Maternal administration of sildenafil citrate alters fetal and placental growth and fetal-placental vascular resistance in the growth restricted ovine fetus

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Abstract

Intrauterine growth restriction (IUGR) is associated with short- and long-term morbidity. Reduced placental perfusion is an important pathogenic component of IUGR; substances which enhance vasodilation in the uterine circulation, such as sildenafil citrate, may improve placental blood flow and fetal growth. The aim of the present study was to examine the effects of sildenafil citrate in the growth-restricted ovine fetus.

Ewes carrying singleton pregnancies underwent insertion of vascular catheters, then were randomised to receive uterine artery embolization (IUGR), or to a control group. Ewes in the IUGR group received a daily infusion of sildenafil citrate (IUGR+SC; n=10) or vehicle (IUGR+V; n=8) for 21 days. The control group received no treatment (n=9). Umbilical artery blood flow was measured using Doppler ultrasound and the resistive index (RI) calculated. Fetal weight, biometry, and placental weight were obtained at post-mortem following completion of treatment.

Umbilical artery RI in IUGR+V fell significantly less than in controls; the RI of IUGR+SC was intermediate to that of the other two groups (mean±SEM for control vs IUGR+V vs IUGR+SC: ΔRI, 0.09±0.03 vs -0.01±0.02 vs 0.03±0.02; F(2,22)=4.21, p=0.03). Compared to controls, lamb and placental weights were reduced in IUGR+V but not IUGR+SC (control vs IUGR+V vs IUGR+SC: fetal weight, 4381±247 vs 3447±235 vs 3687±129 g; F(2,24)=5.49, p=0.01; placental weight: 559.7±35.0 vs 376.2 ±32.5 vs 475.2±42.5 g; F(2,24)=4.64, p=0.01).

Sildenafil may be a useful adjunct in the management of IUGR. An increase in placental weight and fall in fetal-placental resistance suggests that changes to growth are at least partly mediated by changes to placental growth, rather than alterations in placental efficiency.
Introduction

Intrauterine growth restriction (IUGR) occurs when a fetus fails to achieve its genetic growth potential. These fetuses have an increased risk of significant perinatal morbidity and mortality,\textsuperscript{1,2} neurodevelopmental impairment in childhood,\textsuperscript{3} and an increased risk of adult onset chronic diseases.\textsuperscript{4} Currently, there are no clinically available treatments that can improve fetal growth. The management of IUGR pregnancies is limited to intense fetal monitoring in an attempt to determine when the fetus has maximised its time \textit{in utero} and when the risks of hypoxia and death are so high that early delivery is indicated. When IUGR is severe or of early onset, delivery may be indicated at extremely preterm gestations when the risk of perinatal complications are especially high.\textsuperscript{2} Of infants with birth weight $<10^{th}$ centile, those delivered at 30-32 weeks have a more than threefold greater chance of survival free of major morbidity, compared with those delivered at 24-28 weeks.\textsuperscript{5} A therapy facilitating expectant management and safe pregnancy prolongation has the potential to reduce dramatically both short- and long-term health and societal costs.

There are many causes of IUGR; however, the predominant etiology is placental insufficiency, caused by a failure of placental trophoblast to adequately invade and transform maternal spiral arteries in early pregnancy.\textsuperscript{6-8} The net result of this abnormal transformation is increased resistance to maternal blood flow to the placenta resulting in placental under-perfusion.\textsuperscript{7,9} In normal pregnancy, the vasodilator nitric oxide (NO) contributes to the increased vasodilation and reduced vascular resistance seen in the utero-placental circulation.\textsuperscript{10,11} The NO second messenger cGMP is enzymatically degraded by phosphodiesterases. Sildenafil citrate (sildenafil), an inhibitor of phosphodiesterase-5 (an isoform found extensively throughout the reproductive tract\textsuperscript{12,13}), is able to enhance the vasodilatory action of NO. Thus, it is hypothesised that sildenafil will improve uterine and
placental perfusion in compromised pregnancies, increasing placental exchange and fetal growth. This hypothesis is supported by ex-vivo studies of myometrial resistance arteries from growth restricted pregnancies which showed reduced constriction and enhanced relaxation following pre-incubation with sildenafil.\textsuperscript{14}

There is accumulating evidence of efficacy of sildenafil at improving fetal growth, particularly in rodents;\textsuperscript{15-18} however, evidence of an effect of sildenafil treatment on fetal growth in larger species is limited.\textsuperscript{19} It is important to study efficacy in larger species, as duration of pregnancy (and therefore timeframe for treatment) and organ development are more akin to that seen in humans, thereby increasing the potential for identifying detrimental effects, as well as any beneficial effects. The current study was designed to investigate whether maternal administration of sildenafil citrate improves growth of lambs where growth restriction had been induced by uterine artery embolization, and to explore potential mechanisms underlying any improvement in fetal growth.

Methods and Materials

Ethics Statement

All experimentation was conducted in accordance with accepted standards of humane care, with all experiments approved by the University of Auckland Animal Ethics Committee (approval number AEC 001101).

Animals

Time-mated multiparous Romney-cross ewes carrying singleton pregnancies were acclimatised to indoor individual pens. Ewes were randomised preoperatively to a control group (no uterine artery embolization) or an IUGR group that received embolization. Surgery
was performed between 96 – 100 days’ gestational age (dGA, term = 147 dGA). After an overnight fast, general anaesthesia was induced with 30 ml intravenous propofol and maintained with inhaled isofluorane. As previously described, a midline laparotomy and hysterotomy were performed and polyvinyl catheters were placed into both fetal femoral arteries and veins via the tarsal vessels. A single free-floating amniotic fluid catheter was inserted prior to closing the hysterotomy. Maternal uterine veins were catheterised bilaterally with polyvinyl catheters with silicone tips. In the IUGR groups, the main uterine arteries were catheterised via a distal arterial branch. Following closure of the maternal abdomen, a maternal femoral artery and vein were catheterised via the tarsal vessels. The ewe received a single IM dose of 450,000 IU benzathine penicillin and the fetus received 80 mg gentamycin sulphate into the amniotic fluid prior to closing the hysterotomy. All maternal and fetal catheters were checked and flushed for the first three postoperative days, then every other day for the remainder of the experiment. Fetal arterial blood samples were collected in heparinised syringes on ice before performing blood gas analysis and glucose concentrations (arterial blood gas: Alere, Waltham, MA; glucose: Yellow Springs Instruments, Dayton, OH). Fetal samples were collected for the first three days postoperatively, then twice daily during the embolization period, then twice weekly for the remaining duration of the experiment.

**Uterine artery embolization**

From 102 – 107 dGA, growth restriction was induced by up to twice daily embolization of the uterine arteries with polystyrene microspheres 20 - 50 µm diameter (Superose 12, 1:100 dilution, Pharmacia Biotech, Uppsala, Sweden) as described previously. The frequency and volume of injections was titrated against fetal PaO₂ and lactate levels, with embolization withheld if fetal PaO₂ was < 14 mmHg, or fetal arterial lactate was > 4 mmol/L.
Experimental period

Following completion of embolization, ewes in the IUGR group were randomised to treatment with sildenafil citrate (Zhuhai Jiacheng Bio-Tech, Zhuhai City, China) 150 mg/day dissolved in 54 ml sterile water (IUGR+SC group), or a visually indistinguishable infusion of 54 ml vehicle sterile water (IUGR+V group). As there are no published data on the pharmacokinetics of sildenafil citrate in the pregnant ewe, the dose of sildenafil was chosen in line with previous studies where biological effect of sildenafil was apparent. Sildenafil was administered via a continuous subcutaneous infusion over 12 hours, via a portable infusion pump secured to the ewe’s back (WalkMed Infusion, Centennial, CO, USA). The infusion bag of the pump was checked and refilled at the same time each day, and any residual fluid was given as a slow subcutaneous bolus. The infusion site, subcutaneous needle and infusion tubing was changed every third day to minimise the risk of infection and localised pooling of the infusate.

Assessment of umbilical and uterine artery blood flows

Ultrasound assessment of umbilical artery blood flow was performed prior to surgery (96 dGA), and at 107, 119 and 128 dGA. With the ewe non-sedated and standing, a free floating segment of umbilical cord was identified with pulsed wave colour Doppler. Waveforms were recorded during fetal quiescence with the angle of insonation kept to less than 50°. Recorded images were reviewed offline and umbilical artery resistance index was calculated using an average of 3 consecutive waveforms, using the formula Resistive Index (RI) = (S-D)/S, where S is the peak systolic velocity, and D the height of the end diastolic trough. Ultrasound images were obtained and analysed by an investigator blinded to treatment group for the IUGR animals.
At 107, 119, and 128 dGA assessment of uterine artery blood flow was performed via infusion of antipyprine using the Fick principle. A tracer solution containing 160 mg of antipyprine in 20 ml of saline was infused into a fetal vein at 3 ml/hr following a 4 ml bolus. Under these conditions, previous studies have shown that antipyprine reaches a steady state at 90 minutes. From 90 minutes, 4 sets of paired blood samples drawn from a maternal artery and the utero-ovarian vein were collected at 15 minute intervals. Antipyprine concentration was measured by HPLC as described previously, with a Phenomenex Kinetex column (1.7 μm C18(2) 100 Å), dimensions 150 × 2.1 mm (Phenomenex, Torrance, USA). Uterine artery blood flow was then calculated using the antipyrene steady state diffusion method with the application of the Fick principle.

**Tissue collection**

At 132-133 dGA ewes were euthanised with an overdose of intravenous pentobarbitone. The uterus was removed and opened. The fetus was removed, dried, weighed and measured, fetal organs dissected and weighed. Immediately after removing the fetus, a full thickness uterine biopsy was taken from a site proximate to umbilical cord insertion, and myometrial resistance vessels were dissected clean and stored in ice cold physiological saline solution (PSS; in mmol/L: NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgSO₄ 0.6, NaHCO₃ 11.9, KH₂PO₄ 0.5 and glucose 11; pH 7.4). The ruminant placenta is made up of a number of placentomes that develop at uterine attachments sites called caruncles. These placentomes can take a variety of morphological appearances and have been classified as A-D accordingly. Placentomes were dissected from the uterus, sorted into categories A, B, C or D, counted and weighed.
Ex vivo vascular function

Segments of myometrial resistance vessels from each sheep were mounted on a wire myograph system (Multi Myograph System 610 M, Danish Myo Technology A/S, Aarhus, Denmark) and normalised within 8 hours of dissection, as previously described. Vessels were constricted with phenylephrine (PE; 10 μmol/l; Sigma-Aldrich, New Zealand) to confirm viability, washed and equilibrated with PSS for 20 minutes. A second dose of PE 10 μmol/l was given and constriction allowed to plateau before giving a single dose of acetylcholine (Ach; 10 μmol/l; Sigma-Aldrich) to confirm endothelial integrity. Following a further washout and 30 minute equilibration period, a concentration-response curve to PE was constructed (0.1-10 μmol/l). The EC\textsubscript{80} concentration was calculated for individual vessels and used to preconstrict the arteries to construct concentration-response curves to Ach (0.1-10 μmol/l) and then sodium nitroprusside (SNP; Sigma-Aldrich 0.1-10 μmol/l). Finally, a 120mmol/l potassium solution (KPSS; in mmol/l: 10 HEPES, 24 NaCl, 124 KCl, 2.4 MgSO\textsubscript{4}, 4.9 CaCl\textsubscript{2}, 1.18 KH\textsubscript{2}PO\textsubscript{4}, 5.5 glucose; pH 7.4) was added to each vessel and the constriction allowed to plateau.

Data analysis

Statistical analysis was performed using GraphPad Prism (v 6.03) software. Data are presented as mean ± SEM, or median (interquartile range) with significance determined by one-way ANOVA and Tukey’s multiple comparisons test or by the Kruskall-Wallis test and Dunn’s multiple comparisons test as appropriate. For all analyses, a \( p \) value of <0.05 was considered statistically significant.
Fetal weights were normally distributed; histograms were constructed for each group and non-linear regression performed to obtain Gaussian distributions. The 5th percentile of the control group fetal weight was calculated and used to define fetal growth restriction.

Fetal arterial blood gas and glucose concentrations were compared between two groups (control vs IUGR) during embolization and prior to the onset of treatment (102 – 107 dGA), and then three groups (control vs IUGR+V vs IUGR+SC) following completion of embolization (110 – 129 dGA) using the appropriate non-parametric test.

For analysis of wire myography data, sigmoidal curve fitting was used to determine EC50 for each substance.

**Results**

Forty-nine ewes underwent surgery (8 controls vs 41 IUGR). Of these, 20 were euthanized prior to the completion of the study: 14 due to perioperative fetal losses (IUGR group only), 4 fetal losses during embolization (IUGR group only), and 2 fetal losses occurred during the treatment period (one each from control and IUGR+SC group). The IUGR+SC lamb that died during the treatment period had extremely poor blood gasses post embolization (pH 7.2, paCO2 69 mmHg, paO2 8.4 mmHg prior to commencing treatment) and died on the first day of treatment administration. Two ewes in the embolization group were excluded as embolization could not be adequately performed due to technical problems with laboratory equipment in the week of embolization. This left a total of 27 animals in the final analysis: 9 control, 8 IUGR+V and 10 IUGR+SC. There were no differences amongst groups in maternal weight at the beginning of the experimental period or gestational age at surgery.
At post-mortem, mean fetal weight was significantly different amongst groups ($F(2,24) = 5.48$, $p = 0.01$). Mean fetal weight in the IUGR+V group was significantly less than controls ($p = 0.01$), however, IUGR+SC treated fetuses were not significantly different from controls or IUGR+V (control vs IUGR+V vs IUGR+SC: 4,381 ± 247 vs 3,448 ± 236 vs 3,687 ± 129 g, figure 1A). The fifth centile of the control group was 3,162 g; 38% of the IUGR+V lambs and 10% of the IUGR+SC group had a weight below this at post-mortem (figure 1B).

Fetal crown rump length differed amongst groups ($F(2,24) = 4.11; p = 0.03$), with lambs in the IUGR+V group significantly shorter than those in the control group ($p = 0.04$). The CRL of lambs in the IUGR+SC group did not differ from that of either of the other two groups (control vs IUGR+V vs IUGR+SC: 57.8 ± 0.6 vs 45.1 ± 1.0 vs 45.5 ± 0.7 cm). There were no other significant differences in lamb measurements or organ weights between any of the groups when expressed as either absolute weight (table 1) or as a proportion of body weight (data not shown).

Placental weight differed significantly amongst groups ($F(2,24) = 5.52, p = 0.01$). Placental weight was significantly reduced in the IUGR+V group compared with controls ($p < 0.01$), but in the IUGR+SC was not significantly different to either of the other two groups (placental weight control vs IUGR+V vs IUGR+SC: 560 ± 35 vs 376 ± 33 vs 475 ± 43 g) (table 2). The absolute number of placentomes differed between groups ($F(2,24) = 5.22, p = 0.01$), with the IUGR+SC group having significantly more placentomes than IUGR+V ($p = 0.01$), although neither group differed significantly from control (number of placentomes for control vs IUGR+V vs IUGR+SC: 70 ± 5 vs 57 ± 4 vs 80 ± 5). Mean placentome weight differed amongst groups ($F(2,24) = 4.64, p = 0.02$) with placentome weight significantly reduced in IUGR+SC compared to controls ($p = 0.02$), but not compared to IUGR+V (mean
placentomes weight control vs IUGR+V vs IUGR+SC: 8.2 ± 0.6 vs 6.8 ± 0.7 vs 5.9 ± 0.3 g.

The IUGR+SC group had significantly lesser proportion of placentomes weighing >4 g compared to the control group ($p = 0.02$, Table 2), but the proportion of large placentomes did not differ significantly from that in the controls.

All groups had similar proportions of A, B and C placentome types (Table 2). The IUGR+V group had significantly fewer D type placentomes compared with the control group ($p = 0.01$), but not the IUGR+SC group.

**Fetal arterial blood gas and glucose concentration**

Blood sampling from fetal catheters became less reliable with increasing gestation. Due to intermittent catheter function, blood gas and glucose concentrations were not available for every animal at every time point. Prior to the onset of embolization there were no differences amongst groups in fetal arterial blood gas or glucose concentrations. Embolization resulted in a tendency to increased fetal PaCO$_2$ and reduced fetal PaO$_2$ compared to controls; this difference was statistically significant on days 105 and 106 ($p < 0.05$ vs controls day 105; $p < 0.01$ vs controls day 106). During this period, fetal arterial glucose concentrations were also intermittently lower in the embolized group with significantly lower concentrations on day 105 ($p < 0.05$) and 107 ($p < 0.01$) (figure 2D). Fetal arterial pH was significantly lower in the embolized group on day 106 ($p < 0.05$), but otherwise was similar amongst groups (figure 2A). There was no significant effect of sildenafil treatment on fetal arterial blood gas parameters or glucose concentrations (figure 2).
Uterine artery blood flow

Due to complications arising with venous catheter occlusions, only a small number of ewes completed the antipyrine protocol. Median or individual values of blood flow calculated for each of the time points are included as supplementary material.

Umbilical artery blood flow

Mean umbilical artery RI did not differ amongst groups at any individual time point (figure 3A); however, the mean change in RI for each fetus from 107 dGA (end of embolization) to 128 dGA (last measurement recorded) differed significantly amongst groups ($F(2,22) = 4.21$, $p = 0.03$) (figure 3B). Over this time period, RI fell significantly less during the treatment period in the IUGR+V group compared to controls (mean change in RI (107-128dGA) control vs IUGR+V vs IUGR+SC: -0.09 ± 0.03 vs 0.01 ± 0.02 vs -0.03 ± 0.02; $p = 0.03$).

The change in RI for the lambs of sildenafil treated mothers did not differ significantly from either of the other two groups.

Ex vivo vascular function

There was no difference amongst groups in myometrial artery maximum constriction to PE, maximal relaxation to Ach or SNP or sensitivity to any of the 3 agents (as assessed by calculation of the $EC_{50}$; figure 4).

Discussion

Our study adds to the growing body of evidence that sildenafil can improve fetal growth, and suggests that changes in growth are at least partly mediated by increased placental growth and reduced fetal-placental vascular resistance, as opposed to changes in maternal myometrial resistance vessel function.
Lamb weights of the IUGR+V group were significantly less than control group, whereas lamb weight from the IUGR +SC group were not, suggesting maternal sildenafil administration had a beneficial effect on fetal growth. Our study was powered to detect a relatively large (25%) difference in fetal weight between vehicle and sildenafil treated groups; however, the difference in lamb weights in the IUGR+V and IUGR+SC group in our study was only 7%. Although this increase is modest, in the absence of any alternative therapies even a treatment that produced small increases in fetal weight would be clinically useful. Clinically, the babies at the greatest risk of serious morbidity and mortality are those that are the most growth restricted.25 Perhaps most importantly, sildenafil reduced the proportion of fetuses with a weight less than the 5th centile of the control group, suggesting that treatment may have had a greater impact on the growth of the smallest fetuses.

Placental size is a determinant of adequate maternal-fetal exchange capacity, and small placentae are associated with both IUGR and adverse pregnancy outcomes.26,27 Placentae from IUGR pregnancies show pathological findings of acute atherosis, thrombosis, obliteration of the maternal artery lumen, and infarction.28,29 Ovine uterine artery embolization results in blockage of the small maternal arterioles supplying the placenta and results in placental infarction, and thereby provides a paradigm with relevance to human pregnancies affected by IUGR.30 We observed a large drop in total placental weight in the IUGR+V group, whereas placental weight from the IUGR +SC was intermediate to the other two groups, implying that sildenafil increased placental growth. The weight of fetal tissue produced per unit of placental weight was similar amongst groups, consistent with findings in small animal studies,16,17 suggesting that changes in fetal growth are due to an increase in placental mass rather than an increase in placental efficiency. We also observed a relative increase in number of placentomes, and an increase in proportion of smaller placentomes, in the sildenafil treated ewes. We speculate that this could represent sildenafil having a selective
effect on growth at new or relatively underdeveloped sites of placentome attachment, as opposed to increased growth of all placentomes including those that are already well established. From the findings of the present study alone, it is not possible to determine the mechanism through which sildenafil might increase placental (and therefore fetal) weight. However, there is some evidence from in vitro and in vivo small animal work that sildenafil has a pro-angiogenic function: endothelial cells show increased endothelial cell proliferation migration and organisation following culture with sildenafil,\textsuperscript{31} and sildenafil promotes angiogenesis and increased blood flow in cardiomyocytes following cardiac ischaemia reperfusion injury in rats.\textsuperscript{32} These changes were associated with an upregulation of the vascular endothelial growth factor (VEGF) system – a family of growth factors which play a critical role in all stages of placental development. It is feasible that an upregulation of the VEGF system in the placenta could promote angiogenesis, and result in the increase in placental mass observed in this study.

Our other finding of note is the changes in umbilical artery RI; these suggest that maternal sildenafil administration reduced feto-placental resistance. In human pregnancies affected by IUGR, a plateau or increase in the umbilical artery RI is a marker for fetal compromise and correlates with pathologic placental findings such as reduced volume of intermediate and terminal villi, reduced number and diameter of fetal capillaries.\textsuperscript{33,34} In this study, the fall in RI was greatest in the control group and least for the IUGR+V group, with IUGR+SC RI intermediate to the other groups, suggesting that sildenafil may help normalise placental resistance within the fetal – placental circulation. Similar observations have been made in murine studies, where pathologic UmA resistance indices were normalised when dams were treated with sildenafil during pregnancy.\textsuperscript{15} The mechanism behind a reduction in fetal-placental resistance is not clear, but could arise from either an increased sensitivity of the
feto-placental vasculature to dilators or via a pro-angiogenic effect on the fetal vasculature, resulting in increased villous growth.

Contrary to our hypothesis, we did not observe an alteration in myometrial resistance vessel sensitivity to endothelium-dependent vasodilators. This is similar to observations in a small study of preeclamptic women, but differs from that observed in mice. It is possible that sildenafil may have an effect on resistance vessel function that subsides as tissue levels of sildenafil fall. The half-life of sildenafil is relatively short (approximately 4 hours in humans); median collection time for arteries was over 5 hours after last treatment administration in the human study, and over 24 hours in our study. This is in contrast to the murine study where treatment (administered in drinking water) was available up to the point of tissue collection.

Our results differ somewhat from the only other published report of chronic maternal sildenafil exposure in pregnant sheep. Satterfield et al. (2010) used caloric restriction to induce growth restriction, treating pregnant ewes with sildenafil in thrice daily subcutaneous injections from 28 – 115 dGA. A 14% increase in fetal growth was seen with sildenafil treatment 150 mg/day, with no difference in placental weights between groups. The difference in findings between our studies may be consequent upon the different methods of inducing growth restriction, durations of treatment (87 d in Satterfield et al. (2010) vs 21 days in our study), or the different timings of treatment in relation to ovine placental development. It is also possible that the slow subcutaneous infusion used in our experimental design did not result in sufficiently high peak plasma sildenafil concentrations for maximum effect.

Irrespective of these differences, in the context of developing treatments for human pregnancy, it is worthwhile noting that neither study has shown a detrimental effect on fetal growth or fetal organ development. In particular, our study did not show a detrimental effect
on fetal oxygenation. This is in contrast to the observed reduction in fetal oxygenation and MAP accompanying a reduction in uterine blood flow seen in another study following a large intravenous bolus of sildenafil in the pregnant sheep.\textsuperscript{36}

PDE-5 is expressed in tissues other than the uterus,\textsuperscript{12} and the effect of sildenafil on other organ systems should also be considered. Maternal plasma expansion is critical for normal fetal growth, and reduced plasma volume increase is associated with IUGR.\textsuperscript{37-40} A selective increase in PDE-5 activity has been demonstrated in the inner renal medulla of the pregnant rat,\textsuperscript{41,42} where it appears to play an important role in increasing sodium and water retention, resulting in increased plasma volume. In rodents, renal infusion of sildenafil has been associated with blunted anti-natriuresis and increased diuresis,\textsuperscript{42} and oral administration has been associated with reduced maternal plasma volume.\textsuperscript{43} Changes in blood and plasma volumes are minimal in normal ovine gestation,\textsuperscript{44} so the changes described in rats may not be present in sheep. However, as we did not measure the ewe’s blood or plasma volume during this study we are unable to clarify the effect of chronic sildenafil administration on maternal fluid homeostasis. Any future studies should consider incorporating measurements of blood or plasma volume to clarify this point.

**Perspectives**

This study suggests that maternal sildenafil treatment may be associated with a small increase in fetal weight and a modest increase in placental weight in fetal lambs where IUGR has been induced by uterine artery embolization. Treatment with sildenafil was not associated with changes in myometrial resistance artery function, but was associated with a fall in umbilical artery resistance indices, suggesting increased fetal-placental perfusion. These findings, together with the absence of detrimental effect on organ growth, suggest that sildenafil may be a useful adjunct to the management of growth restricted pregnancies.
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Conflicts of interest/disclosure Statement

None

References
Novelty and Significance

What is new?
Rodent studies have suggested a positive effect of the phosphodiesterase inhibitor sildenafil citrate on fetal growth, but the mechanisms of action remain unclear. Furthermore, the duration of treatment in these studies was considerably less than would pertain in human pregnancies, so a continued beneficial effect and the absence of any detrimental effects on organ growth and development have not been proven. We have used a clinically relevant paradigm of IUGR to show a positive effect of sildenafil on fetal growth over a longer (and clinically appropriate) timeframe, and for the first time, we describe effects of medium-term sildenafil exposure on fetal-placental blood flow and myometrial resistance vessel function.

What is relevant?
There are no clinically available treatments that can improve intrauterine growth. This study provides important evidence in support of the efficacy and safety of sildenafil as a new treatment for intrauterine growth restriction.

Summary
In pregnant ewes, uterine artery embolisation resulted in reduced fetal and placental weights, and an increase in fetal-placental vascular resistance (as assessed by Doppler Ultrasound). These changes were all partially ameliorated when ewes were treated with sildenafil. Furthermore, no changes in resistance vessel function were observed, suggesting that changes in growth are at least partly mediated by increased placental growth and reduced fetal-placental vascular resistance, as opposed to changes in maternal myometrial resistance vessel function.
Keywords

Sildenafil Citrate; Fetal Development; Placental Circulation; Doppler Ultrasonography
Figure Legends

**Figure 1.**

Fetal weight at post-mortem was significantly reduced for IUGR + V compared to controls, but not for IUGR + SC compared to controls (A), suggesting a beneficial effect of sildenafil treatment on lamb weight. Distribution of lamb weights for each of the three groups (B); the dashed vertical line represents the 5th centile of fetal weight in the control group. Mean ± SEM; n = 9, 8, 10 (control, IUGR + V, IUGR + SC); *p < 0.05 (1-way ANOVA with Tukey’s multiple comparisons test). —, control; ——, IUGR + V; ---, IUGR + SC

**Figure 2.**

Fetal arterial pH (A), PaO$_2$ (B), PaCO$_2$ (C), and glucose concentrations (D) during maternal uterine artery embolization and treatment periods. Median ± interquartile range. For the embolization period, IUGR + V and IUGR + SC are combined into a single IUGR group (n = 15-18) vs control (n = 5-6). For the treatment period, control (n = 1-6) vs IUGR + V (n = 4-8) vs IUGR + SC (n = 4-9). * p < 0.05, † p <0.01 vs control (Mann-Whitney Test); ‡ p < 0.05 for IUGR + V vs control (Kruskal-Wallis with Dunn’s multiple comparisons test). ● control; ■ IUGR (IUGR + V for treatment period); ▼ IUGR + SC

**Figure 3.**

Fetal Umbilical artery RI fell as gestation advanced (A). The fall in RI between the end of the embolisation period (107 dGA) and the last point measured (128 dGA) was significantly less for IUGR +V compared to controls (p < 0.05) (B). The IUGR+ SC group did not differ significantly from either of the other groups. Mean ± SEM, n = 9, 8, 9 (control, IUGR + V,
IUGR + SC); *p < 0.05 vs control (1-way ANOVA with Tukey’s multiple comparisons test). ● control; ■ IUGR (IUGR + V for treatment period); ▼ IUGR + SC

Figure 4.

There were no differences amongst groups in maternal myometrial artery response to phenylephrine (A), acetylcholine (B) or sodium nitroprusside (C). Mean ± SEM, n = 3-5, 3-5, 4-7 (control, IUGR + V, IUGR + SC). ● control; ■ IUGR (IUGR + V for treatment period); ▼ IUGR + SC
## Tables

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<td>Front limb length (cm)</td>
<td>30.3 ± 0.8</td>
<td>29.2 ± 0.5</td>
<td>29.0 ± 0.5</td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus, placenta, liquor</td>
<td>2,925 (2,273 – 4,333)</td>
<td>2,238 (1,731 – 2,749)</td>
<td>2,585 (1,813 – 3,020)</td>
<td>0.23</td>
</tr>
<tr>
<td>Fetus</td>
<td>4,381 ± 247</td>
<td>3,447 ± 235*</td>
<td>3,687 ± 129</td>
<td>0.01</td>
</tr>
<tr>
<td>Brain</td>
<td>51.0 ± 1.3</td>
<td>47.3 ± 1.2</td>
<td>48.4 ± 1.0</td>
<td>0.09</td>
</tr>
<tr>
<td>Heart</td>
<td>30.0 (26.5 – 35.0)</td>
<td>24.5 (23.1 – 29.8)</td>
<td>29.7 (23.5 – 32.5)</td>
<td>0.13</td>
</tr>
<tr>
<td>Lungs</td>
<td>102.2 ± 9.4</td>
<td>85.6 ± 7.5</td>
<td>92.0 ± 4.2</td>
<td>0.22</td>
</tr>
<tr>
<td>Liver</td>
<td>153.5 (102.8 – 189.3)</td>
<td>118.9 (100.9 – 139.5)</td>
<td>123.9 (87.1 – 150.5)</td>
<td>0.30</td>
</tr>
<tr>
<td>Kidneys</td>
<td>28.4 ± 2.9</td>
<td>22.6 ± 1.9</td>
<td>24.0 ± 1.1</td>
<td>0.18</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.50 ± 0.07</td>
<td>0.36 ± 0.02</td>
<td>0.38 ± 0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Spleen</td>
<td>10.5 (7.0 – 22.5)</td>
<td>8.1 (5.5 – 9.3)</td>
<td>7.8 (6.0 – 12.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Thyroid</td>
<td>1.5 (1.4 – 1.8)</td>
<td>1.3 (1.2 – 1.5)</td>
<td>1.6 (1.3 – 1.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>Organ</td>
<td>Mean ± SEM</td>
<td>Median (IQR)</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>------------</td>
<td>--------------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td>Neck thymus</td>
<td>13.2 ± 2.2</td>
<td>12.4 ± 2.0</td>
<td>14.8 ± 1.8</td>
<td>0.70</td>
</tr>
<tr>
<td>Chest thymus</td>
<td>7.5 (3.3–8.6)</td>
<td>4.4 (3.8–7.1)</td>
<td>5.4 (4.3–6.2)</td>
<td>0.63</td>
</tr>
<tr>
<td>Pancreas</td>
<td>4.7 (3.7–5.0)</td>
<td>3.8 (3.5–4.3)</td>
<td>4.1 (3.0–4.7)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**Table 1. Fetal measurements and organ weights**

Mean ± SEM, or median (inter-quartile range) as appropriate; n = 9, 8, 10 (control, IUGR + V, IUGR + SC). *p < 0.05 compared with controls (1-way ANOVA with Tukey’s multiple comparisons test).
## Table 2 – Placentome weights and morphology

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IUGR + V</th>
<th>IUGR + SC</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental weight (g)</td>
<td>559.7 ± 35.0</td>
<td>376.2 ± 32.5†</td>
<td>475.2 ± 42.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Fetal weight:placental</td>
<td>7.9 (7.1 – 8.3)</td>
<td>9.1 (6.7 – 10.1)</td>
<td>8.8 (7.9 – 9.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean placentome weight (g)</td>
<td>8.2 ± 0.6</td>
<td>6.8 ± 0.7</td>
<td>5.9 ± 0.3*</td>
<td>0.02</td>
</tr>
<tr>
<td>Placentome number</td>
<td>70 ± 5</td>
<td>57 ± 4</td>
<td>80 ± 6‡</td>
<td>0.01</td>
</tr>
</tbody>
</table>

### Distribution of placentomes by morphologic subtype

<table>
<thead>
<tr>
<th></th>
<th>% A</th>
<th>% B</th>
<th>% C</th>
<th>% D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33 (17 – 45)</td>
<td>26 (20 – 39)</td>
<td>19 (8 – 27)</td>
<td>11 (1 – 34)</td>
</tr>
<tr>
<td></td>
<td>41 (28 – 62)</td>
<td>47 (31 – 58)</td>
<td>10 (0 – 20)</td>
<td>0‡ (0 – 0)</td>
</tr>
<tr>
<td></td>
<td>26 (7 – 84)</td>
<td>21 (15 – 43)</td>
<td>31 (0 – 57)</td>
<td>1 (0 – 9)</td>
</tr>
</tbody>
</table>

### Distribution of placentomes by weight

<table>
<thead>
<tr>
<th></th>
<th>% Small (&lt;2 g)</th>
<th>% Medium (2-4 g)</th>
<th>% Large (&gt;4 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7 (4 – 10)</td>
<td>15 (8 – 19)</td>
<td>79 (75 – 84)</td>
</tr>
<tr>
<td></td>
<td>10 (2 – 17)</td>
<td>15 (13 – 25)</td>
<td>70 (60 – 77)</td>
</tr>
<tr>
<td></td>
<td>16 (8 – 25)</td>
<td>23 (19 – 24)</td>
<td>63* (57 – 68)</td>
</tr>
</tbody>
</table>

*†‡: Significant results
Mean ± SEM, or median (inter-quartile range) as appropriate; $n = 9, 8, 10$ (control, IUGR + V, IUGR + SC). *$p < 0.05$, †$p < 0.01$ vs controls (1-way ANOVA with Tukey’s multiple comparisons test, or Kruskal Wallis with Dunn’s multiple comparisons test as appropriate).

‡$p < 0.05$ vs IUGR + V (1-way ANOVA with Tukey’s multiple comparisons test).