Title: Non-invasive High Intensity Focused ultrasound treatment of Twin-Twin Transfusion Syndrome: a preliminary in vivo study

Authors: Caroline J. Shaw\textsuperscript{1,2}, John Civale\textsuperscript{3}, Kimberley J. Botting\textsuperscript{1}, Youguo Niu\textsuperscript{1}, Gail ter Haar\textsuperscript{3}, Ian Rivens\textsuperscript{3}, Dino A. Giussani\textsuperscript{1}, Christoph C. Lees\textsuperscript{2,4*}

Affiliations:

\textsuperscript{1} Department of Physiology, Development and Neuroscience, University of Cambridge, CB2 3EG, UK

\textsuperscript{2} Institute of Reproductive and Developmental Biology, Imperial College London, W12 0HS, UK

\textsuperscript{3} Joint Department of Physics, Institute of Cancer Research, Sutton, SM2 5NG, UK

\textsuperscript{4} Department of Obstetrics and Gynaecology, University Hospitals Leuven, 3000 Leuven, Belgium

*Corresponding author: Christoph.lees@imperial.nhs.uk

One Sentence Summary: High intensity focused ultrasound can ablate blood flow in the sheep placenta and is potentially translatable to human twin twin-transfusion syndrome, where the invasive nature of current treatments limit their value.
Abstract: We investigated the feasibility and short term fetal and maternal effects of High Intensity Focused Ultrasound (HIFU) to non-invasively occlude placental vasculature in the pregnant sheep. Eleven sheep were instrumented and exposed to HIFU (n=5) or sham (n=6) ablation of placental vasculature through exposed uterine surface. Placental vascular flow was occluded in 28/30 targets and histological examination confirmed occlusion in 24/30. In both HIFU and sham exposures, uterine contact reduced maternal uterine artery flow but delivery of oxygen and glucose to the fetal brain remained normal. HIFU can occlude in vivo placental vasculature and ablate blood flow consistently in a pregnant sheep model. Cardiovascular and metabolic fetal responses suggest that the technique is safe in the short-term, and potentially translatable to human pregnancy.
Introduction

High intensity focused ultrasound (HIFU) (1) is a clinically approved therapeutic technique for non-invasive tissue ablation when applied to the treatment of uterine fibroids, and of bony and soft tissue cancers and metastases. Ultrasound waves generated by a shaped piezoelectric ceramic transducer which may be positioned outside the body, produce highly localised tissue destruction at depth, using a combination of thermal and/or pressure effects (2). The converging ultrasound waves pass harmlessly through overlying tissue, only causing damage within the target on which energy is focused, and where the ultrasound intensity (incident energy per unit area) is sufficiently high to create a small lesion, (typically ellipsoidal, 1-2 mm in diameter and 8-15 mm in length). When combined with diagnostic imaging to identify the target tissue and to monitor tissue responses, lesions can be placed adjacent to each-other to destroy larger volumes of tissue. HIFU’s first in-human use in fetal medicine has been described in a complicated multiple pregnancy, where soft tissue ablation of the cord insertion in a compromised fetus with twin reversed arterial perfusion sequence (TRAP) afforded a better prognosis for the surviving twin (3). The same group reported a prior unsuccessful attempt to perform this technique (4); there are currently no other reports of HIFU use in human pregnancy.

Twin-Twin Transfusion syndrome (TTTS) affects 10-15% (5) of monochorionic diamniotic (MCDA) twins, has an untreated mortality >80% (6) and is the leading cause of death and disability in twins (7). It results from abnormal vascular connections (predominantly arterio-venous anastomoses-AVAs) in monochorionic placentae which allow unequal sharing of placental blood flow (8). These AVAs can be identified using colour Doppler ultrasound (9).
Treatment to divide the twin’s circulations is recommended in severe (stage 3-4) TTTS \(^{(10)}\), where fetal compromise has already occurred \(^{(11)}\).

Fetoscopic laser occlusion of placental anastomoses to divide the fetal circulations has been developed over the last 20 years \(^{(12)}\). Although neurological outcomes at 2 years may be improved, meta-analysis has not shown an improvement in survival \(^{(13)}\). Complications are secondary to the invasive nature of the procedure as fetoscopy alone is recognised to worsen neonatal outcomes \(^{(11, 14-16)}\); this limits the use of fetoscopic laser to cases of TTTS where fetal compromise has already occurred. Furthermore, AVAs typically lie deep within the placenta \(^{(17)}\) but laser treatment is limited to surface ablation to a maximum depth of a few millimetres; residual anastomoses are visualised using colour Doppler in 15-30\% cases \(^{(18)}\). This limitation can result in recurrent disease which has a worse overall prognosis \(^{(19)}\) or a related condition, twin anaemia-polycythaemia sequence (TAPS) which is three times more common in laser-treated MCDA twins than in non-laser treated MCDA twin pregnancies \(^{(15)}\). Hence a non-invasive treatment which could divide placental circulations by occluding superficial and deep anastomoses would obviate the risks of invasive fetoscopy while offering the potential for more effective treatment. HIFU could potentially reduce both procedure-related complications and incomplete vascular occlusion, whilst widening the scope of treatment.

We have previously discussed the additional challenges of HIFU-mediated placental vascular, as opposed to soft tissue ablation. The main concerns relate to iatrogenic damage to the fetus or maternal tissues, and the ability to perform retreatments and control vessel haemorrhage \(^{(20)}\). Its use to occlude placental vasculature specifically in human or animal studies represents a different application with the potential to provide a non-invasive treatment for TTTS.
As a precursor to human clinical trials, the aim of this study was to test the feasibility and fetal-maternal safety of ultrasound guided HIFU placental vascular occlusion in a pregnant animal model. While no model can truly represent human twin placentation, the pregnant sheep provides a good substitute. Specific to this project is the requirement for a model of placental vascular anastomoses: unlike the discoid human placenta, the sheep placenta is organised into discrete regions of maternal and fetal tissue called placentomes. Placentomes are formed of enmeshed maternal and fetal villi, in which feto-maternal counter current flow and haemotrophic exchange takes place (21). Fetal umbilical vessels arise from this area of the placentome to run in the allantoic membranes forming the amniotic sac. These vessels are small in diameter (3-5 mm), and run between placentomes before joining together into larger vessels, ultimately forming the two umbilical arteries, vein and umbilical cord. In the human placenta, fetal cotyledons are recognised, with discrete villous trees of fetal blood flow where concurrent flow and haemotrophic materno-fetal exchange occurs, despite the externally continuous nature of the placental surface. The villous trees of both human and sheep placentae are similar in that they contain stem, intermediate and terminal villi of comparable structure and size (22). Hence, while the presentation of the human and sheep placentae initially appear very different, they are functionally comparable and their vasculature is anatomically similar. However, the pregnant sheep cannot provide a true model of TTTS, as vascular anastomoses between allantoic circulations in multiple pregnancy in sheep are rare (23), unlike in human monochorionic placentation where they occur in 90% of cases (24). However, fetal vessels at the point they arise from placentomes, form an acceptable mimic for vascular anastomoses in monochorionic placentae, especially the surface anastomoses which are the target of fetoscopic laser techniques. To this end, the sheep model has previously been used as a model to demonstrate intrauterine
fetoscopic laser ablation techniques (25). Unlike other experimental animal models, sheep tend to have singleton or twin pregnancies and the birth weight of the lamb is similar to that of the human baby. Furthermore, sheep and humans have comparable anatomy of the heart and vasculature, and the temporal development of the cardiovascular system is similar (26). The gestational time for the ewe is 145-150 days, around 50% of the human gestational period, but longer than many other experimental animals, meaning that techniques, timing and duration of experimentation are more easily translated from a research to a clinical context.

In this study, we have used a well-established surgically instrumented pregnant sheep preparation to investigate HIFU applied to the uterine surface to perform placental vascular occlusion, and report the efficacy of this treatment and its effects on the cardiovascular, metabolic and acid-base status of the mother and fetus in late gestation under general anaesthesia.
Results

Efficacy and safety of HIFU placental vascular occlusion

Based on comparison of pre- and post-exposure colour Doppler imaging, HIFU successfully ablated blood flow in 28 of 30 (93.3%) placental vessels in the 5 sheep in the exposed group, (example shown in fig. 1a,b). Of the 28 successful ablations, 27 were achieved using a single exposure series; retreatment was attempted in 2 of the 3 remaining placentomes and resulted in 1 further successful ablation. During exposures, hyperechoic regions (example shown in fig. 1c) were seen to develop at the HIFU focus with harmonic imaging. The appearance of two or more successive hyperechoes in an exposure series was associated with successful ablation of blood flow in 28 of 28 successful HIFU exposure series and 0 of 3 unsuccessful ones. This was a more sensitive marker for monitoring treatment with harmonic imaging than placentome structural change, which was only seen in 15 of 28 successful ablations. Gross pathological changes after HIFU exposure were observed in the central region of all 30 targeted placentomes, most notably tissue darkening (fig. 2a) and tissue pallor (fig. 2b), in some cases extending into the peripheries. Of the 30 placentomes, histological examination (H&E stained) of vessels was possible in 26 cases as damage from HIFU exposures rendered some tissue, particularly in small placentomes, unable to be sectioned. Twenty three of these contained fetal vessels with clot in the lumen, suggestive of occlusion, which was not observed in control placentomes (fig. 2c,d). These treatment outcomes are summarised in table S1.

Treatment success was assessed in this study using 3 measures. The primary measure of treatment success was absence of flow on colour Doppler (“no flow”), as this is the measure that would be available clinically to judge success and guide therapy. The study design allowed secondary confirmation to be sought from (i) macroscopic observation of damage to the targeted
region and, (ii) histological examination of damaged tissue in the targeted region. As already described, treatment success based on comparison of pre and post-exposure colour Doppler imaging indicated a success rate of 93.3%. Macroscopic evidence of damage to placentomes was observed in all 30 of the targeted placentomes, including the 2 in which flow was not successfully occluded. Histological examination of damaged tissue was possible in 26 of 30 placentomes; in 2 placentomes (<2 cm diameter) there was predominantly damaged tissue, rendering them too friable to be sectioned for H&E staining. In the remaining 2 placentomes there was no clear view of the origin of fetal vessels could be seen in the sections. Evidence of clot within fetal vessels was found in 24/26 (92.3%) specimens and was not found in 2/26 (8.7%). These were the 2 placentomes in which colour flow Doppler signals were still present on the post treatment images. A single case of vessel haemorrhage in the 30 placentomes targeted (3.3%) was associated with equipment malfunction. The automated gantry failed to move and delivered 4 exposures to the same position in the vessel wall. We were not able to resolve the haemorrhage non-invasively and the study protocol in this instance precluded invasive repair. All fetuses survived the experimental protocol and no damage to the uterus, adjacent maternal structure or fetus was observed in this study, based on external examination at post mortem.

Maternal cardiovascular, acid-base and metabolic responses to HIFU exposures

In both HIFU and sham ablation studies, there was a reduction in uterine artery blood flow by up to 30% of basal flow, secondary to increased uterine artery vascular resistance during the period during the time the HIFU and sham exposures were being applied; the maternal mean arterial blood pressure and heart rate remained unchanged throughout the procedure (fig. 3).
Given the reduction in blood flow had a similar time of onset and magnitude in both HIFU and sham ablations, the only common potentially causative event which occurred in both groups at this point in the experiment was the gentle handling and manipulation of the uterus to optimise the acoustic window. B-Mode/Doppler ultrasound was used during both the baseline and treatment phases of the experimental protocol so is unlikely to be the causative factor. Values for metabolic and acid-base status were not different between treatment groups at the start of baseline and remained predominantly unchanged during the experimental procedures involving both sham and HIFU exposures (table 1).

**Fetal cardiovascular, acid-base and metabolic responses to HIFU exposures**

The fetal heart rate and mean arterial blood pressure remained constant throughout the experimental procedure (fig. 4). Blood flow to the fetal brain was unchanged in terms of absolute volume, and oxygen and glucose delivery to the fetal brain both remained within expected parameters, and were unaltered during the experimental procedure, despite a reduced partial pressure of oxygen in the fetal blood by the end of the recovery period. (fig. 4, tables 2,3). Overall, by the end of the experimental procedure, there was a gradual deterioration of fetal acid-base and metabolic status, but there was no increase in this effect for HIFU exposures compared to sham exposures, and changes occurred at the same time-points for both groups (table 2).

There was a reduction in fetal femoral artery blood flow with an increase in femoral artery vascular resistance which occurred in conjunction with the reduction in maternal uterine artery blood flow (figs. 3,4). There was also an increase in the ratio of blood flow between the fetal carotid and femoral arteries (fig. 4).
The median duration of anaesthesia at the start of the experimental protocol (start of baseline recording) was 145 min (range 128-180 min) in the sham group and 138 min (range 125-157 min) in the HIFU group. This was not significantly different (p=0.14) and values for maternal and fetal cardiovascular, metabolic and acid-base status during baseline recordings were not significantly different between exposed and sham groups, demonstrating that this difference in anaesthesia time was not clinically significant.
Discussion

This study demonstrates the potential for the use of HIFU as a non-invasive method of placental vascular occlusion in pregnant sheep, an animal model that mimics vascular anastomoses in the monochorionic human placenta. The primary aims of this study were to assess the efficacy and safety of this technique for the mother and fetus. To this end, the recorded maternal and fetal cardiovascular, acid-base and metabolic responses secondary to ultrasound guided HIFU placental vascular occlusion were encouraging.

The main impact on maternal physiology was a modest fall in uterine artery blood flow during the treatment phase of the experimental protocol in both HIFU and sham exposure series. The only experimental feature related temporally to this fall in uterine blood flow was the uterine handling needed to alter uterine position in order to optimise the acoustic window during the treatment phase. This was necessary to optimise the path for the ultrasound beam to follow in order to produce vascular occlusion in the targeted placentomes. The effect of direct intraoperative uterine contact and handling on fetal wellbeing and physiology has not previously been reported. An acute, anaesthetic-related reduction in uterine artery blood flow secondary to maternal bradycardia and arterial hypotension has been linked to isoflurane usage (27-33), however, this response is time-dependent, and all parameters recovered to baseline within 120 minutes of start of anaesthesia in these studies (27, 28). The maternal and fetal cardiovascular parameters in our study were within normal ranges at the start of the experimental protocol (start of baseline) as would be expected based on this previously published work, and isoflurane delivery remained stable throughout the experimental protocol. It is thus unlikely to account for the fall in uterine artery blood flow observed here. Maternal heart rate, arterial blood pressure remained stable through the experimental procedure, so the primary cause of reduced flow in the
branch of the uterine artery blood flow in this setting may be the increased resistance in the uterine artery secondary to local vasospasm, rather than autoregulation due to the system-wide maternal cardiovascular alterations which have been reported under anaesthesia (34).

Fetal peripheral vasoconstriction, although classically understood as part of the response to hypoxia, may also result from fetal acidosis rather than hypoxia (35), primarily mediated by the sympathetic nervous response and maintained by endocrine mediated fetal stress responses (36). Peripheral vasoconstriction has also been described in sheep fetuses as a response to reduced uterine blood flow in the absence of hypoxia (37). Isoflurane sedation does not alter the capacity of fetal sheep to redistribute cerebral and systemic blood flow in response to reduced utero-placental flow or the development of acidosis (31). Accordingly, fetal peripheral vasoconstriction responses of the same magnitude were observed in both groups in response to the reduction in uterine artery blood flow that persisted beyond its normalisation in the recovery period. It is important to note that these responses were not worsened by the addition of HIFU placental vascular occlusion, and there was no corresponding increase in carotid flow during this period to suggest cerebral vasodilation. The cerebral vasodilation aspect of the fetal brain sparing response to hypoxia is under local control and given that the delivery of oxygen and glucose to the fetal brain was preserved within normal limits for the duration of all experiments, and there was no sign of fetal hypoxaemia throughout the procedure, this would not be expected. Therefore, the increase in the ratio of the carotid to femoral blood flow in the fetus is likely to be secondary to the fall in femoral blood flow as a result of increased sympathetic outflow in response to the uterine vasospasm, rather than being representative of cerebral vasodilation and peripheral vasoconstriction in response to fetal hypoxia (38).
While fetal oxygenation remained within normal limits for the duration of the procedure as a result of the elevated oxygen content of gas used for maternal mechanical ventilation, there was a reduction in the fetal partial pressure of arterial oxygen, the saturation level of oxygen and the carotid delivery of oxygen to the brain between the beginning of the baseline and the end of the recovery period. These changes were a gradual trend and not different between HIFU and sham groups; in this context they are more likely to represent gradual fetal deterioration under anaesthetic than a primary effect of HIFU exposures. Mechanical ventilation was used to maintain the ewes in an isocapnic state despite the need for periods of breath holding; however a mixed respiratory and metabolic fetal acidosis still developed. Transfer of oxygen from mother to fetus across the placenta relies on the double Bohr effect, where elimination of carbon dioxide from the fetal circulation drives maternal oxy-haemoglobin disassociation and increases the affinity of fetal haemoglobin for the released oxygen. Anything that reduces fetal elimination of carbon dioxide, resulting in a fetal respiratory acidosis, also reduces the placental transfer of oxygen. Separate to our results, progressive fetal respiratory acidosis secondary to rising fetal partial pressure of carbon dioxide has been reported in the anaesthetised fetus regardless of concomitant operative procedures or fetal challenges (27, 28), and the level of $P_aCO_2$ at the end of the recovery period is comparable to other published values for this duration of isoflurane anaesthesia. It has been reported that this acidosis is fully reversed within 24 hours (27-29, 33, 39). We suggest that these changes in fetal pH are what underlie the trend to reduced oxygenation seen in our results.

Carbon dioxide is generated by the fetus at a steady rate and is eliminated from the fetal circulation by diffusion across the placenta. (40) Elevated maternal $P_aCO_2$ causes steady state equilibration (Fick’s first principle) to reset to a higher baseline, eliminating less CO$_2$ from the
fetus. (40) While there was no increase in maternal \(P_a\)CO\(_2\) observed during the experimental protocol, the maternal levels at baseline were above the normal range of a non-anaesthetised sheep. Ventilating sheep in the recumbent position and their increased alveolar dead space compared to humans make CO\(_2\) elimination less effective from the ovine compared lungs under anaesthesia (41-43) resulting in a mild maternal respiratory acidosis.

The placental exchange rate of CO\(_2\) is also affected by the supra-physiological \(P_a\)O\(_2\) in the mother and fetus. The Haldane effect describes the increased capacity of deoxygenated haemoglobin to buffer CO\(_2\) compared to oxygenated haemoglobin (44), and has been calculated to account for 46% of placental CO\(_2\) exchange (40). The artificially elevated levels of oxygenated haemoglobin in both mother and fetus reduce the magnitude of the Haldane effect in this setting, and so further reduce the elimination of CO\(_2\). CO\(_2\) diffusion across the placenta is limited by uterine blood flow (41) as it is highly soluble (44) so the additive effect of reduced uterine artery blood flow during the period of uterine manipulation accelerates the increase in fetal CO\(_2\) accumulation. Decreases in fetal pH in our results are augmented the peripheral vasoconstriction observed: lactate is a product of anaerobic respiration and is produced in greater quantities by the under-perfused fetal tissues during peripheral vasoconstriction, particularly the muscle bulk of the hind limbs, and was seen to increase by the recovery period of the experiment, contributing to the metabolic acidosis (45).

Collectively, these findings suggest an appropriate fetal defence response allowing compensation for a non-hypoxic intrauterine challenge, rather than evidence of a pathological process or fetal distress resulting from HIFU, or the effect of prolonged anaesthesia. Given that HIFU is already in limited use in human pregnancy for the treatment of TRAP sequence (3) these findings already have relevance to clinical obstetrics. It should be noted that these were healthy
fetuses at the time of HIFU or sham exposure and that the effects on a fetus already compromised by TTTS may be different. However, one aim of developing a non-invasive method to divide fetal circulations is to reduce the risks associated with the invasive nature of current therapies and to allow earlier intervention before such fetal compromise occurs.

This study has demonstrated that placental vessels can be identified using colour Doppler ultrasound in the pregnant sheep model, and this allowed targeting using a HIFU therapy system with integrated diagnostic ultrasound guidance. Non-invasive colour flow Doppler ultrasound has previously been shown to improve the accuracy of targeting the HIFU focus on the vasculature, over that achieved using surgical exposure and visual identification of blood vessels (46). Targeting accuracy worse than 3 mm (47) can lead to failed vascular occlusion and injury to adjacent structures such as bowel (48, 49), nerves (50) or other vessels (51). Our treatment protocol, which places a linear track of 4-7 exposures across each vessel, involves a 6-12 mm linear movement of the automated gantry across the intended target and therefore should be tolerant of a small degree of inaccuracy in targeting placental vessels at their origin within the placentome. Placental vessels can be readily identified by Doppler Ultrasound in sheep, and Doppler velocimetry correlates well with absolute flows measured invasively in these vessels (52, 53). Arterio-arterial and arterio-venous anastomoses in human monochorionic placentae have been successfully identified on ultrasound. Both are located within the placenta using colour Doppler waveform interrogated with pulsed wave Doppler to identify the type of vascular anastomoses. Arterio-arterial anastomoses appear easier to locate with a sensitivity of 85% and specificity of 97.3% when compared to placental injection studies (54). Arterio-venous anastomoses are more challenging, with sensitivity of between 25-50% when compared to placental injection studies (9, 55, 56). In all cases, identification was easier with an anterior
placenta, which is also a more accessible target for HIFU exposures than a posterior placenta due to limitations of the fixed focal of any given HIFU transducer.

The treatment protocol used shows that HIFU can consistently (93.3%) ablate in vivo placental vasculature blood flow in a pregnant sheep model, which has clinically relevant dimensions. While the protocol used did not achieve occlusion in every target, it is strength of our technique that treatment success and failure can be assessed real-time by the same modality (namely colour Doppler) that is used to target HIFU, and residual anastomoses may be suitable for immediate retreatment. Residual anastomoses are identified by colour Doppler imaging in 15-30% of cases following laser therapy (18) and may lead to recurrent disease with a worse overall prognosis (19) or a threefold increased incidence of a related condition, TAPS (15). However these residual anastomoses may not be identified at the same time as laser treatment and would require a further invasive treatment to resolve, which is currently not recommended. Recent developments in fetoscopic laser therapy have moved from selective coagulation of vessels only where they crossed the “vascular equator” toward bipartition of the placenta. In this technique modification, laser ablation of placental tissue is used to join the sites where vessels have been coagulated and create a physical separation between the twins’ circulations. It is reported that this improves neonatal survival and decreases rates of recurrence and TAPS, however they also report an 11.5% double twin loss rate typically related to the invasive nature of fetoscopic laser (57). Although the anatomy of the sheep placental lends itself to selective coagulation of vessels as the placental tissue is discontinuous, the automated gantry used is capable of precision placement of exposures to form a confluent line of tissue destruction along a predetermine track, such as would be required for bipartition of the placenta, making either approach feasible.
HIFU vascular occlusion typically requires higher levels of energy than ablation of soft tissue (20), and carries with it the potential complications of vessel rupture and haemorrhage, attributed to rapid changes in tissue pressure (48, 50, 58) or accumulation of excessive thermal energy in the vessel wall (59-61). This presents the possibility that the levels of energy required to occlude vessels may also cause vessel wall rupture. In our optimisation studies, ultrasound exposure intensities higher than used in this study did produce vessel haemorrhage (62) and maximum thresholds were applied, leading to the optimised protocol presented here. These safeguards meant that the single incidence of vessel wall rupture observed in this study was associated with non-movement of the gantry, resulting in over-exposure of a single region of the vessel wall. This happened only after 4 repeated exposures in the same location, suggesting a large safety margin in the upper dose threshold. By limiting the size of the target volume, and so the total dose delivered to the tissue, our protocol is able to successfully and consistently occlude placental vasculature in this setting, without crossing the threshold at which vascular rupture and haemorrhage occurs. Larger vessels are typically protected from rupture by their higher flow with greater cooling effect (63); one of the treatment failures was an attempt to occlude a larger vessel (8mm diameter), and while unsuccessful was not associated with vascular haemorrhage. Concerns have previously been expressed about repeat exposure of vessels leading to vascular rupture (59-61). In this study, only 2 treatments were repeated, thus limiting our scope to discuss the value and safety of retreatment in this setting. The first was successful, although tissue damage was seen to spread into the peripheries reaching the capsule of the placentome. This might be considered to have breached a theoretical “safety margin” designed to protect adjacent structures. Despite this limitation, there was no maternal, uterine or fetal damage or damage to adjacent placentomes. The second retreatment attempted to ablate flow in a case of vessel
haemorrhage, and was not successful in either ablating flow or in resolving the vessel haemorrhage. This suggests that an additional protocol of HIFU treatment from the one currently used should be applied for the case of inadvertent vascular haemorrhage, and this will be a focus of future studies prior to human application.

The energy levels required to occlude placental vasculature also present the possibility of pre-focal (maternal skin, abdominal fat, uterus) and post-focal (fetus) damage to structures in the path of the ultrasound energy, although there were no such complications in this study. There is also the possibility of lateral thermal spread outside the intended focal zone, as with any energy source that heats tissue, although HIFU exposures of soft tissue typically produce sharply demarcated lesions (64). Again, there were no such complications in this study. There are without doubt important technical considerations with regard to appropriate case selection and careful treatment planning of HIFU exposures to minimise these risks. However, these should be balanced against the potential benefits to mother and fetus of not invading the intra-uterine space. Other potential difficulties still remain to be addressed before a human treatment could be implemented. The protocol, transducer and control software used in these preliminary experiments are not yet optimised for use in human pregnancy, and the need for an adequate acoustic window following surgical instrumentation, meant that HIFU was applied directly through the uterine surface rather than through the maternal skin. Delivering HIFU energy truly non-invasively (through intact skin) to achieve vascular occlusion is an essential challenge still to be met and will need to be the subject of future experimental studies either in the sheep or other large animal model. As previously discussed, placental vascular anastomoses can be detected non-invasively by colour Doppler, and as demonstrated in our results colour Doppler is an appropriate targeting and treatment monitoring modality for HIFU exposures. The work of
Okai et al (3) demonstrates that adequate HIFU energy can be delivered into the intrauterine space to ablate soft tissue at the cord insertion in human pregnancy, demonstrating the feasibility of our intended work.

Another key feature of translating these techniques to human pregnancy will also involve greater understanding of the mechanisms by which vascular occlusion is produced in this protocol, to allow customisation of any potential treatment system for human pregnancy to best exploit them.

Previously published studies demonstrate that HIFU can interact with blood vessels to produce vascular occlusion by thermal mechanisms (20). Tissue heating can cause shrinkage of vessel walls (65), narrowing of vessel lumen (66), and/or fusion of the walls in a closed position (67). HIFU can also damage the vascular endothelium, producing occlusive thrombus that leads to permanent obliteration of the vessel through chronic inflammatory processes (68, 69). The methods used to assess treatment success suggest that tissue heating is an important feature of achieving successful vascular occlusion in this model. Hyperechoic regions, as seen at the HIFU focus in our targets, are associated with bubble formation due to tissue water boiling (70). Development of hyperechoic regions during two or more successive exposures appeared to be a sensitive and specific marker of successful vascular occlusion, compared to the observation of structural change of the placentome, which was not a good indicator of vascular occlusion. Evidence of tissue heating was seen macroscopically where tissue pallor (suggestive of tissue denaturation) occurred in the central region of treated placentomes. Histologically, both shrinkage of vessel lumen and occlusion of vessel lumen with clot were observed. Together, these features suggest that achieving tissue heating within the placentome is an important process in achieving vascular occlusion.
Additional pathological findings related to tissue darkening, suggestive of extravasation of blood from microvasculature into the tissues (71), and histological evidence of extravasation and acellular debris within the HIFU damaged areas of tissue. Both heating and pressure can damage tissue and these effects are visible as regions of tissue darkening, and as acellular debris on histological examination (72). This suggests that mechanical damage of tissue surrounding the vessels targeted may also be occurring, although if so, it is unclear if this would contribute to achieving occlusion, or is a bi-product of energy delivery to the tissue. These mechanistic questions will need to be the subject of further research to aid in future system design.

In summary, data collected to investigate the safety of ultrasound guided HIFU placental vascular occlusion showed an effect on the fetus from both the general anaesthesia, and the uterine manipulation required by the experimental protocol. Neither caused fetal hypoxia or distress. These effects were no different in HIFU and sham exposures. We would not expect to use general anaesthesia or direct uterine contact when translating this technique into human treatments, so we would not expect these effects in human fetuses. Despite the need to apply HIFU non-invasively and observe maternal and fetal outcomes over a longer period, these initial feasibility studies demonstrate the utility of ultrasound-guided HIFU to target and selectively occlude placental blood vessels in vivo with a 93% success rate. This raises the prospect of non-invasive HIFU treatment of TTTS, and other related conditions resulting from abnormal placental vasculature, such as twin reversed arterial perfusion (TRAP) sequence and TAPS in human pregnancy.
Materials and Methods

Study design: This animal study was designed to assess the efficacy, materno-fetal responses and safety of using high intensity focused ultrasound (HIFU) to non-invasively occlude placental vasculature compared to sham treatment in anaesthetised animals. A total of 11 pregnant sheep were used in the study (5 HIFU treated, 6 sham controls) and there was no randomisation or blinding. The study was powered to detect a difference in means of ≥ 2.5 at α = 0.05 with a power of 80%, based on past published data of chronically instrumented sheep fetuses. The primary efficacy endpoint was achieving vascular occlusion; the primary safety endpoints were detection of uterine and fetal burns or placental haemorrhage. Maternal and fetal responses were measured through cardiovascular, acid-base and metabolic criteria. All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and were approved by the Ethical Review Committee of the University of Cambridge.

Surgical preparation: Eleven pregnant Welsh mountain sheep with singleton fetuses at 116±2 days of gestation (0.8 of gestation; term ~147 days) were used in this study. All animals were fasted for 24 hours prior to operation and surgery was performed under aseptic conditions. Anaesthesia was induced with alfaxalone 3mg/kg (Alfaxan©, Jurox) and maintained with isoflurane (1.5-2.5% in 4:1 O$_2$:N$_2$O) throughout the operation. Maternal heart rate, oxygen saturation and end-tidal carbon dioxide (EtCO$_2$) were monitored non-invasively throughout the period of anaesthesia and EtCO$_2$ was maintained at <6% during ventilation. The ewe was maintained in left lateral tilt and maternal mean arterial blood pressure was maintained above 60 mmHg with a crystalloid infusion of 5 ml·kg$^{-1}$·hr$^{-1}$ where indicated. A midline incision was made in the abdomen of the ewe to expose the uterus, and hysterotomy was performed, with attention being paid to conservation of amniotic fluid, as described elsewhere (73) for
instrumentation of the fetus. Fetal arterial catheters (internal diameter 0.86 mm, external
diameter 1.52 mm, Critchley Electrical Products) were introduced intraoperatively via the fetal
carotid and femoral arteries unilaterally, and advanced into the ascending and descending aorta
respectively (38). A third catheter was secured to the fetal hind limb for monitoring of
intrauterine amniotic pressure. Time-transit flow probes were placed around the contralateral
fetal carotid and femoral arteries (2mm aperture, R-series, Transonic Systems Inc.) and on a
main branch of the maternal uterine artery at the level of the cervix (4mm aperture, S-series,
Transonic Systems Inc.). Arterial and venous catheters were introduced into the maternal
femoral artery and vein and advanced into the maternal descending aorta. The hysterotomy
incisions were closed but the rectus sheath remained open to allow direct access for the HIFU
probe to the uterine surface.

**Experimental protocol:** Physiological values of arterial blood pressure and flow were
recorded using a customised data acquisition system, the Cambridge Data Acquisition System (CamDAS). These data were converted into absolute physiological values by IDEEQ data
recording software at a sampling rate of 500 kHz (IDEEQ, Maastricht Instruments), and was
available thereafter for offline data analysis. This system allowed continuous recording of
arterial blood pressure in the fetal ascending and descending aorta, and the maternal descending
aorta, and blood flow in the fetal carotid and femoral arteries and the maternal uterine artery
during the experimental procedure. Mean values for sequential 1 minute epochs throughout the
recording period were generated for cardiovascular data using Labchart 7 Pro (AD Instruments Pty Ltd.). The experimental protocol was performed within 30 minutes of completion of surgery
for maternal and fetal instrumentation while the animal remained under anaesthesia. The
experimental protocol was planned to start 150 minutes after the induction of anaesthesia. The
Experimental protocol was divided sequentially into: (i) a baseline period of 30 minutes during which the uterus was not manipulated and a static water-bag (supported on a gantry) containing approximately 3 L of degassed water and the diagnostic and therapeutic transducers was in contact with the uterine surface, and during which placental vasculature was mapped using B Mode and colour Doppler ultrasound imaging (P10-4, Z. One Zonare or P10-4 Toshiba Powervision 7000); (ii) 30 minutes of HIFU exposure/sham exposure of placental vasculature (a total of 6 placentomes targeted per animal; only a single vessel targeted per placentome). This phase included gentle manipulation of the uterus to optimise the acoustic window; (iii) a 30 minute recovery period, after which the animals were euthanized by terminal anaesthesia (fig. S1).

**HIFU Protocol:** HIFU was applied directly through the uterine surface, through the degassed water filled bag suspended from an arm on a positioning gantry. The Sonic Concepts H148MR transducer used (frequency 1.66 MHz, 64 mm diameter, 63 mm focal length, 19 mm central aperture for ultrasound imaging, focal diameter 1.2 mm, focal length 8.9 mm) was held in position within the water bag on an automated 3D positioning gantry (fig. 5a). A laptop computer was used to run a graphical user interface (GUI) in MATLAB (MATLAB R2013a, Mathworks) to control (i) the automated gantry position (ii) the signal generator settings and (iii) the timing of, and interval between exposures and (iv) to log the signal generator voltage setting, the 3D gantry position, and start time of each exposure. A single line of HIFU exposures was made, using the motorised gantry, across the target vessel in the central region of each placentome (fig. 5b). This was identified using a P 10-4 Zonare ultrasound imaging probe centrally mounted behind the HIFU transducer. Exposure conditions were: 4-7 exposures of 5 s duration, placed 5 s and 2 mm apart at a free-field $I_{SPTA}$ of 5000 ± 750 W.cm$^{-2}$ (table S2). This
reflects a HIFU protocol previously optimised for exposure power, timing and spacing in a preliminary group of experimental sheep, described elsewhere (62). Tissue responses were recorded using tissue harmonic imaging (8.0 MHz B Mode) during exposures. This monitored the development of hyperecho, and allowed observation of structural changes in the placentomes. Three second video clips were recorded (8-10 Hz, ~30 frames) during each exposure for offline analysis. Placental vasculature was assessed immediately before and after HIFU exposure using colour Doppler. Use of the automated positioning gantry ensured that pre- and post-treatment images were collected at the same 3D position, and still image frames were saved for offline comparison. Treatment was deemed to be complete when “no flow” was detectable on colour Doppler post treatment using the lowest velocity scale setting and pre-gain settings (to avoid colour field saturation). This was assessed following the end of each individual exposure series and before a subsequent exposure series was commenced. If occlusion was incomplete, re-ablation of the same target using the same protocol was attempted once, if judged safe to do so. Mechanical ventilation pauses of up to 90 s were required during each HIFU exposure series as respiratory movement could lead to mistargeting. Ventilation was planned to be resumed before the end of a HIFU exposure series if maternal EtCO₂ rose to >8% or SpO₂ fell to <94%, although this did not occur.

**Post mortem and Histology:** Green dye was injected under ultrasound guidance into tissue adjacent to exposed placentomes for post-mortem identification. Animals were sacrificed using pentobarbitone sodium 120mg/kg by rapid intravenous injection (Pentoject®, Animalcare) at the completion of the HIFU protocol (within 4 hours of its start) and a post mortem was conducted to identify exposed placentomes, and any iatrogenic harm to mother (examination of adjacent organs) or fetus (external examination). All treated, and a smaller number of unexposed
control), placentomes were dissected, examined for gross pathological changes, photographed and then immersion fixed in 20% formaldehyde for 5 days prior to embedding in paraffin wax. Ten micrometre sections were stained with Haematoxylin and Eosin and these were examined under the microscope for morphological changes.

**Blood sampling regime:** Blood samples were taken from the maternal femoral artery, and the fetal femoral and carotid arteries: (i) at the start of baseline (-30 min); (ii) at the start of HIFU/sham placental vascular ablation (0 min); (iii) during and after exposures (15 min and 30 min) and (iv) at the end of the recovery phase (60 min) (fig. S1). These were used to determine acid-base status, partial pressures of oxygen and carbon dioxide (ABL5 Blood Gas Analyzer, Radiometer), and haemoglobin, haematocrit and oxygen saturation of the blood (ABL80 Flex, Radiometer). Blood glucose and lactate concentrations were obtained from an automated analyser (YSI 2300 Stat Plus, Yellow Springs Instruments). Knowledge of arterial blood flow in a vessel (ml·min\(^{-1}\)), of glucose and of its calculated oxygen content at the corresponding time point allow calculation of oxygen and glucose delivery by that blood vessel at that instant, as previously described (74).

**Statistical analyses:** Continuously recorded cardiovascular data were converted into mean values for each sequential minute (“minute means”) of the experimental protocol. Minute means and absolute values from blood sampling are expressed as mean ± standard error of the mean (SEM). Summary measure analysis was applied to the cardiovascular data to generate areas under the curve for statistical analysis (75). Normality was assessed using the Shapiro Wilks test, and a repeated measure, two-way ANOVA (variables of time and treatment group) for parametric values and Kruskall-Wallis test for non-parametric values was applied. In the repeated measure ANOVA, if a significant interaction was demonstrated for time or treatment,
post hoc Tukey’s or Sidak’s test was applied. Statistical significance was accepted when p < 0.05.

List of Supplementary Materials:

Figure S1: Summary of experimental timeline

Table S1: Summary of treatment outcomes

Table S2: Summary of exposure conditions
References:

Acknowledgments:

Funding: supported by Action Medical Research grant no. GN2052, the Isaac Newton Trust, Genesis Research Trust. G.t.H. and I.R. are supported by Focused Ultrasound Foundation Centre of Excellence. Professor D. A. Giussani is supported by the British Heart Foundation. Dr C.C. Lees is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Author Contributions: C.J.S wrote paper, designed and performed all experiments. D.A.G. and I.R. designed and performed all experiments and contributed to paper. G.t.H. and C.C.L. designed experiments and contributed to paper. K.J.B., Y.N. and J.C. performed all experiments.

Competing interests: None
**Fig. 1. Colour Doppler and B-Mode ultrasound imaging of placental vascular ablation**

(A) Example of pre-treatment colour Doppler ultrasound imaging of a placentome with a cursor marking the position of the HIFU focal zone overlying the targeted vessel; (B) post-treatment colour Doppler imaging of the same placentome demonstrating “no flow” within the targeted vessel; (C) B-mode harmonic ultrasound imaging of tissue responses during treatment with an example of an hyperecho region within the HIFU focal zone.
Fig. 2. Macroscopic and microscopic results of HIFU exposure of placental vasculature

(A) Tissue darkening across the centre of a bisected placentome; (B) Tissue pallor involving the central area of a bisected placentome; (C) H&E section (x2.5 magnification) of origin of fetal vessels in unexposed placentome; (D) H&E section (x2.5 magnification) of origin of fetal vessels in HIFU exposed placentomes showing clot filled vessel lumen.
Figure 3: Maternal cardiovascular responses to HIFU / Sham placental vascular ablation

Values represent mean values for each sequential minute ± SEM of percentage change from baseline during the baseline (-30-0 mins), HIFU or sham ablation of placental vasculature (dashed box; 0-30 mins) and recovery (30-60 mins) while under general anaesthesia. Black bar represent a significant difference from baseline when a statistical summary method (area under the curve) is applied to the cardiovascular data. HIFU n=5, Sham n=6. Significant differences: * p<0.05 time vs. baseline, repeated measures two way ANOVA with post hoc Tukey test.
Figure 4: Fetal cardiovascular responses to HIFU / Sham placental vascular ablation

Values represent mean values for each sequential minute ± SEM of percentage change from baseline during the baseline (-30-0 mins), HIFU or sham ablation of placental vasculature (dashed box; 0-30 mins) and recovery (30-60 mins) while under general anaesthesia. Black bar represent a significant difference from baseline when a statistical summary method (area under the curve) is applied to the cardiovascular data. HIFU n=5, Sham n=6. Significant differences: * p<0.05 time vs. baseline repeated measures two way ANOVA with post hoc Tukey test.
Figure 5: Diagram of side view of equipment setup and HIFU exposure placement

(A) Setup of the ring shaped HIFU transducer and central diagnostic ultrasound probe within a bag of degassed water, which is in contact with the uterine surface; (B) diagram of a bisected placentome with grey ellipses representing the HIFU focuses placed in a linear track of positions across the central region of the placentome and the origin of the fetal vessels.
**Table 1: Maternal arterial acid base and metabolic status**

Values represent mean ± SEM of maternal femoral arterial blood sampled at the start of the baseline period (-30 mins), the start, middle and end of the HIFU or sham exposure series (0, 15, 30 mins) and the end of the recovery period (60 mins). HIFU n=5, Sham n=6. Significant differences *p<0.05 effect of time vs. baseline; †p<0.05 effect of treatment group, repeated measures two-way ANOVA with post hoc Tukey test.

**Table 2: Fetal arterial acid base and metabolic status**

Values represent mean ± SEM of fetal carotid arterial blood sampled at the start of the baseline period (-30 mins), the start, middle and end of the HIFU or sham exposure series (0, 15, 30 mins) and the end of the recovery period (60 mins). HIFU n=5, Sham n=6. Significant differences *p<0.05 effect of time vs. baseline, repeated measures two-way ANOVA with post hoc Tukey test.

**Table 3: Fetal substrate delivery**

Values represent mean ± SEM of fetal carotid and femoral arterial blood sampled at the start of the baseline period (-30 mins), the start, middle and end of the HIFU or sham exposure series (0, 15, 30 mins) and the end of the recovery period (60 mins). HIFU n=5, Sham n=6. Significant differences *p<0.05 effect of time vs. baseline, repeated measures two-way ANOVA with post hoc Tukey test.
Table 1: Maternal arterial acid base and metabolic status
<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment Group</th>
<th>Baseline (-30 min)</th>
<th>Exposure Series (0 min)</th>
<th>Exposure Series (15 min)</th>
<th>Exposure Series (30 min)</th>
<th>Recovery (60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIFU</td>
<td>7.27 ± 0.01</td>
<td>7.26 ± 0.02</td>
<td>7.23 ± 0.01</td>
<td>7.18 ± 0.02*</td>
<td>7.15 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7.21 ± 0.03</td>
<td>7.18 ± 0.02</td>
<td>7.17 ± 0.02*</td>
<td>7.15 ± 0.03*</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>HIFU</td>
<td>7.21 ± 0.02</td>
<td>7.20 ± 0.02</td>
<td>7.19 ± 0.02</td>
<td>7.18 ± 0.02*</td>
<td>7.17 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>7.21 ± 0.03</td>
<td>7.20 ± 0.02</td>
<td>7.19 ± 0.02</td>
<td>7.18 ± 0.02*</td>
<td>7.17 ± 0.03*</td>
</tr>
<tr>
<td>Arterial Base Excess (mmol.L⁻¹)</td>
<td>HIFU</td>
<td>-0.4 ± 0.9</td>
<td>-0.6 ± 0.7</td>
<td>-1.8 ± 0.4</td>
<td>-4.4 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>-0.6 ± 1.2</td>
<td>-1.6 ± 0.6</td>
<td>-2.0 ± 0.8</td>
<td>-2.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>pCO₂ (mmHg)</td>
<td>HIFU</td>
<td>62.3 ± 3.0</td>
<td>70.2 ± 9.3</td>
<td>69.0 ± 6.0</td>
<td>77.2 ± 5.6*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>78.6 ± 7.6</td>
<td>83.2 ± 6.5</td>
<td>83.6 ± 7.3</td>
<td>90.6 ± 12.2*</td>
<td></td>
</tr>
<tr>
<td>Lactate (mmol.L⁻¹)</td>
<td>HIFU</td>
<td>2.3 ± 0.4</td>
<td>2.2 ± 0.3</td>
<td>2.8 ± 0.4</td>
<td>3.0 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>1.5 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>2.2 ± 0.3*</td>
<td>2.2 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>HCO₃⁻ (mEq.L⁻¹)</td>
<td>HIFU</td>
<td>25.7 ± 1.1</td>
<td>25.5 ± 0.8</td>
<td>25.3 ± 0.6</td>
<td>24.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>28.1 ± 1.2</td>
<td>27.8 ± 1.0</td>
<td>27.5 ± 1.0</td>
<td>28.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>HIFU</td>
<td>25.2 ± 1.1</td>
<td>23.8 ± 3.3</td>
<td>23.0 ± 3.2</td>
<td>18.4 ± 0.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>28.0 ± 1.1</td>
<td>27.8 ± 1.6</td>
<td>28.2 ± 1.4</td>
<td>22.2 ± 2.2*</td>
<td></td>
</tr>
<tr>
<td>Sat.Hb (%)</td>
<td>HIFU</td>
<td>72.8 ± 1.1</td>
<td>69.3 ± 8.3</td>
<td>66.3 ± 7.4</td>
<td>46.8 ± 2.8*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>73.9 ± 6.8</td>
<td>65.5 ± 3.5</td>
<td>65.4 ± 4.8</td>
<td>50.8 ± 9.6*</td>
<td></td>
</tr>
<tr>
<td>Hb (g.dL⁻¹)</td>
<td>HIFU</td>
<td>9.8 ± 0.2</td>
<td>10.5 ± 0.3</td>
<td>10.9 ± 0.4</td>
<td>11.1 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>9.4 ± 0.3</td>
<td>9.8 ± 0.2</td>
<td>10.0 ± 0.2</td>
<td>10.7 ± 0.4*</td>
<td></td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>HIFU</td>
<td>0.32 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.35 ± 0.01*</td>
<td>0.35 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sham</td>
<td>0.27 ± 0.02</td>
<td>0.34 ± 0.02*</td>
<td>0.34 ± 0.02*</td>
<td>0.33 ± 0.03*</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Fetal arterial acid base and metabolic status
<table>
<thead>
<tr>
<th></th>
<th>HIFU</th>
<th>Sham</th>
<th>HIFU</th>
<th>Sham</th>
<th>HIFU</th>
<th>Sham</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid Arterial Oxygen Delivery (mmol.min⁻¹)</td>
<td>383 ± 30</td>
<td>377 ± 81</td>
<td>374 ± 39</td>
<td>387 ± 38</td>
<td>373 ± 41</td>
<td>336 ± 29</td>
</tr>
<tr>
<td></td>
<td>352 ± 28</td>
<td>355 ± 42</td>
<td>283 ± 19</td>
<td>262 ± 40</td>
<td>352 ± 28</td>
<td>283 ± 19</td>
</tr>
<tr>
<td>Femoral Arterial Oxygen Delivery (mmol.min⁻¹)</td>
<td>116 ± 9</td>
<td>112 ± 15</td>
<td>116 ± 13</td>
<td>120 ± 13</td>
<td>73 ± 15 *</td>
<td>82 ± 10 *</td>
</tr>
<tr>
<td></td>
<td>67 ± 12 *</td>
<td>99 ± 10 *</td>
<td>55 ± 8 *</td>
<td>85 ± 18 *</td>
<td>116 ± 13</td>
<td>120 ± 13</td>
</tr>
<tr>
<td>Carotid : Femoral Oxygen Delivery Ratio</td>
<td>3.1 ± 0.4</td>
<td>3.7 ± 1.0</td>
<td>3.1 ± 0.2</td>
<td>3.4 ± 0.4</td>
<td>4.8 ± 0.7</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>5.2 ± 1.1*</td>
<td>3.9 ± 0.7</td>
<td>4.6 ± 0.3</td>
<td>3.5 ± 0.6</td>
<td>3.1 ± 0.4</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Carotid Arterial Glucose Delivery (µmol.min⁻¹)</td>
<td>61 ± 14</td>
<td>78 ± 14</td>
<td>57 ± 16</td>
<td>76 ± 12</td>
<td>64 ± 15</td>
<td>79 ± 14</td>
</tr>
<tr>
<td></td>
<td>77 ± 19</td>
<td>77 ± 16</td>
<td>72 ± 13</td>
<td>80 ± 19</td>
<td>77 ± 19</td>
<td>72 ± 13</td>
</tr>
<tr>
<td>Femoral Arterial Glucose Delivery (µmol.min⁻¹)</td>
<td>21 ± 3</td>
<td>28 ± 5</td>
<td>19 ± 3</td>
<td>26 ± 3</td>
<td>15 ± 2</td>
<td>19 ± 3 *</td>
</tr>
<tr>
<td></td>
<td>16 ± 3</td>
<td>24 ± 4</td>
<td>19 ± 4</td>
<td>24 ± 3</td>
<td>15 ± 2</td>
<td>19 ± 3 *</td>
</tr>
<tr>
<td>Carotid : Femoral Glucose Delivery Ratio</td>
<td>3.0 ± 0.4</td>
<td>3.0 ± 0.4</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.4</td>
<td>4.2 ± 0.8 *</td>
<td>4.4 ± 0.8 *</td>
</tr>
<tr>
<td></td>
<td>4.7 ± 0.7 *</td>
<td>3.5 ± 8</td>
<td>4.0 ± 0.2</td>
<td>3.5 ± 0.7</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.4</td>
</tr>
</tbody>
</table>

Table 3: Fetal substrate delivery