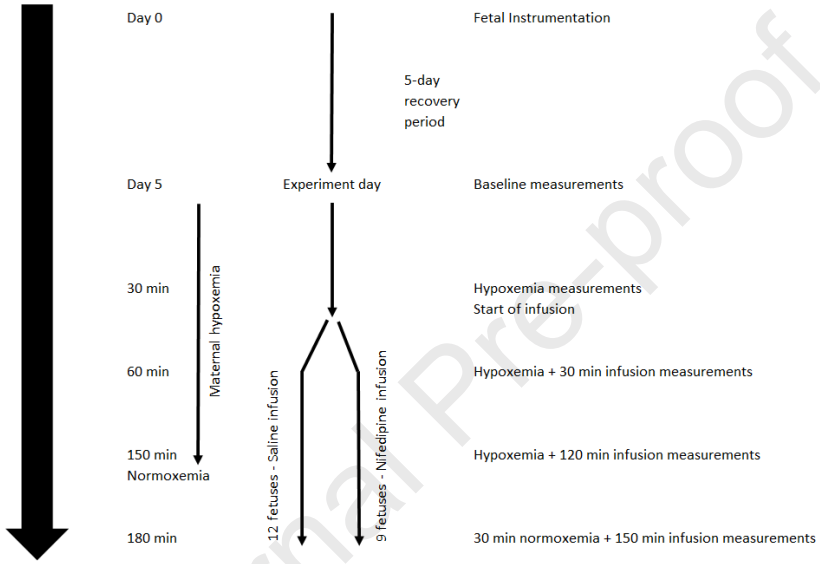

Figure 1. Timeline of the experiment