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Title: Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

Article Type: Original article

Keywords: blood flow; fetus; physiology; pregnancy; ultrasound

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Abstract: Objective: We hypothesized that nifedipine and sildenafil would have no detrimental effects on placental hemodynamics and gas exchange under fetal hypoxemia.
Methods: In 33 chronically instrumented fetal sheep, placental volume blood flow (QPlac) and umbilical artery (UA) vascular impedance were measured by Doppler ultrasonography. Fetal carotid artery blood pressure and blood gas values were monitored. After baseline data collection, maternal and fetal hypoxemia were induced. Following hypoxemia phase data collection, 12 fetuses received sildenafil and 9 fetuses nifedipine infusion, and 12 fetuses served as controls receiving saline infusion. Data were collected 30 and 120 minutes after infusion was started. Then maternal oxygenation was normalized and normoxemia phase data were collected, while infusion was continued.
Results: Hypoxemia significantly decreased fetal pO2 and blood pressure. In the sildenafil group at 30 and 120 minutes hypoxemia + infusion phases, fetal blood pressure and QPlac were significantly lower and pCO2 higher than at baseline without returning to baseline level at normoxemia + infusion phase. In hypoxemia, nifedipine did not affect fetal blood pressure or placental hemodynamics. Both in the sildenafil and nifedipine groups, fetal pO2 remained significantly lower at normoxemia + infusion phase than in the control group. Umbilical artery vascular impedance did not change during the experiment.
Conclusions: In fetal hypoxemia, sildenafil had detrimental effects on placental hemodynamics that disturbed placental gas exchange. Nifedipine did not alter placental hemodynamics in hypoxemia but disturbed placental gas exchange upon returning to normoxemia. Umbilical artery vascular impedance did not reflect alterations in placental hemodynamics.

Response to Reviewers

Reviewer #2: Understanding the mechanisms, and possible therapeutics, for at-risk pregnancies via placental insufficiency is a very important area of research. This study investigated the role that nifedipine and sildenafil could play in placental function in a state of hypoxia.

Overall, this study is very interesting and offers clarification to mechanisms that have been questioned. There are some important details that need to be added to the manuscript.

We thank the reviewer for providing excellent comments to improve our manuscript.

In the experimental design, it is not clear how long the final "normoemia + infusion" lasted. It was stated that 30 minutes after normoxia was achieved, the infusion started, but how long did the infusion last? How long did it take for the maternal O2 to return to normal? Was the end of the experiment the same for all treatment groups? At the time of the last infusion, what was the final dose?

The infusion was started after hypoxemia phase measurements were obtained, and the infusion continued until normoxemia 30min+infusion phase (final phase of the experiment) measurements were collected. We have now revised the Figure 1 in order to present the timeline of the experiment more explicit.

Maternal normoxemia was restored within 1-3 minutes in each case. We have added this information to the revised manuscript (page 6, line 22).

The end of the experiment was similar in each group.

We have provided the total sildenafil and nifedipine doses in the revised manuscript (page 6, lines 24-25)

Page 3: line 3: Insert "would" after sildenafil

This has been corrected in the revised manuscript.

Page 6: line 14-15: how were the doses determined?

We used similar sildenafil dose as in the the study by Jaillard et al that explored the effect of sildenafil on fetal sheep pulmonary vascular reactivity. The nifedipine dose was based on the studies by Blea et al and Nugent et al that investigated the the effect of nifedipine on fetal and maternal hemodynamics and blood gases in a sheep model, and pharmacokinetics and pharmacodynamics of nifedipine in pregnant sheep. This information is now provided in the revised manuscript (page 6, lines 15-18).

Table 1: Base excess—correct the spelling please.

This has been corrected in the revised manuscript.

Reviewer #3: The study by Alanne et al investigates the vasoactive agents sildenafil citrate and nifedipine on placental hemodynamics under hypoxic conditions in a chronically instrumented sheep model. They showed that sildenafil application under hypoxic conditions decreased fetal arterial blood pressure and placental volume blood flow accompanied by rising fetal pCO2 levels which could not be observed in the presence of nifedipine. However, both substances led to constant lower fetal pO2 values after reoxygenation whereas umbilical artery vascular impedance remained unaffected. They conclude that sildenafil citrate has harmful effects on the hemodynamics of the placenta due to decreasing fetal blood pressure and placental volume blood flow resulting in aberrant placental gas exchange.
The study is well conducted and written and the statistical analyses adequate. There are some minor points that need to be clarified before publication:

We thank the reviewer for providing excellent comments to improve our manuscript.

1.) How do the authors explain the differing effects of nifedipine and sildenafil?

In the revised manuscript, we have provided possible explanations for differing effects of nifedipine and sildenafil (page 10, lines 10-12 and page 11, lines 10-12).

2.) Fig 1 needs clarification:

- Why is fetal "Hypoxemia" indicated in line with maternal "30 min"? I understood that maternal (and also fetal) hypoxemia has been induced for 30 minutes at that time point. Should it not better be called "start of infusion under hypoxemia"?

It means that hypoxemia phase data collection was at that point, we have now clarified this issue in the revised Figure 1.

- Reversion to normoxic condition should be indicated on the maternal (left) side

We have revised Figure 1 according to reviewer's comments.

- Please indicate in the corresponding section Material and Methods how long it has taken until maternal normoxic (baseline) condition was achieved.

Maternal normoxemia was achieved within 1-3 minutes. This information has been added to revised manuscript (page 6, line 22).

- Please indicate in the figure and text how long normoxemia + infusion have lasted.

We have revised Figure 1 according to reviewer's suggestion.

3.) Table 2: Heart Rate and Qplac: Time p-Values are significant. Why is there no asterisk indicated? Line 16 says that Qplac were significantly lower compared to baseline in the sildenafil group. Moreover it seems that the heart rate of the sildenafil group does not return to baseline levels.

We thank the reviewer for this correction and we have modified Table 2 accordingly.

4.) Line 25 Please correct: pCO2 higher than….

This has been corrected in the revised manuscript.

5.) Discussion can be tightened as there are repetitive sections e.g. line 8-12+21-23+11-13

This has been corrected in the revised manuscript.

6.) Discussion line 6 (page 11): Which other metabolic mechanisms of the fetus could explain the low pO2 levels?

In the revised manuscript, we have provided possible mechanisms for the low fetal pO_2 levels (page 11, lines 10-12).

Alanne et al; Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

Author Agreement

Leena Alanne, Amarnath Bhide, Jonna Hoffren, Juulia Lantto, Heikki Huhta, Merja Kokki[f] Mervi Haapsamo, Ganesh Acharya, Juha Räsänen

have agreed the final submission.

Alanne et al; Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

Conflicts of interest

None declared.

Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

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Abstract

Introduction: We hypothesized that nifedipine and sildenafil would have no detrimental effects on placental hemodynamics and gas exchange under fetal hypoxemia.

Methods: In 33 chronically instrumented fetal sheep, placental volume blood flow (Q_{Plac}) and umbilical artery (UA) vascular impedance were measured by Doppler ultrasonography. Fetal carotid artery blood pressure and blood gas values were monitored. After baseline data collection, maternal and fetal hypoxemia were induced. Following hypoxemia phase data collection, 12 fetuses received sildenafil and 9 fetuses nifedipine infusion, and 12 fetuses served as controls receiving saline infusion. Data were collected 30 and 120 minutes after infusion was started. Then maternal oxygenation was normalized and normoxemia phase data were collected, while infusion was continued.

Results: Hypoxemia significantly decreased fetal pO_2 and blood pressure. In the sildenafil group at 30- and 120-minutes hypoxemia + infusion phases, fetal blood pressure and Q_{Plac} were significantly lower and pCO_2 higher than at baseline without returning to baseline level at normoxemia + infusion phase. In hypoxemia, nifedipine did not affect fetal blood pressure or placental hemodynamics. Both in the sildenafil and nifedipine groups, fetal pO_2 remained significantly lower at normoxemia + infusion phase than in the control group. Umbilical artery vascular impedance did not change during the experiment.

Discussion: In fetal hypoxemia, sildenafil had detrimental effects on placental hemodynamics that disturbed placental gas exchange. Nifedipine did not alter placental hemodynamics in hypoxemia but disturbed placental gas exchange upon returning to normoxemia. Umbilical artery vascular impedance did not reflect alterations in placental hemodynamics.

Alanne et al; Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

Highlights

- Sildenafil decreased fetal blood pressure and placental blood flow in hypoxemia.
- Nifedipine did not disturb placental blood flow in fetal hypoxemia.
- Umbilical artery vascular impedance did not reflect placental hemodynamic changes.

Effects of nifedipine and sildenafil on placental hemodynamics and gas exchange during fetal hypoxemia in a chronic sheep model

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Highlights

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Abstract

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Methods: In 33 chronically instrumented fetal sheep, placental volume blood flow (Q_{Plac}) and umbilical artery (UA) vascular impedance were measured by Doppler ultrasonography. Fetal carotid artery blood pressure and blood gas values were monitored. After baseline data collection, maternal and fetal hypoxemia were induced. Following hypoxemia phase data collection, 12 fetuses received sildenafil and 9 fetuses nifedipine infusion, and 12 fetuses served as controls receiving saline infusion. Data were collected 30 and 120 minutes after infusion was started. Then maternal oxygenation was normalized and normoxemia phase data were collected, while infusion was continued.

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Discussion: In fetal hypoxemia, sildenafil had detrimental effects on placental hemodynamics that disturbed placental gas exchange. Nifedipine did not alter placental hemodynamics in hypoxemia but disturbed placental gas exchange upon returning to normoxemia. Umbilical artery vascular impedance did not reflect alterations in placental hemodynamics.

Keywords: blood flow; fetus; physiology; pregnancy; ultrasound

Abbreviations: $Q_{Plac,}$ placental volume blood flow; UA, umbilical artery; PI, pulsatility index; UV, umbilical venous; $R_{plac,}$ placental vascular resistance; MAP, mean arterial pressure: LMM, Linear Mixed Model

1. Introduction

In pregnancies complicated by maternal hypertensive disorder, antihypertensive medication is often indicated. Calcium channel blockers, i.e. nifedipine, are commonly used in pregnancy to control maternal blood pressure. In addition, calcium channel blockers have a tocolytic effect on uterine smooth muscle and therefore they are widely used to delay delivery in preterm labor [1]. It has been shown that nifedipine does not lower fetal blood pressure [2]. However, there is some evidence that nifedipine could impair uterine blood flow, potentially even resulting in fetal hypoxemia and acidemia [3]. In human pregnancies, studies have shown that maternal nifedipine administration either does not change uterine artery hemodynamics or may cause a short-term decrease in uterine artery vascular impedance, while umbilical artery vascular impedance is unaffected [4, 5].

Recently sildenafil citrate (sildenafil), an inhibitor of phosphodiesterase-5, has gained a lot of interest, especially as a potential treatment in pregnancies complicated by early onset severe fetal growth restriction and placental insufficiency. Sildenafil has shown promise in studies of fetal growth restriction, preeclampsia, as well as in animal studies [6-13]. However, recent randomized trials revealed disappointing results suggesting that sildenafil does not prolong pregnancy or improve pregnancy outcomes in severe early-onset fetal growth restriction [14, 15]. There is even alarming evidence that maternally administered sildenafil could be harmful to the newborns by increasing the incidence of persistent pulmonary hypertension [16].

Both nifedipine [17] and sildenafil [18] cross the placenta with fetal concentrations close to those observed in maternal blood. In addition, maternal hypertensive disorders are often associated with

placental insufficiency and suboptimal gas exchange exposing the fetus to hypoxemia. Therefore, we developed a fetal sheep model to investigate the effects of nifedipine and sildenafil on placental hemodynamics when fetus is hypoxemic. We hypothesized that nifedipine and sildenafil would not detrimentally affect placental hemodynamics and gas exchange under hypoxemic conditions. The specific aims of the present study were to explore the effects of nifedipine and sildenafil on 1) fetal arterial blood pressure and placental volume blood flow, 2) fetal arterial blood gas values, and 3) umbilical artery blood flow velocity waveform pattern.

2. Materials and 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 [19] and the EU Directive 2010/63/EU [20]. A total of 33 sheep of Finnish breed with time-dated pregnancies were included in this study.

2.1 Surgical protocol

Sheep underwent surgery at 115-129 gestational days (term 145 days) for fetal instrumentation under general anesthesia that was induced with intravenous Propofol (4–7 mg/kg) and maintained with isoflurane (1.5–2.5%) in an oxygen–air mixture delivered via an endotracheal tube. Fentanyl (0.05–0.15 mg) was given as intravenous boluses when required. After laparotomy, the fetal head and neck were delivered through a small hysterotomy incision. Polyvinyl catheters were introduced into the internal jugular vein and the carotid artery. A 3-lead 28-gauge silver coated copper electrocardiogram wire (New England Wire Tech., Lisbon, NH) was inserted subcutaneously on the fetal chest. A 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 epidural catheter that was placed to the ewe before the surgery.

2.2 Experimental protocol

Following a 4-5-day recovery period, at 119-133 gestational days, the experiments were performed under general anesthesia induced with propofol and maintained by isoflurane in an oxygen/air mixture. A 16-gauge polyurethane catheter was inserted into the maternal femoral artery. Anesthesia was titrated to minimize its effect on maternal heart rate and blood pressure and allow for ultrasound examination without discomfort. The ewe was placed supine with a right lateral tilt and allowed to stabilize for 30 minutes before obtaining the baseline measurements. Thereafter, the ewe was connected to a re-breathing circuit to induce maternal and fetal hypoxemia. Maternal FiO_2 was reduced to reach the oxygen saturation level of 80%. This was verified by maternal arterial blood gas values. Hypoxemia phase data (hypoxemia) were collected 30 minutes after desired maternal oxygen saturation level was reached. After hypoxemia data collection, 12 fetuses were allocated to receive sildenafil infusion into the internal jugular vein (sildenafil citrate 0.8mg/ml) that was diluted 1:1 in saline and infused at a rate of 2.5ml/h (1.0mg/h). The sildenafil dose was selected from the study by Jaillard et al [22]. Nine fetuses were allocated to receive nifedipine infusion at a rate of 1.0 ml/h (700 µg/ml) (5 µg/kg/min). The nifedipine dose was based on the studies by Blea et al [2] and Nugent et al [23]. The control group consisted of 12 fetuses that received saline infusion, respectively. Data were collected at 30 (hypoxemia + 30 min) and 120 (hypoxemia + 120 min) minutes following commencement of infusion. After hypoxemia+120 min infusion data collection was completed, maternal oxygenation was returned to baseline level while infusion was continued. Maternal normoxemia was achieved within 1-3 minutes. Recovery phase data collection (normoxemia + infusion) was started 30 minutes after maternal normoxemia was restored (Figure 1). The infusion time was about 150 minutes in each group, and the calculated total mean dose of sildenafil was 2.5 mg and that of nifedipine was 1.75 mg, respectively. 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.

2.3 Monitoring protocol

Maternal and fetal blood pressures were continuously monitored with disposable pressure transducers (DT-XX, Ohmeda, Hatfield, UK). Fetal arterial and venous blood pressures were referenced to intra-amniotic pressure. Heart rates were determined from the arterial pressure waveforms. Fetal electrocardiogram leads were connected to the ultrasound equipment. 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).

Doppler ultrasonography of placental hemodynamics was performed at the end of each phase by a single investigator (J.R.) using the 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. Umbilical artery pulsatility index (PI) values were calculated ([peak systolic velocity – end diastolic velocity]/time-averaged maximum velocity over the cardiac cycle). Three consecutive cardiac cycles were measured, and the mean value was used for further analysis. Placental volume blood flow (Q_{plac}) was estimated by calculating umbilical venous (UV) volume blood flow as follows: 0,5 × UV maximum velocity (cm/s) ×π (UV diameter (cm)/2) ² ×60. [21] Placental volume blood flow was weight indexed. Placental vascular resistance (R_{plac}) was calculated by dividing fetal MAP by Q_{plac}.

2.4 Statistical analysis

Linear Mixed Model (LMM) was used for repeatedly measured data. Phase of the experiment and treatment versus saline were included as fixed effects, an interaction term, and individual fetus as 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: IBM Corp.). Data are presented as mean and standard deviation (SD) unless stated otherwise. Two-tailed p value < 0.05 was considered statistically significant.

3. Results

The experiments were performed at the mean (SD) gestational age of 128 (2), 126 (5) and 128 (3) days in the control, nifedipine and sildenafil groups (F=0.88, p=0.42). Maternal weight was comparable between the groups. Maternal heart rate and blood pressures did not differ between the groups during the experiment (data not shown). Mean (SD) fetal weight was 2.44 (0.28), 2.52 (0.24) and 2.38 (0.34) kg in the control, nifedipine and sildenafil groups (F=0.58, p=0.56).

During hypoxemia phase, fetal pO_2 and mean arterial pressure (MAP) decreased significantly compared to baseline with no difference between the groups. Other fetal blood gas values, as well as placental hemodynamic parameters and UA PI values were comparable to baseline (Tables 1 and 2).

At hypoxemia + 30 min infusion and hypoxemia + 120 min infusion phases, fetal MAP and weight-indexed Q_{Plac} were significantly lower compared to baseline in the sildenafil group (Table 2, Figures 2 and 3). Weight-indexed R_{Plac} did not change significantly. Furthermore, fetal pCO_2 increased significantly at hypoxemia + 120 min infusion phase in the sildenafil group (Table 1). In the control and nifedipine groups, fetal MAP, placental hemodynamic parameters and pCO_2 remained stable during the hypoxemia + infusion phases. In addition, in each group, fetal pH and base excess decreased and lactate concentration increased significantly during hypoxemia +

infusion phases with no differences between the groups. Umbilical artery PI values did not change during the hypoxemia + infusion phases in any of the groups.

During normoxemia + infusion phase, fetal MAP and Q_{Plac} remained significantly lower and fetal pCO_2 higher than at baseline in the sildenafil group (Table 2, Figures 2 and 3). In addition, in the sildenafil group fetal pCO_2 was significantly higher than in the control and nifedipine groups. At normoxemia + infusion phase, fetal pO_2 values remained significantly lower in the sildenafil and nifedipine groups than in the control group (Table 1). Fetal pH, base excess and lactate values were lower than at baseline in each group. Umbilical artery PI values and R_{Plac} were comparable to baseline values with no difference between the groups.

4. Discussion

In the present study using a chronically instrumented sheep model with intact placental circulation and fetal hypoxemia, we found that sildenafil, when given directly to the fetus, significantly decreased fetal arterial blood pressure and placental volume blood flow that were associated with a rise in fetal pCO_2 indicating abnormal placental perfusion. On the other hand, fetal nifedipine infusion did not significantly alter placental volume blood flow or fetal arterial blood pressure. However, during normoxemia + infusion phase fetal pO_2 did not return to baseline level in nifedipine and sildenafil groups. Despite significant changes in fetal blood gas values, placental volume blood flow and fetal blood pressure in the sildenafil group, umbilical artery vascular impedance did not reflect any of these alterations.

Both sildenafil and nifedipine cross the placenta and their concentrations in fetal blood are close to those values in maternal blood. In the present study, we wanted to explore the direct effects of sildenafil and nifedipine on the fetoplacental circulation rather than those secondary to changes in maternal hemodynamics. Therefore, we decided to give both drugs directly into the fetal circulation

during hypoxemia in relevant concentrations [22, 23]. Previously, we have shown that fetal sildenafil infusion does not alter cardiac function or central hemodynamics in hypoxemia [24]. Experimental studies have shown that umbilicoplacental circulation has no significant autoregulative capacity and perfusion pressure has a critical role in the maintenance of placental volume blood flow [25]. Therefore, a drop in fetal arterial blood pressure can have a profound detrimental effect on placental volume blood flow and gas exchange. On the other hand, it has been shown that sildenafil infusion into the fetal circulation did not affect fetal arterial blood pressure under normoxemic environment [22]. In addition, a previous sheep study with fetal intrauterine growth restriction induced by single umbilical artery ligation showed that maternally administered sildenafil significantly reduced uterine blood flow and decreased fetal pO_2 as well as arterial blood pressure [26]. We propose that sildenafil could interfere with hypoxemia induced fetal peripheral chemoreflex causing a drop in fetal blood pressure and placental volume blood flow. Altogether, evidence from experimental studies including the present study point out that sildenafil can have significant detrimental effects on both utero- and umbilicoplacental hemodynamics, even with intact placental circulation.

We did not find any significant change in fetal arterial blood pressure or placental volume blood flow during nifedipine infusion under fetal hypoxemia. However, during normoxemia + infusion phase fetal pO_2 remained lower than in the control group. Our observations are in agreement with a study that found only a transient reduction in uteroplacental blood flow with no change in fetoplacental blood flow by using radioactively-labeled microsphere technique when nifedipine was administered to the ewe [2]. In addition, nifedipine did not alter volume blood flows and vascular resistances of the fetal organs, except for the adrenal blood flow that increased during nifedipine administration. However, conflicting results have been reported. Maternal nifedipine infusion has been shown to increase fetal lung and skeletal muscle blood flow, while blood flow to carcass decreases, suggesting a redistribution of fetal circulation [27]. In addition, significant increases in total and regional fetal cerebral blood flow have been demonstrated with maternal nifedipine infusion [3].

It seems that at least some of these hemodynamic changes are related to nifedipine dose, because with higher doses also a reduction in uterine blood flow and fetal arterial oxygen content have been demonstrated [3]. In the present study, nifedipine infusion did not alter fetal blood gas values during hypoxemia, most likely reflecting mean arterial blood pressure and placental volume blood flow that were maintained comparable to the control group fetuses. Interestingly in nifedipine group, upon returning to normoxemia + infusion phase, fetal pO_2 did not return to baseline level that was observed in the control group fetuses. This finding is similar to that published previously [2]. The authors observed fetal acidosis and hypoxia without evident temporal blood flow changes and a relative lack of maternal hemodynamic changes strongly suggesting that other fetal metabolic mechanisms could explain this finding. It has been speculated that local discrepancies in placental metabolism caused by cellular calcium entry blockade or increased fetal oxygen utilization could lead to decreased arterial oxygen content [2].

Sildenafil and nifedipine had no significant effect on umbilical artery PI values. This agrees with a study, in which fetal growth restriction was induced by uterine artery embolization [28]. In a group with uterine artery embolization and maternal sildenafil, umbilical artery resistance index values were comparable to the control group fetuses. In contrast, clinical studies on human pregnancies complicated by maternal preeclampsia or intrauterine fetal growth restriction have reported improvement in the umbilical artery blood flow velocity waveform during maternal sildenafil therapy [6, 8, 9]. Our findings with significantly reduced placental volume blood flow, disturbed gas exchange and significant fetal metabolic acidosis combined with unaffected umbilical artery PI demonstrate the limitations of umbilical artery blood flow velocity waveform to detect significant changes in placental circulatory physiology.

Our experimental study has several clinical implications. Maternally administered vasoactive agents can also affect fetal and placental circulatory physiology. Sildenafil has shown some promise in the treatment of severe early-onset placental insufficiency. However, recent data suggests that sildenafil

when administered during pregnancy could increase the incidence of persistent pulmonary hypertension in newborns [16]. We designed this study to explore the effects of sildenafil and nifedipine on placental hemodynamics under hypoxemic conditions. Fetal hypoxemia often complicates severe placental insufficiency. The observations that sildenafil decreases fetal arterial blood pressure and placental volume blood flow and disturbs gas exchange are clinically important. These detrimental alterations occurred even in the presence of histologically unaffected placental circulation. Placental insufficiency is usually associated with reduced tertiary villous artery capacity. Furthermore, our study shows that the effects of vasoactive drugs on placental circulatory physiology cannot be explored by only investigating umbilical artery vascular impedance.

STRIDER trials conducted in different countries have revealed partially inconsistent results, but especially concerning possible harmful effects of in utero exposure to sildenafil on newborn pulmonary circulatory physiology [14, 16]. This becomes even more clinically important, because animal studies suggest that in fetal congenital diaphragmatic hernia in utero sildenafil improves lung vasculature [29]. However, in fetuses without diaphragmatic hernia sildenafil can reduce pulmonary vascular branching. Therefore, more understanding about the action of sildenafil in different pregnancy complications is urgently needed. Furthermore, as our study shows, umbilical artery blood flow velocity waveform has a limited capacity to reflect changes in the placental circulatory physiology. Future studies exploring the possible drug effects on placental hemodynamics should consider the results of the present study.

5. Limitations

The fetuses underwent surgical intervention that could constitute a major stress. However, the recovery period after surgery is long enough for recovery of fetal circulatory physiology as evidenced by normal blood gas values at baseline [30]. We performed the experiments under general anesthesia that could modify fetal cardiovascular adaptation. There is evidence that cardiovascular system of the newborn lamb can increase oxygen delivery in response to hypoxemic stress during

isoflurane anesthesia. Therefore, 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 response to hypoxemia [31]. There are some differences in placental circulatory physiology and anatomy between human and sheep fetuses. However, sheep experiments have provided invaluable information on placental hemodynamics. Validation studies in sheep fetuses have proven that invasive and Doppler echocardiographic volume blood flow calculations correlate well [32]. 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 [33, 34].

6. Conclusions

Our study shows that in hypoxemic fetus with intact placental circulation, sildenafil had detrimental effects on placental hemodynamics by decreasing fetal blood pressure and placental volume blood flow that led to disturbed placental gas exchange. On the other hand, under fetal hypoxemia nifedipine did not alter placental hemodynamics. However, it disturbed placental gas exchange when oxygenation was normalized. Umbilical artery vascular impedance did not change despite significant alterations in placental hemodynamics.

Conflicts of interest

None declared.

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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 it critically for important intellectual content

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

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

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

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

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

GA - Conception and design of the work, acquisition, analysis and interpretation of data for the work, revising it critically for important intellectual content

JR - Conception and design of the work, acquisition, analysis and interpretation of data for the work, revising it critically for important intellectual content

• All the authors approved the final version of the manuscript.

• All the authors 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

References

1. Smith P, Anthony J, Johanson R. Nifedipine in pregnancy. BJOG 2000;107:299-307.

2. Blea, CW, Barnard, JM, Magness, RR, Phernetton, TM, Hendricks, SK. Effect of nifedipine on fetal and maternal hemodynamics and blood gases in the pregnant ewe. Am J Obstet Gynecol 1997;176:922-30.

3. Harake B, Gilbert RD, Ashwal S, Power GG. Nifedipine: Effects on fetal and maternal hemodynamics in pregnant sheep. Am J Obstet Gynecol 1987;157:1003-8.

4. Guclu S, Gol M, Saygili U, Demir N, Sezer O, Baschat AA. Nifedipine therapy for preterm labor: effects on placental, fetal cerebral and atrioventricular Doppler parameters in the first 48 hours. Ultrasound Obstet Gynecol 2006;27:403-8.

5. Ulubasoglu H, Özmen Bayar U, Kaya C, Ungan B. The effect of nifedipine tocolysis on Doppler indices of the uterine and umbilical arteries. J Clin Ultrasound 2015;43:322-6.

6. Dastjerdi MV, Hosseini S, Bayani L. Sildenafil citrate and uteroplacental perfusion in fetal growth restriction. J Res Med Sci 2012;17:632-6.

7. von Dadelszen P, Dwinnell S, Magee LA, et al. Sildenafil citrate therapy for severe early-onset intrauterine growth restriction. BJOG 2011;118:624-8.

8. El-Sayed MA, Saleh SA, Maher MA, Khidre AM. Utero-placental perfusion Doppler indices in growth restricted fetuses: effect of sildenafil citrate. J Matern Fetal Neonatal Med 2018;31:1045-50.

9. Trapani A Jr, Goncalves LF, Trapani TF, Franco MJ, Galluzzo RN, Pires MM. Comparison between transdermal nitroglycerin and sildenafil citrate in intrauterine growth restriction: Effects on

uterine, umbilical and fetal middle cerebral artery pulsatility indices. Ultrasound Obstet Gynecol 2016;48:61-5.

10. Samangaya RA, Mires G, Shennan A, et al. A randomised, double-blinded, placebo-controlled study of the phosphodiesterase type 5 inhibitor sildenafil for the treatment of preeclampsia. Hypertension in Pregnancy 2009;28:369-82.

11. Trapani A Jr, Goncalves LF, Trapani TF, Vieira S, Pires M, Pires MM. Perinatal and hemodynamic evaluation of sildenafil citrate for preeclampsia treatment: A randomized controlled trial. Obstet Gynecol. 2016;128:253-9.

12. Wareing M, Myers JE, O'Hara M, Baker PN. Sildenafil citrate (viagra) enhances vasodilatation in fetal growth restriction. J Clin Endocrinol Metab 2005;90:2550-5.

13. Stanley JL, Andersson IJ, Poudel R, et al. Sildenafil citrate rescues fetal growth in the catechol-O-methyl transferase knockout mouse model. Hypertension 2012;59:1021-8.

14. Sharp A, Cornforth C, Jackson R, et al. Maternal sildenafil for severe fetal growth restriction (STRIDER): A multicentre, randomised, placebo-controlled, double-blind trial. Lancet Child Adolesc Health. 2018;2:93-102.

15. Groom KM, McCowan LM, Mackay LK, et al. STRIDER NZAus: a multicentre randomised controlled trial of sildenafil therapy in early-onset fetal growth restriction. BJOG 2019 Feb 19.

16. Groom KM, Ganzevoort W, Alfirevic Z, Lim K, Papageorghiou AT. Clinicians should stop prescribing sildenafil for fetal growth restriction (FGR): Comment from the STRIDER consortium. Ultrasound Obstet Gynecol 2018;52:295-6.

17. Prevost RR, Akl SA, Whybrew WD, Sibai BM. Oral nifedipine pharmacokinetics in pregnancy-induced hypertension. Pharmacotherapy 1992;12:174-7.

18. Russo FM, Conings S, Allegaert K, et al. Sildenafil crosses the placenta at therapeutic levels in a dually perfused human cotyledon model. Am J Obstet Gynecol 2018;219:619.e1-e10.

19. Finnish Government. Decree on the Protection of Animals Used for Scientific or Educational Purposes. 564/2013.

20. The European Parliament and the Council of the European Union. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes, 2010.

21. Acharya G, Wilsgaard T, Rosvold Berntsen G K, Maltau JM, Kiserud T. Reference ranges for umbilical vein blood flow in the second half of pregnancy based on longitudinal data. Prenatal Diagnosis 2005; 25:99-111.

22. Jaillard S, Larrue B, Deruelle P, et al. Effects of phosphodiesterase 5 inhibitor on pulmonary vascular reactivity in the fetal lamb. Ann Thorac Surg 2006;81:935-42.

23. Nugent CE, Hill M, Hursey T, Abramson FP. Pharmacokinetics and pharmacodynamics of nifedipine in pregnant sheep. Drug Metab Dispos 1991;19:754-7.

24. Bhide A, Alanne L, Rasanen J, et al. Effect of sildenafil on pulmonary circulation and cardiovascular function in near-term fetal sheep during hypoxemia. Reprod Sci 2019; 26:337-47.

25. Berman W Jr, Goodlin RC, Heymann MA, Rudolph AM. Relationships between pressure and flow in the umbilical and uterine circulations of the sheep. Circ Res 1976;38:262-6.

26. Miller SL, Loose JM, Jenkin G, Wallace EM. The effects of sildenafil citrate (viagra) on uterine blood flow and well being in the intrauterine growth-restricted fetus. Am J Obstet Gynecol 2009;200:102.e1-e7.

27. Princzkel E, Vojcek L, Lampé LG, Turnbull AC. The redistribution of the blood flow under nifedipine treatment in the sheep foetuses. Acta Paediatr Hung 1991;31:321-36.

28. Oyston C, Stanley JL, Oliver MH, Bloomfield FH, Baker PN. Maternal Administration of Sildenafil Citrate Alters Fetal and Placental Growth and Fetal-Placental Vascular Resistance in the Growth-Restricted Ovine Fetus. Hypertension 2016;68:760-7.

29. Mous DS, Kool HM, Buscop-van Kempen MJ, et al. Clinically relevant timing of antenatal sildenafil treatment reduces pulmonary vascular remodeling in congenital diaphragmatic hernia. Am J Physiol Lung Cell Mol Physiol 2016;311:L734–42.

30. De Muylder X, Fouron JC, Bard H, Urfer FN. Changes in the systolic time intervals of the fetal heart after surgical manipulation of the fetus. Am J Obstet Gynecol 1983;147:285-8.

31. Brett CM, Teitel DF, Heymann MA, Rudolph AM. The young lamb can increase cardiovascular performance during isoflurane anesthesia. Anesthesiology 1989;71:751-6.

32. Schmidt KG, Di Tommaso M, Silverman NH, Rudolph AM. Doppler echocardiographic assessment of fetal descending aortic and umbilical blood flows. validation studies in fetal lambs. Circulation 1991;83:1731-7.

33. Bernard LS, Hashima JN, Hohimer AR, et al. Myocardial performance and its acute response to angiotensin II infusion in fetal sheep adapted to chronic anemia. Reproductive Sciences 2012;19: 173-80.

34. Rasanen J, Wood DC, Debbs RH, Cohen J, Weiner S, Huhta JC. Reactivity of the human fetal pulmonary circulation to maternal hyperoxygenation increases during the second half of pregnancy: A randomized study. Circulation 1998;97:257-62.

Table 1. Fetal arterial blood gas parameters

Parameter	Baseline	Hypoxemia	Group	Hypoxemia + 30 min infusion	Hypoxemia + 120 min infusion	Normoxemia + infusion	Group *p*-value	Time *p*-value	Group x time *p*-value
pO2 (kPa)			**C**	1.5 (0.4)	1.5 (0.2)	2.8 (0.4)			
	2.8 (0.6)	1.6 (0.4)	**N**	1.6 (0.4)	1.6 (0.5)	2.3 (0.3)*	0.59	0.001	0.030
			S	1.5 (0.3)	1.5 (0.5)	2.1 (0.4)**			
Base excess			**C**	-7.0 (5.1)	-10.2 (6.0)	-9.0 (3.6)			
(mmol/l)	-1.4 (3.4)	1.9 (3.2)	**N**	-6.0 (6.1)	-8.9 (5.5)	-7.3 (4.8)	0.99	0.001	0.57
			S	-6.1 (4.6)	-11.6 (7.6)	-10.6 (7.9)			
pH			**C**	7.21 (0.11)	7.15 (0.12)	7.18 (0.06)			
	7.29 (0.05)	7.28 (0.05)	**N**	7.21 (0.11)	7.14 (0.14)	7.19 (0.10)	0.54	0.001	0.72
			S	7.19 (0.08)	7.06 (0.16)	7.09 (0.16)			
Lactate (mmol/l)			**C**	7.7 (3.5)	9.7 (4.0)	9.6 (4.0)			
	0.4 (0.2)	0.5 (0.4)	**N**	7.5 (2.8)	10.4 (3.4)	10.4 (3.5)	0.97	0.001	0.59
			S	6.4 (3.0)	10.3 (4.0)	10.8 (4.6)			
pCO2 (kPa)			**C**	6.9 (0.8)	7.0 (1.0)	6.8 (0.4)	0.007	0.001	0.41
	7.0 (1.1)	7.0 (0.8)	**N**	7.1 (0.7)	7.8 (1.5)	7.3 (1.0)			
			S	7.7 (1.0)	8.7 (2.4)**	8.4 (1.1)*			

Values are mean and (SD). Group p-value indicates the level of difference between the control (C), nifedipine (N) and sildenafil (S) groups, Time p-value indicates the change in measurements over time. Group x time p-value indicates the group x time interaction. *=<0.05 between groups in pairwise comparisons, **=<0.005 between groups in pairwise comparisons.

Table 2. Fetal heart rate, blood pressure and placental hemodynamic parameters.

Parameter	Baseline	Hypoxemia	Group	Hypoxemia + 30 min infusion	Hypoxemia + 120 min infusion	Normoxemia + infusion	Group *p*-value	Time *p*-value	Group x time *p*-value
Heart rate (bpm)	171 (27)	168 (27)	**C**	162 (27)	176 (16)	144 (25)¤	0.72	0.004	0.49
			N	156 (22)	158 (32)	159 (28)			
			S	159 (20)	150 (42)	138 (35)¤			
MAP (mmHg)	40 (8)	36 (7)	**C**	38 (13)	35 (9)	39 (7)	0.53	0.001	0.07
			N	36 (4)	32 (7)	32 (5)			
			S	33 (8)	28 (5)	28 (6)*****			
Qplac (ml/min/kg)	85 (34)	73 (33)	**C**	66 (32)	64 (29)	67 (15)	0.81	0.001	0.81
			N	64 (28)	62 (25)	64 (32)			
			S	55 (20)¤	52 (13)¤	55 (20)¤			
Rplac(mmHg/ ml/min / kg)	0.54 (0.22)	0.61 (0.30)	**C**	0.67 (0.33)	0.67 (0.39)	0.61 (0.20)	0.93	0.08	0.93
			N	0.75 (0.50)	0.60 (0.25)	0.58 (0.22)			
			S	0.69 (0.33)	0.60 (0.15)	0.62 (0.30)			
UA PI	1.6 (1.6)	1.7 (1.0)	**C**	2.8 (3.2)	2.4 (1.5)	2.2 (1.3)	0.08	0.15	0.46
			N	1.7 (0.4)	1.7 (0.5)	1.7 (0.5)			
			S	1.5 (0.3)	1.6 (0.5)	1.6 (0.7)			

Values are mean (SD). Group p-value indicates the level of difference between the control (C), nifedipine (N) and sildenafil (S) groups, Time p-value indicates the change in measurements over time. Group x time p-value indicates the group x time interaction. *=<0.05 between groups in pairwise comparisons. ¤ (p<0.05) comparison to baseline. *MAP*, mean arterial pressure; *Qplac*, placental volume blood flow; *Rplac*, placental vascular resistance; *UA PI*, Umbilical artery pulsatility index.

Figure legends:

Figure 1. Timeline of the experiment

Figure 2. Fetal mean arterial pressure (MAP) during the experiment. Data are presented as mean (SD). ¤ Indicates significant difference (p<0.001) to baseline in the sildenafil group. * Indicates significant difference between the control and sildenafil groups (p=0.03).

Figure 3. Weight-indexed placental volume blood flow (Q_{plac}) during the experiment. Data are presented as mean (SD). ¤ Indicates significant difference (p=0.007) to baseline in the sildenafil group.

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Figure 1. Timeline of the experiment

Figure 2. Fetal mean arterial pressure (MAP) during the experiment. Data are presented as mean (SD). ¤ Indicates significant difference ($p<0.001$) to baseline in the sildenafil group. * Indicates significant difference between the control and sildenafil groups ($p=0.03$).

Figure 3. Weight-indexed placental volume blood flow (Q_{plac}) during the experiment. Data are presented as mean (SD). ¤ Indicates significant difference ($p=0.007$) to baseline in the sildenafil group.

Tables 1 and 2

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			N	1.7 (0.4)	1.7 (0.5)	1.7 (0.5)			
			S	1.5 (0.3)	1.6 (0.5)	1.6 (0.7)			

Values are mean (SD). Group p-value indicates the level of difference between the control (C), nifedipine (N) and sildenafil (S) groups, Time p-value indicates the change in measurements over time. Group x time p-value indicates the group x time interaction. *=<0.05 between groups in pairwise comparisons. ¤ (p<0.05) comparison to baseline. *MAP*, mean arterial pressure; *Qplac*, placental volume blood flow; *Rplac*, placental vascular resistance; *UA PI*, Umbilical artery pulsatility index.

Figure 1.
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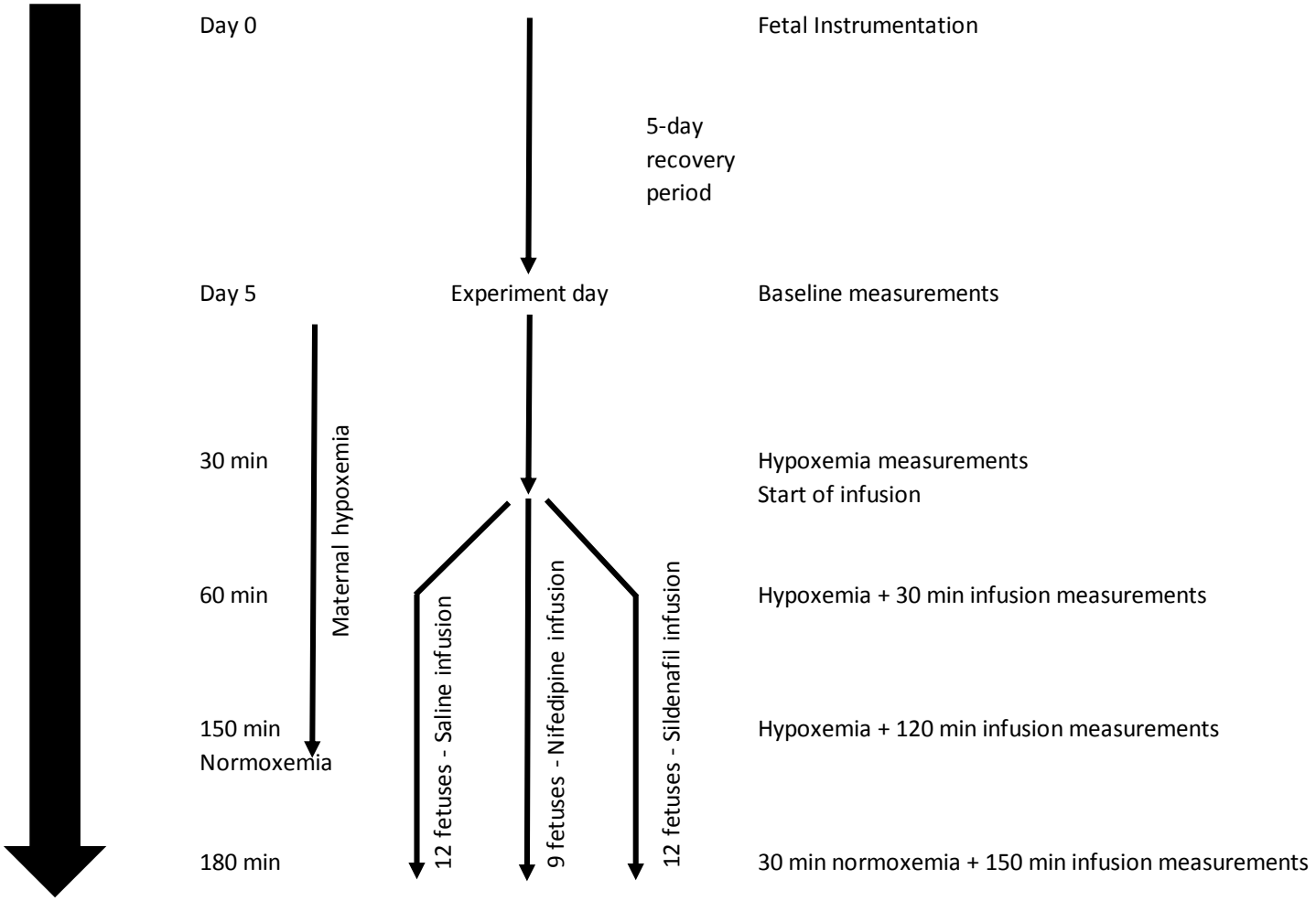

Figure 1. Timeline of the experiment

Figure 2.
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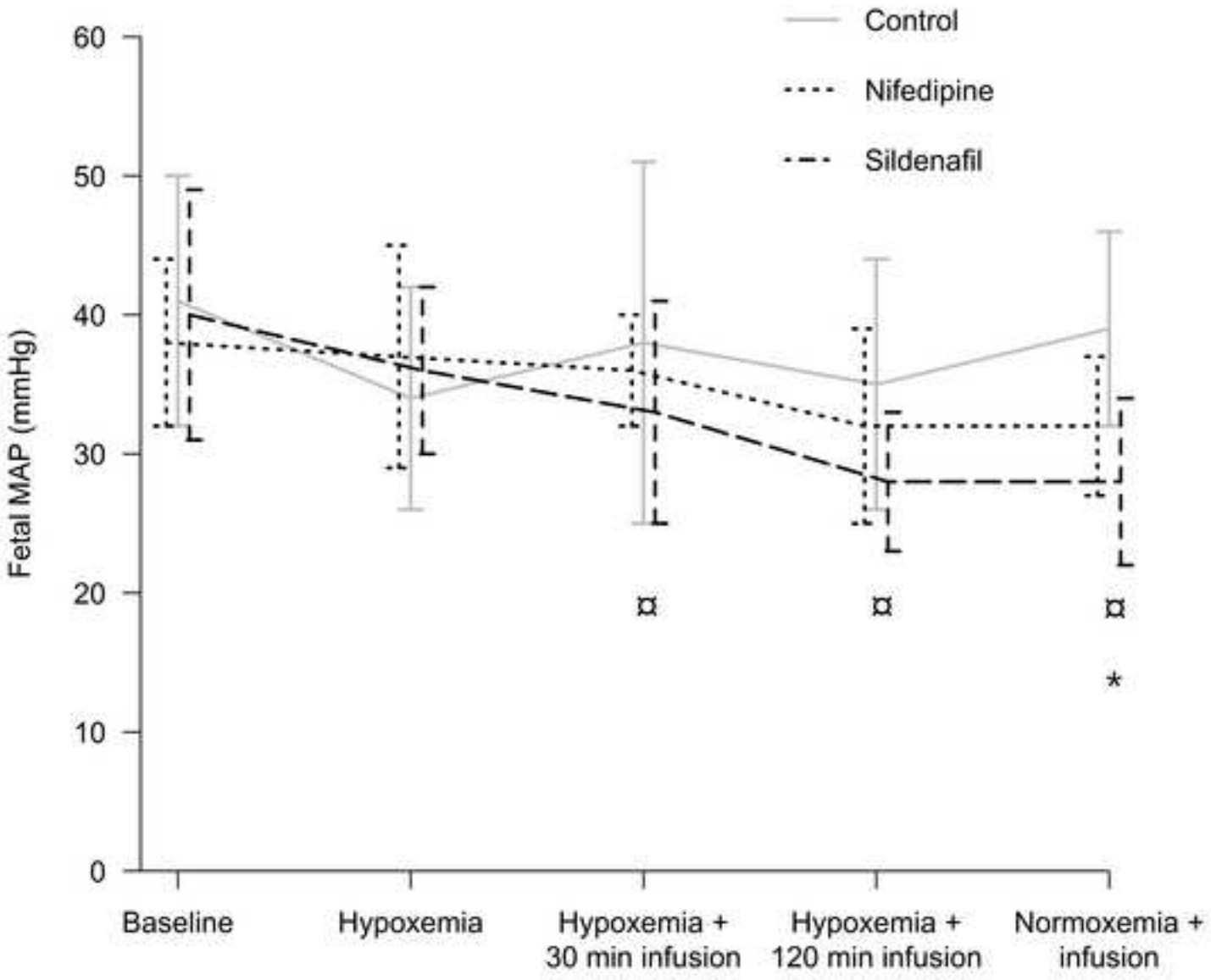

Figure 3.

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