TITLE

FETOSCOPIC INSUFFLATION OF HEATED-HUMIDIFIED CARBON DIOXIDE DURING SIMULATED SPINA BIFIDA REPAIR IS SAFE UNDER CONTROLLED ANESTHESIA IN THE FETAL LAMB

Running Head

Fetoscopic insufflation of heated-humidified carbon dioxide is safe

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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/pd.6093.

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Bulleted statements

What's already known about this topic?

There is experimental concern that Partial-Amniotic-CO_2-Insufflation (PACI) could induce fetal acidosis and hypercapnia as well as damage to the fetal membranes and brain. Heated-humidified PACI is clinically used for fetoscopic spina bifida (SB) repair and has been shown to lessen fetal acidosis and hypercapnia in the fetal lamb.

What does this study add?

In the fetal lamb, heated-humidified PACI during simulated fetoscopic SB repair through an exteriorized uterus under controlled anesthesia avoids significant fetal acidosis and hypercapnia. Moreover it does not induce substantial changes in fetal brain and membranes histology.

Keywords: Spina bifida, myelomeningocele, fetal surgery, fetoscopy, safety, carbon dioxide insufflation, fetal acidosis, fetal hypercapnia.

ABSTRACT

Objective: to assess the safety of Partial-Amniotic-Insufflation-of-heated-humidified-CO_2 (hPACI) during fetoscopic spina bifida repair (fSB-repair).

Method: a simulated fSB-repair through an exteriorized uterus under hPACI was performed in 100-day fetal lambs (term=145 days) under a laboratory anesthesia protocol (n=5;group 1) which is known to induce maternal-fetal acidosis and hypercapnia. Since these may not occur clinically, we applied a clinical anesthesia protocol (n=5;group 2), keeping maternal parameters within physiological conditions, i.e. controlled maternal arterial CO_2 pressure (pCO_2=30mmHg), blood pressure (≥67mmHg), and temperature (37.1-39.8°C). Our superiority study used fetal pH as the primary outcome.

Results: Compared to group 1, controlled anesthesia normalized fetal pH (7.23±0.02 vs. 7.36±0.02,p<0.001), pCO_2 (70.0±9.1 vs. 43.0±1.0mmHg, p=0.011) and bicarbonate (27.8±1.1 vs. 24.0±0.9mmol/L, p=0.071) at baseline. It kept them within clinically acceptable limits (pH≥7.23, pCO_2≤70mmHg, bicarbonate≤30mm/L) for ≥120min of hPACI as opposed to ≤30min in group 1. Fetal pO_2 and lactate were comparable between groups and generally within normal range. Fetal brain histology demonstrated fewer apoptotic cells and higher neuronal density in the prefrontal cortex in group 2. There was no difference in fetal membrane inflammation, which was mild.

Conclusion: fetoscopic insufflation of heated-humidified CO_2 during simulated fSB-repair through an exteriorized uterus can be done safely under controlled anesthesia.

INTRODUCTION

Fetoscopic alternatives for the open fetal repair of spina bifida aperta (SBA)[1-3] are currently being offered[4-6]. Fetoscopic SBA repair uses amniotic gas insufflation and is a complex procedure with a steep learning curve.[7] Compared to working in a liquid-filled environment, gas insufflation improves visualization, fetal immobilization, bleeding control and maneuverability of surgical instruments.[8] Carbon dioxide (CO_2) is the preferred gas because of its chemical stability, high solubility and low embolization and combustion risks.[9] However, early animal studies have raised concerns about partial amniotic CO_2 insufflation (PACI) inducing fetal acidosis and hypercapnia[10-13], potentially leading to asphyxia-ischemia when associated with hypotension and hypoxia[14,15]. PACI may also damage the fetal membranes due to inflammation of the choriodecidua.[16]

To decrease the metabolic impact of CO_2, one can lower the insufflation pressure[17,18] and/or use heated-and-humidified CO_2[17,19]. In adults, the latter increases the core body temperature and decreases peritoneal injury during laparoscopic surgery.[20] Experimentally, heated-and-humidified PACI (hPACI) partially mitigates fetal acidosis and hypercapnia in catheterized and monitored fetal lambs exposed to 3 hours of pneumamnion.[16] Several mechanisms could explain this, mainly the 40% reduction of CO_2 solubility, improved placental CO_2 absorption and clearance, and the normal biological activity of fetal carbonic anhydrase between 35 and 45°C.[16,21-23] Nonetheless, that experiment did not simulate fetoscopic SBA repair and the uterus was neither exteriorized during insufflation nor cannulated with multiple ports.

In the present experiment, those variables were introduced and we expanded the metabolic outcomes with brain and membrane histology. We also reduced the insufflation pressure from 15 to ≤ 10 mmHg, as clinically implemented.[6,24] There is early clinical evidence that under those conditions, the fetal pH remains within safe limits.[25,26] In both studies totaling ten fetuses, cordocentesis of the umbilical vein was performed before hPACI and/or a few minutes after hPACI when the uterus was closed, yet not during insufflation. Moreover since blood from the umbilical vein has already been processed through the placenta and fetal CO_2 partially removed[8], fetal pCO_2 might have be underestimated. Additionally, no measurable effects on term human fetal membranes have been reported.[27] Therefore we assessed maternal and fetal safety - in terms of maternal-fetal metabolic effects using arterial blood samples, as well as fetal membranes and brain effects - of hPACI combined with simulated fetoscopic SBA repair in the fetal lamb model prior to clinical implementation of a fetoscopic program in our center.[28,29]

METHODS

Ethics statement

This experiment was approved by the Animal Ethics Committee of the KU Leuven (P164-2017). It followed the NC3Rs (National Center for the Replacement, Refinement, and Reduction of Animals in Research) and the ARRIVE (Animals in Research Reporting In Vivo Experiments) guidelines for animal research.[30,31]

Study design

A ten-step fetoscopic SBA repair[32-34] was simulated in fetal lambs at 100 days of gestation (term=145)[11,16] (Figure 1; Supplementary Methods 1). We used an anesthesia protocol for pregnant sheep as previously described referred as "laboratory anesthesia".[11,16,35] Fetal safety was defined by comprehensive and clinically relevant maternal and fetal metabolic and hemodynamic outcomes as well as fetal histological outcomes based on previous animal studies.[8,11,12,16,23,36] The primary outcome measure was fetal pH. Secondary outcomes were fetal survival, fetal and maternal acid-base status (arterial pressure of carbon dioxide (pCO_2) and oxygen (pO_2), bicarbonate and lactate), and hemodynamics (heart rate and blood pressure), as well as fetal membranes and brain histology. The lowest clinically acceptable limit of the maternal or fetal variables were based on available data in awake pregnant ewes with chronically instrumented fetal lambs with mild hypoxia at 100 days of gestation.[14,37-39]

For standardization, procedures were performed by a team consisting of two board certified laparoscopic surgeons (LJ and JD) with an experience of >50

open fetal SBA repairs, and one board certified obstetrician and maternal-fetal specialist (DB) monitoring and manipulating the position of the fetus. Our surgical team had fixed roles in this experiment and was previously trained on a high-fidelity rabbit model.[34] To ensure homogeneity in the fetoscopic SBA repair, we assessed the watertightness of the skin closure, Objective Structured Assessment of Technical Skill (OSATS) score, operation time, and total insufflation volume (Supplementary Methods 2).[34] All measurements were done by independent observers (YK, EC).

Despite using hPACI, the laboratory anesthesia group had a drop in fetal pH values since the beginning of insufflation as observed with a previous experiment.[16] It persisted for the entire experiment duration. Moreover, the ewes and fetuses displayed acidosis and hypercapnia at baseline prior to hPACI. Therefore, we reconsidered the experimental conditions and developed the “clinical anesthesia” protocol with the aid of our clinical fetal anesthesiology team. The experiment was repeated using the same outcome measures.

A power calculation was made to demonstrate that the fetal pH would raise at 120 min of hPACI from 7.12 ± 0.04 under laboratory anesthesia to ≥ 7.23 ± 0.04 under clinical anesthesia. The latter pH has been shown to be safe and prevent brain damage in fetal lambs.[14] We used a superiority design with 5% significance level and 90% power (Sealed Envelope tool[40]) resulting in at least 3 animals required. Accounting for potential fetal deaths and absence of randomization, we eventually operated on 5 lambs in each group.

Experimental anesthesia procedures

Time-dated pregnant Swifter ewes were provided by the university farm and fasted 12 hours with access to water ad libitum. Two different protocols of general anesthesia were consecutively implemented (Supplementary Table 1). Maternal and fetal arterial blood gas samples as well as maternal and fetal hemodynamic parameters were collected simultaneously at different time-points before, during and after insufflation (Supplementary Methods 3).

Laboratory anesthesia

This protocol has been previously reported.[11,16,35] First, ewes were sedated with intra-muscular xylazine injection (0.3 mg/Kg XYL-M 2%, VMD, Arendonk, Belgium). Thirty minutes later, general anesthesia was induced with 1% propofol (4mg/Kg Propovet Multidose 10mg/mL, Abbot, Breda, The Netherlands) using an 18 Ga peripheral venous catheter placed in the right internal jugular vein. Ewes were ventilated via an 8 mm endotracheal tube and general anesthesia was maintained with 1.5-2.5% inhaled isoflurane (Iso-Vet 1000mg/g, Dechra, Northwich, UK) in 100% oxygen (5L/min).[11,35] The endotracheal position of the tube was confirmed by thoracic movements. End-tidal CO_2 was measured using capnography. A pulse oximeter was placed on the ear.

A 22 Ga arterial cannula was placed in the ear artery to measure blood pressure and to obtain maternal arterial blood samples at three time points: before insufflation, after 120 min of insufflation and at the end of the surgery when the uterus and skin were closed. One liter of Hartmann (Braun, Melsungen, Germany) was infused in bolus before positioning the ewe in 20° left lateral position to avoid aortocaval compression. The latter can indeed cause a drop in placental gas exchange and fetal hypoxemia.[41] 1.5 g cefazolin (Cefazolin

Sandoz 1g, Sandoz Novartis, Holzkirchen, Germany) and 0.02 mg/kg buprenorphine (Vetergesic multidose 0.3mg/mL 10mL, Ecuphar, Oostkamp, Belgium) was given intravenously. Ewes were placed on a heating pad to maintain body temperature at around 37°C. Temperature was measured using a rectal thermometer every 30 min. An orogastric tube was inserted to decompress the stomach. Maternal ventilation settings were adjusted to maintain a maternal end tidal CO_2 between 35-45 mmHg.

Clinical anesthesia

An intravenous catheter was placed, and the ewe was slightly sedated with 0.5-1 mg/kg of 2% propofol (Diprivan 2%, Aspen, Dublin, Ireland) to allow gentle application of a face mask while maintaining voluntary movements. The ewe was pre-oxygenated with 100% oxygen for 3 minutes. The pulse oximeter was placed on the ear. 3 mg/kg of 2% propofol was given intravenously before the endotracheal intubation (8 mm diameter tube). The ewe was ventilated with a RIMAS 2000 anesthesia engine (Dräger, Lübeck, Germany) with 30% inspiratory oxygen starting with a tidal volume of 8 mL/kg and a respiratory rate of 15/min, both titrated to achieve a target arterial pCO2 of 30 mmHg which is the mean value in awake pregnant ewes under physiologic conditions.[38,39] Arterial samples were obtained from an ear artery. Isoflurane was given at an end tidal concentration of 1.5 Vol-%. A 3-lumen central venous catheter was placed in the right jugular vein. A fluid bolus of 10 mL/kg Hartmann (Braun, Melsungen, Germany) was infused before positioning the sheep in 20° left lateral position. Cefazoline 1.5 g and buprenorphine 0.02 mg/Kg were given intravenously. To maintain maternal temperature between 37.1 and 39.8°C as measured with an esophageal probe to avoid severe fetal hypothermia, a

heating pad and forced air warmer (Bair Hugger, Courtelary, Switzerland) were used.[42] Standard "American Society of Anesthesiologists" monitoring was applied using the Datex-Ohmeda monitor (S5, Helsinki, Finland): ECG, invasive arterial blood pressure, pulse oximetry, end tidal CO_2, inspiratory and expiratory oxygen and isoflurane and esophageal temperature. The mean arterial pressure was maintained above 67 mmHg (80% of awake value) using intravenous norepinephrine (Levophed, Hospira, Brussels, Belgium).[38,39] When the dose of norepinephrine exceeded 0.2 µg/kg/min, 0.01-0.06 units/kg/hour vasopressin (Fresenius Kabi, Bad Homburg, Germany) were added.[43] A gastric tube and bladder catheter were inserted. During laparotomy, 5 mL/kg/hour Hartmann's solution was infused intravenously. Colloids were given (Isogelo, BBraun, Melsungen, Germany) when hypovolemia and hypotension were resistant to fluid administration. Every hour, an arterial blood gas sample was obtained to adjust ventilation, the lungs were recruited to maintain 40 cmH_2O of ventilation pressure and the urine output was recorded. The last fetal blood sample was collected at the end of the procedure after the closure of the abdominal wall. Afterwards, the ewe and the fetus were euthanized.

Histological analysis

Fetal membranes and brain specimens from operated fetuses in both groups and non-operated twin fetuses (negative controls) were harvested and processed (Supplementary Methods 4). Fetal membranes sections were prepared for hematoxylin and eosin (H&E) and brain sections for Nissl staining.[35] Finally, immunofluorescence staining was done to assess acute

inflammation (antibodies against FITC neutrophils, CD45 leucocytes and iNOS macrophages) and apoptosis (cleaved Caspase 3).[16,27]

Slides were digitized using the Zeiss AxioScan Z1 imaging platform (AxioScan Slide Scanner, Carl Zeiss Microscopy). Fetal membranes cell counts were performed using the Qupath freeware (qupath.github.io)[44] in three randomly selected, non-overlapping areas with a length of 200µm. The number of positive cells in the amnion epithelium, the connective tissue underneath, and the chorion (inner chorion and trophoblast) were manually counted by a single blinded observer (SV). The cell count average per fetus was calculated and adjusted per mm^2. For the brain, automated counting of neuron and caspase positive apoptotic cells was performed on the whole-slide digitized images by a single blinded observer (LT) using QuPath.

Statistical analysis

Statistical analysis was done using GraphPad Prism (version 7 for MacOs X, GraphPad, La Jolla, CA, USA). Interpretation and reporting was done according to the guidelines for improving statistical interpretation and reporting.[45] P values <0.05 were considered statistically significant.

Continuous variables were tested for normal distribution using the Shapiro-Wilk test and the Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefor p value ($n<10$, $p>0.05$). The normally-distributed variables were presented as mean and standard deviation (SD) and compared with unpaired student t test. Continuous variables that were not normally distributed, were expressed as median and interquartile range (IQR) and compared with the Mann-Whitney

test. Binomial and ordinal variables were expressed as percentage and score, respectively.

For the maternal and fetal longitudinal data, normal distribution was assessed using a QQ-plot. For comparison of the longitudinal data, we used a repeated measures two-way ANOVA test and a post-hoc Sidak's multiple comparisons test for different timepoints analysis. In case of missing values, the mixed-effects ANOVA model was used.

RESULTS

Ten fetal lambs from ten pregnant ewes were instrumented and underwent simulated fetoscopic SBA repair, first under laboratory anesthesia (n=5) and subsequentially under clinical anesthesia (n=5). Gestational age at surgery was 102 ± 4 days. The indicators of surgical performance were comparable between both groups (Table 1).

Maternal effects

Maternal outcomes were different between the clinical and laboratory anesthesia groups. At baseline maternal pH and pCO_2 were significantly better and in the normal range under clinical anesthesia (Table 2). Maternal pH, pCO_2 and body temperature were in the normal range of awake pregnant ewes at 120 min of hPACI (Table 2) and throughout the surgery (Figure 2). In contrast acidosis, hypercapnia and hypothermia were observed under laboratory anesthesia. Maternal lactate and bicarbonate were comparable at 120 min and within normal range during the procedure in both groups. However maternal lactate in the clinical protocol was significantly higher than in the laboratory protocol. pO_2, heart rate and mean arterial pressure were not significantly different between the groups, yet they were within normal range only under clinical anesthesia (Figure 2).

Fetal effects

Outcomes were improved under the clinical anesthesia protocol compared to the laboratory protocol. At baseline, fetal pH and pCO_2 were significantly better

and within normal range under clinical anesthesia (Table 2). At 120 min of hPACI, fetal pH was also significantly higher and within the clinically acceptable range (7.24 ± 0.05 vs. 7.12 ± 0.04, $p<0.001$) in the clinical anesthesia group (Table 2). It also remained in a clinically acceptable range throughout the entire procedure, although a transient drop <7.23 for less than 60 min out of 180 min of hPACI was noted (Figure 3). Fetal pCO_2 and bicarbonate were better and within a clinically acceptable range at 120 min.

When anesthetizing under the laboratory protocol, fetal acidosis started from 30 min onwards and persisted until and beyond the end of the fetal surgery procedure. In addition, fetal pCO_2 and bicarbonate increased significantly and fetal heart rate dropped at some point after the start of hPACI (Figure 3).

Overall pH, pCO_2 and bicarbonate were within clinically acceptable limits (pH ≥ 7.23, pCO_2 ≤ 70 mmHg, bicarbonate ≤ 30 mm/L) for ≥ 120 min of hPACI under clinical anesthesia protocol as opposed to ≤ 30 min under laboratory anesthesia. Conversely, fetal pO_2 and lactate were comparable between groups throughout the whole experiment. Fetal pO_2 was within the normal range, yet fetal lactate was above the normal range in the clinical anesthesia protocol from catheter insertion until 30 minutes of insufflation.

Histology of the fetal membranes and fetal brain

There was no significant difference in counts of apoptotic cells, neutrophils, macrophages or leucocytes in the fetal membranes (Figure 4A). Conversely, the neuronal density was higher, and the number of apoptotic cells lower in the prefrontal cortex of lambs anesthetized according to the clinical protocol (Figure

4B). No changes in apoptosis, inflammation or neuronal density were found in the other brain regions (caudate nucleus, hippocampus and corpus callosum).

DISCUSSION

Fetoscopic insufflation of heated and humidified CO_2 during simulated fetoscopic SBA repair under general anesthesia with strict maintenance of maternal arterial pCO_2, blood pressure, and temperature within physiological limits results in maternal and fetal pH, pCO_2, pO_2, bicarbonate, and lactate within a clinically acceptable range.

In a previous study conducted in a different research center, we demonstrated that hPACI partly mitigated fetal acidosis and hypercapnia. Conventional dry (0-5% humidity) and cold (22°C) PACI was associated with a pH of 6.75 at 120 min, whereas hPACI was associated with a pH of 7.10.[16] Though better, that is still not physiological. At closer look, the fetal blood gas values at baseline were neither normal in this previous study (pH=7.24 and pCO_2=67.4 mmHg)[16] as well as in ours (pH=7.23 and pCO2=70.0 mmHg). We therefore, reviewed our anesthesia protocol for factors present, apart from hPACI, that may explain these findings.

Keeping a similar insufflation duration of 180 min, we lowered the insufflation pressure from 15[16] to 5-7 mmHg. Both pressure and duration were selected based on the averages from human series of fetoscopic SBA repair insufflating the uterus exteriorized through a laparotomy.[6,46] Lowering pressure decreases the partial pressure for CO_2, thereby reducing placental CO_2 absorption and clearance and thus the severity of subsequent fetal hypercapnia and acidosis, as shown in fetal lambs assessed in the extrauterine environment for neonatal development (EXTEND).[23] It also avoids uterine overdistension and therefore does not compromise the uterine and umbilical artery flow leading to fetal hypoxia.[8]

When converting to an exact mimic of our clinical anesthesia protocol used during standard SBA repair, i.e. using the same drugs though with rigorous control of maternal ventilation, blood pressure, and temperature, both pH and pCO_2 baseline values were normal, as well as within clinically acceptable limits during surgery [47,48]. The lowest mean fetal pH recorded was 7.20 for an epoch that did not last longer than 60 min. Also, all other metabolic and hemodynamic parameters were within clinically acceptable range. An experiment in chronically instrumented fetal lambs demonstrated that the fetal pH needs to stay below 7.23 for at least 120 min and be associated with hypoxia and hypotension to cause brain changes.[14] We did not test the combination of the clinical anesthesia protocol in combination with insufflation of dry and cold CO_2. However, we think that is not necessary as it is unlikely to result in fetal parameters in an acceptable range at baseline and during insufflation. Two previous sheep studies have indeed shown that maternal hyperventilation targeting a pCO_2 of 30 mmHg, to increase the fetal-maternal CO_2 gradient, attenuated but could not prevent significant fetal hypercapnia and acidosis.[10,11] In this experiment, we also assessed the fetal brain. The Food and Drug Administration (FDA) recently raised concerns about lengthy anesthesia in pregnant women and children under three years.[49] In chronically instrumented fetal lambs, the presence of fetal acidosis (pH < 7.23) and hypoxia (mean arterial oxygen saturation of 28%) for two hours induces changes in cerebral metabolism, evidenced by proton magnetic resonance spectroscopy of cerebrospinal fluid samples.[14] Such hypoxemic insult lasting ≥ 6 hours, in combination with fetal acidosis (pH ≤ 7.10) can cause neuronal death in the hippocampus and to a lesser extent in the cerebral cortex and cerebellum on

electron microscopy.[50] To exclude effects on the brain, we applied validated morphometric analysis[35,51] combined with immunochemistry assessment of apoptosis. This showed very low numbers of apoptotic cells and neuronal density within normal range in the prefrontal cortex under clinical anesthesia and hPACI.[35] Conversely, none of six lambs exposed to dry and cold PACI showed demonstrable abnormal macroscopical or histological finding brain changes, even though the methodology was different and rather qualitative.[52]

We acknowledge some limitations. First, we only have arterial and no venous measurements or fetal temperature, hence lack information on the full fetal metabolic status. Second, despite being within normal range, maternal lactate in the clinical protocol was significantly higher than in the laboratory protocol. This may be caused by infusion of norepinephrine only in the clinical protocol. Since norepinephrine induces vasoconstriction, it can lead to tissue hypoperfusion and anaerobic glycolysis and subsequentially release lactate. Moreover, several animal and clinical studies have demonstrated that norepinephrine induces a transient release of lactate mainly from skeletal muscles through stimulation of aerobic glycolysis.[53-55] Third, the instrumentation of the lambs, the lack of standardization of the laboratory anesthesia protocol. Fourth the inadequate heating system of the laboratory protocol may have induced metabolic disturbances[16,56]. Fifth, we did not measure the effect of changes in fetal temperature, insufflation pressure or reduction of the uterus and closing of the abdomen like in percutaneous fetoscopy.

There are also limitations to the choice of the model. For instance, we used fetuses with a skin-defect rather than with a surgically induced or spontaneous

SBA.[32] In the latter scenario, the fetal effects of the surgery may be different. Overall, though sheep have many similarities with humans, such as a lengthy gestation, comparable placental development, metabolic function and nutrient transport, there are also some striking differences. The overall anatomical structure of the placenta is different and the fetal membranes are never attached.[57] Also, this was a terminal experiment, so we did not investigate effects on the longer term, not on the fetus or its membranes.

Our study has also some strengths. It followed the international guidelines for animal research and for validation of animal disease models[58,59] as well as guidelines for improving statistical interpretation and reporting[45]. Secondly, our study was post-hoc adequately 90% powered. The model, as well as the experiment, were standardized, ruling out the variability one may have in clinical circumstances or when using fetal lambs with SBA of different severity.[25,33] Fetal surgery was done at 100 days, which corresponds in size and weight to 25-26 weeks of gestation in humans.[60] This is currently the typical time point for fetal SBA repair.[1,4,6,46] More importantly, to mimic clinical reality we used the anesthesia protocol which is clinically applied for this procedure and could be implemented in other fetal centers.[47,48]

In summary, fetoscopic insufflation of heated and humidified CO_2 during simulated fetoscopic SBA repair through an exteriorized uterus can be safely performed in fetal lamb under controlled general anesthesia maintaining maternal physiological homeostasis. Any experiment in fetal lambs, for this and other conditions, that would study the effects of fetal surgery and fetal organ development and function, should be done under these conditions. These

observations are reassuring for fetal centers that already offer a fetoscopic program and implement a clinical anesthesia protocol.

ADDITIONAL INFORMATION

Data availability

The data that support the findings of this study are available from the corresponding authors, upon reasonable request.

Funding

LJ and YK are supported by an Innovative Engineering for Health award by the Wellcome Trust (WT101957) and the Engineering and Physical Sciences Research Council (EPSRC) (NS/A000027/1). LJ and SV are supported by a TBM-FWO grant. DB and LT are and LVdV was funded with support of the Erasmus+Programme of the European Union (Framework Agreement number: 2013-0040). This publication reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein. FDB is supported by the Belgian Fulbright commission, the Belgian American Education Foundation, the Sofina-Boel Foundation and the Flanders Research Foundation (FWO: 1S31720N). JD was a Clinical Researcher of the Flanders Research Foundation (FWO Vlaanderen; 1.8.012.07) and is currently supported by the Great Ormond Street Hospital Charity fund. PDC is supported by the National Institute for Health Research (NIHR-RP-2014-04-046) and the BRC NIHR GOSH. The HumiGard™ insufflation system was generously provided free of charge by Fisher-Paykel Healthcare, Auckland, New Zealand.

Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing

of the manuscript, or in the decision to publish the results. All the surgical instrumentation were part of a research loan from the company KARL STORZ (agreement 1705230948). Above mentioned support of KARL STORZ is in no way affiliated with any other service or procurement decisions on the part of the contractual parties.

Acknowledgements

We are grateful to Dr. Wouter Merckx and Stijn Massart from the Zootechnical Institute of KU Leuven for preparing and caring for the pregnant ewes, and to Dr. S. De Vleeschauwer from the KU Leuven animal research center and Rosita Kinnart from the KU Leuven Center for Surgical Technologies for their precious help in anesthetizing and caring for the animals. We also thank the following KU Leuven colleagues for their technical support: Anna-Maria Mazzer, Ozgur Can Kilinc, Imke Loret, Francisco Vitale and Francesca Russo. Finally we thank Dr. David Nittner from the VIB CCB Histopathology Expertise Center of KU Leuven for all the support regarding protocols and slides scanning.

Authorship

All authors contributed substantially to the submitted manuscript. LJ designed the entire study with the help of DB and JD. LJ, DB and JD performed the surgeries and animal preparations with help of LVdV, SV, YK, LP, EC, AI. The clinical anesthesia protocol was designed by TB and SR and anesthesia was conducted by TB. FDB independently analyzed and rated the videos of the surgeries and scored them using the OSATS rating scale. Histological processing and analysis were performed by LJ, SV and LT. LJ performed the statistical analysis of the data and wrote the manuscript. LJ and JD drafted and edited the manuscript. All co-authors made substantial contributions to the

study design, acquisition and interpretation of data, critically revised and approved the final submitted manuscript.

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Figures and tables legends

***Figure 1 – Three-port fetoscopic repair**. (A) Set-up of operation room; (B) port placement; (C) simulation of a lumbar SBA defect prior repair; (D) status after resection and skin undermining; (E) paraspinal muscle flap dissection and suture of first layer; (F) second layer with collagen patch; (G) third layer closure of the skin and (H) watertightness test. Images copyright by UZ Leuven, Belgium and reproduced with permission.*

***Figure 2 – Maternal acid-base status and hemodynamics.** Abbreviations: labGA, laboratory anesthesia group; clinGA, clinical anesthesia group; pO_2, partial arterial partial pressure of Oxygen; pCO_2, partial arterial partial pressure of carbon dioxide; HR, heart rate; MAP, mean arterial pressure; Mean, Max and Min are respectively the average level, highest clinically acceptable threshold and lowest clinically acceptable threshold in the awake pregnant sheep based on reference published data. Significance: * $0.01<p\leq0.05$; ** $0.001<p\leq0.01$; *** $0.0001<p\leq0.0001$; **** $p\leq0.0001$.*

***Figure 3 – Fetal acid-base status and hemodynamics.** Abbreviations: labGA, laboratory anesthesia group; clinGA, clinical anesthesia group; pO_2, partial arterial partial pressure of oxygen; pCO_2, partial arterial partial pressure of carbon dioxide; HR, heart rate; Mean, Max and Min are respectively the average level, highest clinically acceptable threshold and lowest clinically acceptable threshold in the awake pregnant sheep based on reference published data. Significance: * $0.01<p\leq0.05$; ** $0.001<p\leq0.01$; *** $0.0001<p\leq0.0001$; **** $p\leq0.0001$.*

***Figure 4 – Quantification of selected cell types in the fetal membranes and fetal brain.** Abbreviations: labGA, laboratory anesthesia group; clinGA, clinical anesthesia group; Am, amnion; CT, connective tissue; Ch, chorion. Significance: * $0.01<p\leq0.05$; ** $0.001<p\leq0.01$; *** $0.0001<p\leq0.0001$; **** $p\leq0.0001$.*

Table 1 – Surgical performance characteristics. *Abbreviations: GA, general anesthesia; OSATS, Objective Structured Assessment of Technical Skill) score; hPACI, Partial Amniotic heated-humidified CO_2 Insufflation.*

	Laboratory GA	**Clinical GA**	P value
Number	**N=5**	**N=5**	
Watertight repair	60% (3/5)	80% (4/5)	1.000
OSATS (/25)	21.4 ± 2.3	20.6 ± 4.7	0.742
Duration of fetal repair (min)	126 ± 18	145 ± 34	0.298
Duration of hPACI (min)	200 ± 19	196 ± 25	0.793

Table 2 – Fetal-maternal blood gas and hemodynamics at baseline and 120 min of insufflation. *Abbreviations: GA, general anesthesia; hPACI, partial amniotic heated-humidified CO_2 insufflation; pCO_2, arterial partial pressure of carbon dioxide; pO_2, arterial partial pressure of Oxygen; MAP, mean arterial pressure. All results reported as mean ± standard deviation.*

Parameters	**At baseline**			**At 120 min of hPACI**		
	Laboratory GA	**Clinical GA**	P value	**Laboratory GA**	**Clinical GA**	P value
Fetal						
pH	7.23 ± 0.02	7.36 ± 0.02	**<0.001**	7.12 ± 0.04	7.24 ± 0.05	**<0.001**
pCO_2 (mmHg)	70.0 ± 9.1	43.0 ± 1.0	**0.011**	106.9 ± 7.5	66.2 ± 9.7	**<0.001**
pO_2 (mmHg)	18.0 ± 3.6	16.4 ± 5.0	1.000	22.6 ± 6.5	20.72 ± 8.8	1.000
Bicarbonate (mmol/L)	27.8 ± 1.1	24.0 ± 0.9	0.071	34.6 ± 0.9	27.2 ± 3.0	**<0.001**
Lactate (mmol/L)	5.0 ± 2.2	5.8 ± 0.8	0.994	2.9 ± 1.1	4.2 ± 1.3	0.846
Heart rate (/min)	138 ± 13	130 ± 10	0.959	123.3 ± 5.8	133.3 ± 11.5	0.968
Maternal						
pH	7.37 ± 0.03	7.54 ± 0.04	**<0.001**	7.32 ± 0.06	7.52 ± 0.03	**<0.001**
pCO2 (mmHg)	47.6 ± 3.7	29.5 ± 2.2	**<0.001**	51.9 ± 7.4	30.9 ± 1.6	**<0.001**
pO2 (mmHg)	178.5 ± 102.3	112.4 ± 32.1	0.162	176.6 ± 75.4	112.4 ± 34.8	0.182
Bicarbonate (mmol/L)	27.4 ± 2.0	25.2 ± 2.5	0.767	27.0 ± 4.7	25.1 ± 2.6	0.858
Lactate (mmol/L)	1.2 ± 0.7	2.8 ± 1.0	**0.044**	0.7 ± 0.5	2.9 ± 1.4	**0.005**
Heart rate (/min)	66 ± 8	78 ± 11	0.226	69.6 ± 5.4	84.0 ± 16.3	0.097
MAP (mmHg)	60.2 ± 9.1	75.2 ± 3.1	0.577	55.6 ± 22.0	70.6 ± 6.8	0.577