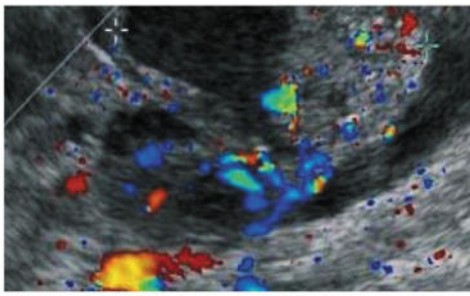
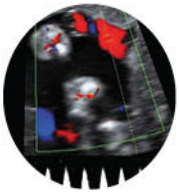
ISUOG Education 2019/20



ISUOG delivers quality education courses internationally in Ultrasound in Obstetrics and Gynecology, providing up-to-date research and clinical guidance for a range of topics and professional levels. Our courses are dynamic, interactive and provide delegates with practical tips as well as excellent networking opportunities.

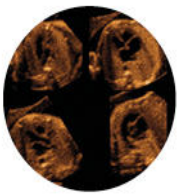


Modern management in twins: All you need to know

4 February 2020
Texas, USA and live streamed worldwide

Course Chairs: Lorraine Dugoff (USA), Asma Khalil (UK), Magda Sanz (USA), Joanne Stone (USA)

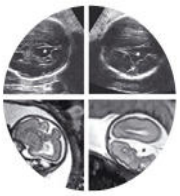
In association with Society for Maternal-Fetal Medicine



Advances in fetal echocardiography

21-22 February 2020
London, UK and live streamed worldwide

Course Chairs: Dario Paladini (Italy), Julene Carvalho (UK)



State of the art ultrasound in pregnancy

16 - 18 April 2020
Washington, USA and live streamed worldwide

Course Chairs: Lorraine Dugoff (USA), Aris Papageorghiou (UK), Laurent Salomon (France), Lynn Simpson (USA), Joanne Stone (USA)

In association with Society for Maternal-Fetal Medicine

Please note the speakers are subject to change at any time.

ISUOG EDUCATION ISUOG EDUCATION ISUOG EDUCATION ISUOG EDUCATION

isuog.org

Our previous delegates said:

"Very relevant to clinical practice"

"Well organised, good speakers"

"Great course, especially the fact that it was live streamed"

Register now at: isuog.org/events

Discounts available for ISUOG members

Find out more at **isuog.org/events.html**

congress@isuog.org | +44 (0)20 7471 9955

isuog.org

Placental gas exchange during amniotic carbon dioxide insufflation in sheep

BJ Amberg[1,2], PLJ DeKoninck[1,2,3], AJ Kashyap[1,2], SM Skinner[1], KA Rodgers[1], EV McGillick[1,2], JA Deprest[4,5], SB Hooper[1,2], KJ Crossley[1,2,#] and RJ Hodges[1,2,#]

1. The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, Victoria, Australia
2. The Department of Obstetrics and Gynaecology, Monash University, Melbourne, Victoria, Australia
3. Department of Obstetrics and Gynaecology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands
4. Department of Obstetrics and Gynaecology, Division Woman and Child, University, Hospitals Leuven, Leuven, Belgium
5. Department of Development and Regeneration, Cluster Organ Systems, Faculty of Medicine, KU Leuven, Belgium

KJC and RJH share joint senior authorship

Short Title: Carbon dioxide insufflation and placental gas exchange

Keywords: carbon dioxide, amniotic insufflation, fetal therapy, placenta, gas exchange

Corresponding Author: Associate Professor Ryan Hodges – The Ritchie Centre, Hudson Institute of Medical Research, 27-31 Wright St, Clayton, Victoria, 3168. ryan.hodges@monash.edu.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/uog.21933

Contribution:

What are the novel findings of this work?

This is the first study to show that amniotic CO2 insufflation causes reductions in placental gas exchange irrespective of gas temperature and humidity. It also showed that heated humidified insufflation reduces fetal blood gas disturbances seen when using cold dry gas by reducing fetal CO2 absorption from the uterus.

What are the clinical implications of this work?

This study provides physiological support for the use of heated humidified CO2 and lower uterine distension pressures as a way of reducing the fetal effects of amniotic insufflation during fetal surgery.

Abstract:

Objectives: Insufflation of the amniotic cavity with carbon dioxide (CO_2) is used clinically to improve visibility during complex fetoscopic surgery. Insufflation with heated, humidified CO_2 has recently been shown to reduce fetal hypercapnia and acidosis in sheep, but the underlying mechanisms are unclear. We have investigated whether differences in placental CO_2 and oxygen (O_2) exchange could explain these findings.

Methods: Fetal lambs at 105 days gestation (term 146 days) were instrumented with an umbilical artery and vein catheter and common umbilical vein flow probe. Arterial and venous catheters and flow probes were also inserted into the maternal uterine circulation. Six ewes were insufflated with cold, dry CO_2 (22^{o}C, 0-5% humidity) and seven with heated, humidified CO_2 (40^{o}C, 95-100% humidity) at 15mmHg for 180 minutes. Blood flow recordings and paired arterial and venous blood gasses were sampled from uterine and umbilical vessels. Rates of placental CO_2 and O_2 exchange were calculated. Data are presented as mean±SEM.

Results: After 180 minutes of insufflation fetal survival was 33% (2/6) using cold, dry CO_2 and 71% (5/7) using heated, humidified CO_2. By 120 minutes, fetuses insufflated with heated, humidified CO_2 had lower arterial CO_2 levels and higher pH compared to those insufflated with cold, dry gas. Insufflation significantly decreased placental gas exchange in both groups as measured by rates of both (i) fetal CO_2 clearance and O_2 uptake and (ii) maternal O_2 delivery and CO_2 uptake from the fetal compartment.

Conclusions: Lower arterial CO_2 and higher pH levels in fetuses insufflated with heated, humidified, compared to cold, dry CO_2, could not be explained by differences in placental gas exchange. Instead, heated humidified insufflation appeared to

reduce fetal CO_2 absorption from the uterus supporting its use in preference to cold, dry CO_2.

Introduction:

Detection of congenital anomalies, such as myelomeningocele, during mid-gestation ultrasound provides a window of opportunity for surgical intervention to reduce infant morbidity and improve their long-term outcomes.[1] To minimise the impact on the mother, many of these surgeries are performed under video-guidance through ports in the uterine wall (fetoscopy).[2-4] Complex fetoscopic procedures require gaseous distension of the amniotic space with carbon dioxide (CO_2) to see clearly and manipulate the fetus during surgery.[4]

Carbon dioxide is a logical gas for insufflation given its low cost and extensive use in laparoscopic surgery with minimal adverse effects. Additionally, its high solubility allows gas from the amniotic space to dissolve into fetal and maternal blood rather than forming gas emboli which are potentially fatal.[5] However, this additional dissolved CO_2 must be removed from maternal and fetal blood to avoid lowering blood pH (acidosis). The mother easily eliminates excess CO_2 from her lungs, however the fetal lungs play no role in gas exchange.[6] Instead, excess CO_2 is eliminated from the fetal compartment in the placenta where it enters the maternal circulation (Figure 1).[6] As fetal placental gas exchange is less efficient than maternal respiratory CO_2 elimination, the fetus may be vulnerable to accumulating CO_2 during insufflation, which is consistent with several animal studies.[7-12]

Our recent sheep study showed that using heated, humidified CO_2 for insufflation greatly reduces fetal CO_2 levels and partially mitigates the associated acidosis compared to unheated, unhumidified (cold, dry) insufflation.[8] While these findings are consistent with trends in small human case series, the mechanisms explaining the fetal benefits remain unclear.[13] Understanding these mechanisms will

allow refinement of insufflation parameters to further minimise the fetal effects of amniotic insufflation during a time of significant physiological stress.

We hypothesised that cold, dry amniotic insufflation impairs fetal CO_2 elimination by reducing umbilical blood flow and placental gas exchange. Additionally, we hypothesised that heated, humidified insufflation mitigates the decrease in umbilical blood flow and, thereby explains lower fetal CO_2 and higher pH levels. Our aim was therefore, to compare rates of placental gas exchange during cold, dry and heated, humidified amniotic insufflation in sheep.

Methods:

Surgical Instrumentation:

The experimental protocol was approved by the Monash Medical Centre Animal Ethics Committee and followed a series of similar insufflation experiments in our laboratory.[8, 12] Thirteen pregnant, Merino-Border Leicester ewes (103-106 days gestation) were anaesthetized with Sodium Thiopentone (i.v. Pentothal, Boehringer Ingelheim, Warriewood, NSW, Australia) and intubated with an endotracheal tube. General anaesthesia was maintained with 1.5-2% inhaled isoflurane (Isoflow, Abbot Pty Ltd, North Chicago, IL, USA) in air/oxygen. The ewe's ventilation rate and tidal volume were continuously adjusted to maintain arterial CO_2 levels between 35-45mmHg. Polyvinyl catheters (Internal Diameter (ID) 2.6mm, Dural Plastics, Sydney, NSW, Australia) were inserted into the ewe's carotid artery and jugular vein. Ewes were administered a continuous infusion of saline solution throughout the experiment via the jugular vein.

Fetuses were partially exteriorised via a midline laparotomy and hysterotomy incision made near the tip of the pregnant uterine horn. During instrumentation the exteriorised fetus and uterus were covered with warmed Hartmans solution to prevent heat loss and vessel constriction. A flow probe (Transonic Systems, Ithaca, NY, USA, Size 4) was placed around the fetal common umbilical vein and a temperature probe inserted into the fetal oesophagus. Fetuses were then returned to the amniotic space and a cotyledon close to the placental origin of the umbilical cord was identified. Heparinised saline-filled polyvinyl catheters (internal diameter, 0.86 mm) where introduced into large branches of the cotyledonary artery and vein and the tips advanced into the common umbilical vessels within the cord (Figure 1). Insufflation tubing, a second temperature probe and an amniotic drainage catheter

were inserted through the uterine wall into the amniotic sac. All catheters, temperature probes and flow probes were exteriorised and the hysterotomy incision closed in three layers to maintain an airtight seal of the uterus for insufflation as previously described.[8, 12]

A flow probe was placed around a large branch of the uterine artery supplying the pregnant horn and a polyvinyl catheter (internal diameter, 0.86 mm) introduced into the common uterine vein. The uterus was then returned to the maternal abdomen and the laparotomy incision closed to allow uterine insufflation within the abdominal cavity as previously performed in sheep and clinically in humans.[14-16]

Amniotic CO_2 Insufflation:

Paired maternal (carotid artery and uterine vein) and fetal (umbilical artery and vein) blood gases were sampled immediately after surgery and then after a 10-minute stabilisation period (post-operative and baseline samples). Maternal (ventilation rate, amniotic temperature, heart rate and uterine artery flow) and fetal (temperature, umbilical artery and vein pressure and common umbilical vein flow) physiological parameters were continuously recorded using LabChart and PowerLab data acquisition system (ADInstruments, Bella Vista, NSW, Australia).

The amniotic fluid was drained, and ewes were randomly allocated to cold, dry (22ºC, 0-5% humidity) or heated, humidified (40ºC, 100% humidity) amniotic insufflation. Intra-amniotic pressures were increased with an insufflator (40L High-Performance Insufflator, Stryker South Pacific, Australia) over five minutes to 15mmHg and maintained for 180 minutes with a flow rate of 0.5L per minute. This pressure and duration were chosen to replicate averages from human case series and our previous ovine study showing a benefit to heated, humidified insufflation.[8,

[14-16] In ewes receiving heated, humidified insufflation, the gas was passed through a laparoscopic humidification system (MR860, Fisher and Paykel Healthcare, Auckland, New-Zealand) before entering the uterus.

During insufflation, paired maternal blood gases were sampled every ten minutes. Paired fetal gases were sampled every 10 minutes for the first 30 minutes and every 30 minutes thereafter. The uterus was desufflated after 180 minutes in surviving fetuses and monitoring continued for a further 20 minutes. Both the ewe and fetus were then euthanized using intravenous Pentobarbitone (Lethobarb, Virbac Pty Ltd, Peakhurst, Australia) and post mortem examination conducted.

Data analysis and statistics:

Maternal and fetal physiological parameters were assessed every five minutes by determining the mean of each parameter over twenty second epochs. Umbilical vein blood flow was adjusted for fetal bodyweight.

Total blood CO_2 and O_2 content was calculated from each blood gas sample to account for differences in bound and dissolved gas in arterial and venous blood (table 1, equations 1 and 2).[17, 18] Uteroplacental production of CO_2, the rate of CO_2 elimination from the umbilical circulation (fetal CO_2 clearance) and CO_2 uptake by maternal uterine blood (maternal CO_2 uptake) was calculated using equations 3-5 respectively. Similarly, the rate of O_2 loss from the uterine circulation (maternal O_2 delivery) and uptake by fetal umbilical blood (fetal O_2 uptake) was calculated using equations 6 and 7. These equations have been used extensively to calculate rates of placental gas exchange in sheep.[19-22] Values are presented as mean ± standard error of mean (SEM). Blood gas and physiological data were normally distributed and compared at matched time points using a repeated-measures mixed analysis of

variance (ANOVA) with Sidak post hoc analysis. Analysis of variance was also used to compare each time point to baseline values within each group.

Linear regression and correlation analysis were used to assess the relationship between umbilical vein blood flow and fetal placental gas exchange as well as uterine artery blood flow and maternal placental gas exchange.

Results:

Of the thirteen pregnant ewes undergoing surgery and amniotic insufflation, six were insufflated with cold, dry CO_2 and seven with heated, humidified CO_2. Fetal weight and baseline blood gasses values, physiological parameters and placental gas exchange data were similar between groups. Fetal survival over 180 minutes of insufflation was 33% (2/6) with cold, dry CO_2 and 71% (5/7) with heated, humidified CO_2 (Figure 2a.). 120 minutes was chosen as the timepoint for comparison because of limited survival in the cold, dry group beyond this point.

Fetal Effects

By 120 minutes of cold, dry and heated, humidified insufflation, the partial pressure of CO_2 in the umbilical artery ($PaCO_2$, Figure 2b), CO_2 content in the umbilical vein (Figure 2c) and blood lactate level (Figure 2d) increased while the pH decreased (Figure 2e) from baseline. However, compared to cold, dry insufflation at 120 minutes, fetuses insufflated with heated, humidified CO_2 had lower $PaCO_2$ (99.5 ±14.6 vs.167.0 ±5.0 mmHg, $P<0.01$) and blood lactate (3.2 ±1.1 vs. 7.2 ±2.1mmol/L, $P<0.01$) and higher arterial pH (pH 7.02 ±0.08 vs. 6.78 ±0.10, $P<0.01$). Within both insufflation groups, umbilical artery oxygen saturation decreased (Figure 2f) from baseline while the PaO_2 remained stable (Figure 2g). During cold, dry insufflation, uteroplacental production of CO_2 increased from baseline and was higher than the heated, humidified group after 120 minutes (Figure 2h).

During both cold, dry and heated, humidified insufflation, fetal temperature remained unchanged (Figure 3a.) while umbilical artery (Figure 3b) and vein (Figure 3c) pressure increased from baseline immediately after starting insufflation. Pressures remained elevated in the umbilical vein and gradually returned to baseline

in the umbilical artery over 120 minutes. In both groups, the rate of umbilical vein blood flow (Figure 3d), placental CO_2 clearance (Figure 3e) and O_2 uptake (Figure 3f) decreased from baseline in response to insufflation. As there were no differences between groups, umbilical blood flow and fetal placental gas exchange data was pooled for linear regression analysis. This showed that rates of fetal CO_2 clearance ($r=0.43$, $P=<0.01$, Figure 3g.) and O_2 uptake ($r=0.64$, $P=<0.01$, Figure 3h.) were positively correlated with umbilical vein blood flow.

Maternal Effects

No changes in amniotic temperature were observed during heated, humidified or cold, dry insufflation (Figure 4a). Maternal ventilation rates (Figure 4b) required to maintain maternal $PaCO_2$ between 35 and 45mmHg (Figure 4c) were also similar between groups throughout the experiment.

Rates of uterine artery blood flow (Figure 4d), CO_2 uptake (Figure 4e) and O_2 loss (Figure 4f) from the uterine circulation decreased in both groups over 120 minutes of insufflation. As there were no differences between groups, uterine blood flow and maternal placental gas exchange data were pooled for linear regression analysis. This showed that both maternal CO_2 uptake ($r=0.44$, $P<0.01$, Figure 4g) and O_2 delivery ($r=0.57$, $P<0.01$, Figure 4h) were positively correlated with uterine artery blood flow.

Discussion:

This is the first study to simultaneously monitor fetal blood gasses and rates of placental gas exchange during cold, dry and heated, humidified amniotic insufflation. Fetal $PaCO_2$ progressively increased and arterial pH decreased during cold, dry insufflation. These changes were partially mitigated by 90-120 minutes when the insufflated CO_2 was heated and humidified. Interestingly, we found that reductions in fetal and maternal placental gas exchange induced by insufflation were similar in both groups. Fetal and maternal placental gas exchange positively correlated with umbilical and uterine blood flow respectively.

These results confirm previous observations that fetal hypercapnic acidosis caused by cold, dry insufflation can be mostly mitigated over clinically relevant insufflation durations (120min) by heating and humidifying the CO_2.[7-12] Like others, we observed a rapid (within 10min) and sustained reduction in fetal arterial oxygen saturation which was associated with a non-significant rise in fetal PaO_2.[11, 23] This was likely a pH-induced decrease in the affinity of fetal haemoglobin for oxygen.[24] The slightly lower baseline fetal PaO_2 and pH values measured in this study, compared to our previous study, are simply due to the collection of fetal blood from a post-ductal artery (umbilical artery) instead of pre-ductal (carotid artery).[8]

We suggest that increasing the amniotic pressure by 15mmHg caused the reductions in placental gas exchange. As ovine umbilical venous pressure is normally less than 15mmHg, amniotic insufflation would have compressed umbilical veins, potentially causing them to collapse. Vessel pressure must therefore increase during insufflation to re-expand the vessels and restore blood flow. This would explain the immediate increase in umbilical arterial and venous pressure during insufflation and the associated reduction in umbilical venous blood flow. Our data,

like others, has shown that umbilical blood flow is essential to maintain CO_2 and O_2 diffusion between the fetal and maternal compartments.[25] As insufflation pressures were the same in both groups, this explains why reductions in umbilical blood flow and placental gas exchange were independent of CO_2 temperature and humidity.

These results provide a physiological explanation for ultrasound and histological findings in human fetuses undergoing amniotic insufflation suggestive of increased placental vascular resistance and fetal under-perfusion of the placenta.[26, 27] Additionally, they provide an important rationale for using insufflation pressures below human umbilical venous pressure (3-7mmHg) as a way to minimise the physiological effects of insufflation on the fetus.[12, 28] As such, ongoing efforts to avoid high insufflation pressures during human surgery by partially exteriorising the uterus appear physiologically justified.[12, 29] Future studies should investigate if lower insufflation pressures can maintain both placental gas exchange and adequate visualization during fetoscopic surgery.

Increasing amniotic pressure was also associated with lower uterine artery blood flow. Lower uterine artery blood flow appeared to decrease maternal CO_2 uptake from the fetus which likely contributed to progressive fetal hypercapnia. In both humans and sheep there is normally significant reserve in uteroplacental perfusion to avoid fetal blood gas changes during fluctuations in placental blood flow.[30, 31] However, this reserve may be reduced in the context of amniotic insufflation where fetal placental gas exchange is also compromised.

Contrary to our hypothesis we found no differences in umbilical venous blood flow or placental gas exchange to explain the fetal benefits of heated, humidified insufflation. This suggests that cold, dry CO_2 did not induce umbilical vasospasm like *in vitro* studies using temperatures <28ºC.[32, 33] Although the entry temperature of

insufflated CO_2 was very different (22 vs 40ºC), intra-amniotic temperatures were similar between groups. This similarity could be caused by the temperature probe lying against the fetus, uterine wall or residual amniotic fluid in both groups. This seems likely as there was little difference between the amniotic and fetal temperatures at all stages of the experiment. However, we also consider it likely that the insufflation gas was progressively heated within the uterus, particularly as insufflation flow rates were relatively low (0.5L/minute).

Instead of conveying acid base benefits by improving placental gas exchange, heated, humidified insufflation may slow fetal CO_2 absorption from the uterus. This is consistent with lower uteroplacental CO_2 production and the trend for lower umbilical vein CO_2 content during heated, humidified insufflation. Heating CO_2 gas from 22 to 40^{o}c nearly halves its solubility and would reduce fetal absorption, but as discussed, this effect may be short lived. The addition of water vapour (humidification) lowers the partial pressure of CO_2 within the insufflated gas from ~740 to ~685mmHg.[34-36] While this 7% reduction would also reduce fetal CO_2 absorption during heated, humidified insufflation, we do not believe it completely explains the fetal acid base benefits. We hypothesize that cold, dry insufflation damages the fetal membranes, increasing the rate of fetal CO_2 absorption. Indeed, we have previously shown that cold, dry insufflation increases neutrophil recruitment into the fetal membranes.[8]

Although monitoring human fetuses during insufflation is technically challenging, human fetuses clearly tolerate insufflation better than sheep. Rates of human fetal survival are high and, reassuringly, post-surgical follow up has not identified neurological sequelae attributable to severe intra-operative acidosis.[13-16, 29, 37, 38] On the other hand, fetal sheep clearly develop severe hypercapnic acidosis that impacts survival.[8] These differences could be attributed to the cotyledonary

arrangement of the sheep placenta that may increase the surface area for fetal CO_2 absorption or the thinner sheep myometrium that may over-stretch and occlude uterine arteries when insufflated. Additionally, the partially keratinized skin of the 103-106-day sheep fetus may more readily absorb CO_2 from the uterus compared the 24-26-week human fetus.[39, 40] While these differences suggest that human fetuses are not experiencing the same severity of disturbances, the principals of reducing fetal CO_2 absorption using heated, humidified CO_2 and avoiding high insufflation pressures to maintain placental gas exchange cannot be ignored. These principals should provide the rationale for future studies aiming to confirm the fetal safety of amniotic insufflation.

This study showed that amniotic insufflation reduced placental gas exchange in sheep, independent of CO_2 temperature and humidity. Interestingly, the fetal benefits of heated, humidified insufflation could not be explained by differences in placental gas exchange. Instead, heated humidified insufflation appeared to reduce fetal CO_2 absorption from the uterus.

Acknowledgements:

This work is supported by the Victorian Government's Operational Infrastructure Support Program and also received funding from Monash Health (Emerging researcher fellowship, Dr Sasha Skinner).

Disclosure statement: The authors report no conflict of interest

References:

1. Adzick NS, Thom EA, Spong CY, Brock JW, 3rd, Burrows PK, Johnson MP, Howell LJ, Farrell JA, Dabrowiak ME, Sutton LN, Gupta N, Tulipan NB, D'Alton ME, Farmer DL, Investigators M. A randomized trial of prenatal versus postnatal repair of myelomeningocele. N Engl J Med. 2011;364(11):993-1004.
2. Kabagambe SK, Jensen GW, Chen YJ, Vanover MA, Farmer DL. Fetal Surgery for Myelomeningocele: A Systematic Review and Meta-Analysis of Outcomes in Fetoscopic versus Open Repair. Fetal Diagn Ther. 2018;43(3):161-74.
3. Joyeux L, Engels AC, Russo FM, Jimenez J, Van Mieghem T, De Coppi P, Van Calenbergh F, Deprest J. Fetoscopic versus Open Repair for Spina Bifida Aperta: A Systematic Review of Outcomes. Fetal Diagn Ther. 2016;39(3):161-71.
4. Kohl T, Tchatcheva K, Weinbach J, Hering R, Kozlowski P, Stressig R, Gembruch U. Partial amniotic carbon dioxide insufflation (PACI) during minimally invasive fetoscopic surgery: early clinical experience in humans. Surg Endosc. 2010;24(2):432-44.
5. Menes T, Spivak H. Laparoscopy: searching for the proper insufflation gas. Surg Endosc. 2000;14(11):1050-6.
6. Carter AM. Placental Gas Exchange and the Oxygen Supply to the Fetus. Comprehensive Physiology. 2015;5(3):1381-403.
7. Gratacos E, Wu J, Devlieger R, Van de Velde M, Deprest JA. Effects of amniodistention with carbon dioxide on fetal acid-base status during fetoscopic surgery in a sheep model. Surg Endosc. 2001;15(4):368-72.
8. Amberg B, Hodges R, Kashyap A, Skinner S, Rodgers K, McGillick E, Deprest J, Hooper S, Crossley K, Dekoninck P. Physiological effects of cold-dry versus heated-humidified partial amniotic carbon dioxide insufflation in sheep. Ultrasound Obstet Gynecol. 2018.
9. Luks FI, Deprest J, Marcus M, Vandenberghe K, Vertommen JD, Lerut T, Brosens I. Carbon dioxide pneumoamnios causes acidosis in fetal lamb. Fetal Diagn Ther. 1994;9(2):105-9.
10. Pelletier GJ, Srinathan SK, Langer JC. Effects of intraamniotic helium, carbon dioxide, and water on fetal lambs. J Pediatr Surg. 1995;30(8):1155-8.
11. Saiki Y, Litwin DE, Bigras JL, Waddell J, Konig A, Baik S, Navsarikar A, Rebeyka IM. Reducing the deleterious effects of intrauterine CO2 during fetoscopic surgery. J Surg Res. 1997;69(1):51-4.
12. Skinner S, Crossley K, Amberg B, Kashyap A, Hooper S, Deprest JA, Hodges R, DeKoninck P. The effects of partial amniotic carbon dioxide insufflation in an ovine model. Prenatal diagnosis. 2018;38(13):994-1003.
13. Baschat AA, Ahn ES, Murphy J, Miller JL. Fetal blood-gas values during fetoscopic myelomeningocele repair performed under carbon dioxide insufflation. Ultrasound Obstet Gynecol. 2018.
14. Kohl T. Percutaneous minimally invasive fetoscopic surgery for spina bifida aperta. Part I: surgical technique and perioperative outcome. Ultrasound Obstet Gynecol. 2014;44(5):515-24.
15. Lapa Pedreira DA, Acacio GL, Gonçalves RT, Sá RAM, Brandt RA, Chmait R, Kontopoulos E, Quintero RA. Percutaneous fetoscopic closure of large open spina bifida using a bilaminar skin substitute. Ultrasound in Obstetrics & Gynecology. 2018;0(ja).
16. Pedreira DA, Zanon N, Nishikuni K, Moreira de Sa RA, Acacio GL, Chmait RH, Kontopoulos EV, Quintero RA. Endoscopic surgery for the antenatal treatment

of myelomeningocele: the CECAM trial. Am J Obstet Gynecol. 2016;214(1):111 e1-e11.
17. Gardner DS, Giussani DA, Fowden AL. Hindlimb glucose and lactate metabolism during umbilical cord compression and acute hypoxemia in the late-gestation ovine fetus. American journal of physiology Regulatory, integrative and comparative physiology. 2003;284(4):R954-64.
18. Gattinoni L, Samaja M. Acid-base equilibrium in the blood of sheep. Experientia. 1979;35(10):1347-8.
19. Meschia G, Cotter JR, Makowski EL, Barron DH. SIMULTANEOUS MEASUREMENT OF UTERINE AND UMBILICAL BLOOD FLOWS AND OXYGEN UPTAKES. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences. 1967;52(1):1-18.
20. Kastendieck E, Kunzel W, Kurz CS. Placental clearance of lactate and bicarbonate in sheep. Gynecologic and obstetric investigation. 1979;10(1):9-22.
21. Battaglia FC. PLACENTAL CLEARANCE AND FETAL OXYGENATION. Pediatrics. 1970;45(4):563.
22. Ward JW, Wooding FB, Fowden AL. Ovine feto-placental metabolism. J Physiol. 2004;554(Pt 2):529-41.
23. Pickering BG, Palahniuk RJ, Cote J, Wade JG, Pash MG. Cerebral vascular responses to ketamine and thiopentone during foetal acidosis. Can Anaesth Soc J. 1982;29(5):463-7.
24. Antonini E, Wyman J, Brunori M, Fronticelli C, Bucci E, Reichlin M, Fanelli AR. The oxygen Bohr effect of human fetal hemoglobin. Archives of Biochemistry and Biophysics. 1964;108(3):569-72.
25. Adamson SL. Arterial pressure, vascular input impedance, and resistance as determinants of pulsatile blood flow in the umbilical artery. European journal of obstetrics, gynecology, and reproductive biology. 1999;84(2):119-25.
26. Kassir E, Belfort MA, Shamshirsaz AA, Sanz Cortes M, Whitehead WE, Espinoza J. Doppler changes in umbilical artery and ductus venosus during fetoscopic prenatal surgical repair of myelomeningocele. Ultrasound Obstet Gynecol. 2018.
27. Sanz Cortes M, Castro E, Sharhan D, Torres P, Yepez M, Espinoza J, Shamshirsaz AA, Nassr AA, Popek E, Whitehead W, Belfort MA. Amniotic membrane and placental histopathological findings after open and fetoscopic prenatal neural tube defect repair. Prenatal diagnosis. 2019.
28. Weiner CP, Heilskov J, Pelzer G, Grant S, Wenstrom K, Williamson RA. Normal values for human umbilical venous and amniotic fluid pressures and their alteration by fetal disease. American Journal of Obstetrics & Gynecology. 1989;161(3):714-7.
29. Belfort MA, Whitehead WE, Shamshirsaz AA, Bateni ZH, Olutoye OO, Olutoye OA, Mann DG, Espinoza J, Williams E, Lee TC, Keswani SG, Ayres N, Cassady CI, Mehollin-Ray AR, Sanz Cortes M, Carreras E, Peiro JL, Ruano R, Cass DL. Fetoscopic Open Neural Tube Defect Repair: Development and Refinement of a Two-Port, Carbon Dioxide Insufflation Technique. Obstetrics and gynecology. 2017;129(4):734-43.
30. Wilkening RB, Meschia G. Fetal oxygen uptake, oxygenation, and acid-base balance as a function of uterine blood flow. The American journal of physiology. 1983;244(6):H749-55.

31. Hooper SB, Walker DW, Harding R. Oxygen, glucose, and lactate uptake by fetus and placenta during prolonged hypoxemia. The American journal of physiology. 1995;268(2 Pt 2):R303-9.
32. Atalik KE, Kilic M, Nurullahoglu ZU, Dogan N. Effects of cooling and warming on 5-hydroxytryptamine- and acetylcholine-induced contractions of human umbilical vessels: role of nitric oxide. Fundamental & clinical pharmacology. 2008;22(1):37-44.
33. Boura AL, Boyle L, Sinnathuray TA, Walters WA. Release of prostaglandins during contraction of the human umbilical vein on reduction of temperature. British journal of pharmacology. 1979;65(3):360-2.
34. Carroll JJ, Slupsky JD, Mather AE. The Solubility of Carbon Dioxide in Water at Low Pressure. Journal of Physical and Chemical Reference Data. 1991;20(6):1201-9.
35. Dutton FB. Dalton's law of partial pressures. Journal of Chemical Education. 1961;38(8):A545.
36. Sammour T, Kahokehr A, Hill AG. Independent testing of the Fisher & Paykel Healthcare MR860 Laparoscopic Humidification System. Minim Invasive Ther Allied Technol. 2010;19(4):219-23.
37. Gine C, Arevalo S, Maiz N, Rodo C, Manrique S, Poca A, Molino JA, Carreras E, Lopez M. Fetoscopic two-layer closure of open neural tube defects. Ultrasound Obstet Gynecol. 2018;52(4):452-7.
38. Cortes MS, Torres P, Yepez M, Guimaraes C, Zarutskie A, Shetty A, Hsiao A, Pyarali M, Davila I, Espinoza J, Shamshirsaz AA, Nassr A, Whitehead W, Lee W, Belfort MA. Comparison of brain microstructure after prenatal spina bifida repair by either laparotomy assisted fetoscopic or open approach. Ultrasound Obstet Gynecol. 2019.
39. Coolen NA, Schouten KCWM, Middelkoop E, Ulrich MMW. Comparison between human fetal and adult skin. Arch Dermatol Res. 2010;302(1):47-55.
40. Lyne AG, Hollis DE. The structure and development of the epidermis in sheep fetuses. Journal of Ultrastructure Research. 1972;38(5):444-58.

Figure legends

Figure 1: Gas exchange in the placenta and instrumentation of the fetal and maternal placental compartments

Fetal blood is cleared of carbon dioxide (CO_2) and re-oxygenated by diffusion of dissolved gasses between the fetal and maternal compartments of the placenta. To measure rates of placental gas exchange in sheep the fetus was instrumented with an umbilical artery and vein catheter, umbilical vein flow probe and oesophageal temperature probe. The ewe was instrumented with a carotid artery catheter, uterine vein catheter and uterine artery flow probe. A temperature probe was also inserted into the amniotic space.

Figure 2: Fetal survival and blood gas changes

Fetal survival was 33% (2/6) with cold, dry CO_2 (■) and 71% (5/7) with heated, humidified CO_2 (▲) (a.). By 120 minutes in both groups, dissolved CO_2 in the umbilical artery ($PaCO_2$) (b), umbilical vein CO_2 content (c) and blood lactate level (d) had increased while the pH had decreased (e) from baseline. Umbilical artery $PaCO_2$ and lactate levels were higher and umbilical artery pH lower in the heated humidified group by 120 minutes. In both groups, umbilical artery oxygen saturation decreased (f) while dissolved oxygen levels remained stable (g). Uteroplacental production of CO_2 increased from baseline and was higher than the heated, humidified group by 120 minutes (h). Pre insufflation recordings were recorded immediately after surgery (post-surgery) and immediately before drainage of the amniotic fluid and insufflating the uterus (baseline). Data are presented as mean ± SEM. (*) represents $p<0.05$ vs. heated, humidified CO_2. (↑) and (↓) represent a

significant (P<0.05) increase or decrease compared to baseline values within each group.

Figure 3: Fetal physiology and placental gas exchange

Fetal temperature (a.) remained stable from baseline during cold, dry (■) and heated, humidified (▲) insufflation. Umbilical artery (b.) and vein (c.) pressure increased while umbilical vein blood flow (d.), fetal CO_2 clearance (e.) and fetal O_2 uptake (f.) decreased in both groups independent of CO_2 temperature and humidity. Umbilical vein blood flow negatively correlated with fetal CO_2 clearance (g.) and oxygen uptake (h.). Pre insufflation recordings were recorded immediately after surgery (post-surgery) and immediately before drainage of the amniotic fluid and insufflating the uterus (baseline). Data are presented as mean ± SEM. (*) represents p<0.05 vs. heated, humidified CO_2. (↑) and (↓) represent a significant (P<0.05) increase or decrease compared to baseline values within each group.

Figure 4: Maternal physiology and placental gas exchange

Amniotic temperature (a.), maternal ventilation rate (b.) and arterial CO_2 levels (c.) were the same during cold, dry (■) and heated, humidified (▲) insufflation and remained stable from baseline. Uterine artery blood flow (d.), maternal placental CO_2 uptake (e.) and placental O_2 delivery (f.) decreased in both groups independent of CO_2 temperature and humidity. Uterine artery blood flow negatively correlated with maternal CO_2 clearance (g.) and oxygen uptake (h.) Pre insufflation recordings were recorded immediately after surgery (post-surgery) and immediately before drainage of the amniotic fluid and insufflating the uterus (baseline). Data are presented as

mean ± SEM. (*) represents $p<0.05$ vs. heated, humidified CO_2. (↑) and (↓) represent a significant ($P<0.05$) increase or decrease compared to baseline values within each group.

Table 1: Calculations of blood gas content and placental gas exchange

Name	Equation
1. Total Blood CO_2 Content (mmol/L)[18]	$= PCO_2 \times 10^{(0.91 \times pH - 6.99)}$
2. Total Blood O_2 Content (mmol/L)[17]	$= \frac{Hb \times SaO_2}{100} \times 0.62$
3. Uteroplacental CO_2 production[22]	$= (UtV\ PCO_2 - UtA\ PCO_2) + (UmV\ PCO_2 - UmA\ PCO_2)$
4. Fetal Placental CO_2 Clearance (mmol/L/kg/min)[19-21]	$= \frac{(UmV\ flow \times 0.95)\ (UmA\ TCO_2 - UmV\ TCO_2)}{UmA\ PCO_2 - UtA\ PCO_2}$
5. Maternal Placental CO_2 Uptake (mmol/L/Kg/min)[19-21]	$= \frac{(UtA\ flow \times 0.84)\ (UtV\ TCO_2 - UtA\ TCO_2)}{UmA\ PCO_2 - UtA\ PCO_2}$
6. Maternal Placental O_2 Delivery (mmol/L/Kg/min)[19-21]	$= \frac{(UtA\ flow \times 0.84)\ (UtA\ TO_2 - UtV\ TO_2)}{UtA\ PO_2 - UmA\ PO_2}$
7. Fetal Placental O_2 Uptake (mmol/L/kg/min)[19-21]	$= \frac{(UmV\ flow \times 0.95)\ (UV\ TO_2 - UmA\ TO_2)}{UtA\ PO_2 - UmA\ PO_2}$

PCO_2 – partial pressure of carbon dioxide, PO_2 – partial pressure of oxygen, SaO_2 – arterial haemoglobin oxygen saturation, TCO_2 – total carbon dioxide content, TO_2 – total blood oxygen content, UmA – umbilical artery, UmV – umbilical vein, UtA – uterine artery (maternal carotid artery blood samples have been used provide these values), UtV – uterine vein.