Title: Partial amniotic carbon dioxide insufflation for fetal surgery

Running title: CO₂ amnio-insufflation and fetoscopy

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Bulleted statements

What's already known about this topic?

- PACI causes fetal hypercapnia and acidosis in sheep
- Early case series of fetoscopic myelomeningocele repair using PACI reported high rates of PPROM and preterm birth, however recent case series describe improved pregnancy outcomes.
- Limited clinical series using PACI do not show abnormal neurological development in operated fetuses

What does this study add?

- Comprehensive review of literature from animal and clinical studies relating to PACI
- Discuss possible mechanisms and effects of PACI insufflation pressure, duration, humidity and maternal hyperventilation on fetal physiology and wellbeing

Abstract

Partial amniotic carbon dioxide insufflation (PACI) involves insufflating the amniotic sac with carbon dioxide (CO₂) and, in some cases, draining some of the amniotic fluid. The creation of a gaseous intra-amniotic compartment improves visualization, even in the presence of limited bleeding, and creates the work space required for complex fetoscopic procedures. Clinically, PACI is mostly used to perform fetoscopic myelomeningocele (MMC) repair, enabling a minimally invasive alternative to open fetal surgery. However, evidence of the fetal safety of PACI is limited. Previous animal experiments in sheep demonstrate that PACI induces fetal hypercapnia and acidosis with largely unknown short and longer-term implications. In this review, we examine the literature for the physiological effects of intrauterine insufflation pressure, duration, humidity and the role of maternal hyperventilation on fetal physiology and wellbeing.

Introduction

One of the challenges of fetoscopic surgery is the confined liquid-filled intra-uterine environment, which limits visibility, fetal immobilization and maneuverability of surgical instruments. Partial amniotic carbon dioxide insufflation (PACI) was proposed to overcome these limitations by draining a portion of amniotic fluid and insufflating the uterus with CO₂.^{1,2} Gaseous uterine distension improves visualization, allows better control of bleeding and enables use of the required surgical tools in a more conducive workspace^{3,4}. PACI thus enables complex fetoscopic surgeries, such as myelomeningocele (MMC) repair.⁵⁻⁹ However, there are safety concerns about PACI as in sheep it causes severe fetal hypercapnia and acidosis.¹⁰⁻¹³ The impact of PACI at clinically relevant pressures and duration on utero-placental circulation, uterine membranes and the fetal brain are not well described.^{1,10-13} Furthermore, there is no consensus on optimal maternal ventilation targets during PACI to offset fetal hypercapnia.¹⁰⁻¹³ Given that antenatal MMC repair by hysterotomy is beneficial, there is much interest in optimizing this operation using fetoscopy to avoid maternal morbidity associated with hysterotomy.¹⁴ In this review, we summarize the available literature concerning PACI, as scarcity of data and fundamentally different study designs prevent rigorous systematic review. We further discuss the physiological principles relevant to CO₂ insufflation and define novel research questions relating to the clinical use of PACI.

Fetal surgery for myelomeningocele

Myelomeningocele (MMC) is characterized by a vertebral column defect that exposes the underlying spinal cord to the intra-uterine environment and allows cerebrospinal fluid leakage, resulting in prenatal hindbrain herniation, changes in ventricular size and

progressive neurological impairment.¹⁵⁻¹⁹ The “Management of Myelomeningocele Study” demonstrated that antenatal MMC repair via open fetal surgery improves neuromotor outcomes and reduces hindbrain herniation and postnatal shunt rate compared to postnatal repair.¹⁴ However, open fetal surgery is associated with high rates of premature delivery, significant maternal complications and implications for future pregnancies.^{14,20,21}

Fetoscopic MMC repair using PACI was therefore proposed as a minimally invasive alternative to reduce preterm birth and maternal morbidity. However, initial experience resulted in high rates of PPRM, preterm delivery, pregnancy complications and perinatal death compared to open fetal surgery.^{5,8,22} Additionally, animal studies demonstrating severe fetal hypercapnia and acidosis during PACI increased safety concerns for the developing fetus.¹⁰⁻¹³

On the other hand, recent clinical series report more promising maternal and fetal outcomes.⁹ A recent preliminary case series of three fetuses undergoing fetoscopic MMC repair using PACI suggests that fetal hypercapnia in humans may not be as severe as described in animal models.²³ However, umbilical vein blood samples were collected after PACI, during the recovery phase. Interestingly, the pH reduction was least in the fetus exposed to heated-humidified PACI.²³ This could be explained by reduced fetal CO₂ absorption due to lower CO₂ solubility at higher temperatures and reduced CO₂ partial pressures due to higher water vapor pressures.²⁴

While case series report no perinatal deaths or adverse outcomes directly linked to PACI, concerns remain for the potential of fetal neurological injury.^{5-9,25} Indeed, adverse neurological outcomes relating to PACI may be difficult to distinguish from other causes given multiple confounding factors that may contribute to neurologic disability in this patient group. This includes the natural history of MMC, success and

timing of repair, duration of anesthesia and iatrogenic prematurity. Therefore, robust animal studies are required to thoroughly assess the independent neurological risk relating to PACI use.

Multiple procedure related factors could contribute to high PPROM rates following fetoscopic MMC repair, such as long operating times, dehydration, cooling and using multiple trocars.^{5-9,26} However, it is unclear whether PACI influences PPROM risk by prolonged CO₂ exposure of the uterine membranes, insufflation pressure or gas temperature and humidity.^{5-8,14,22} Interestingly, lower PPROM rates have been reported when heated-humidified, low-pressure, low-flow PACI is used in an exteriorized uterus, permitting surgical closure of the fetal membranes.⁹

The debate concerning the optimal surgical approach for fetoscopic MMC repair is ongoing, as discussed in several recent papers on this subject, and is thus not the focus of this review.^{25,27-29} However, optimization of the PACI technique may also influence clinical outcomes and is potentially relevant to other surgeries, such as fetoscopic laser-coagulation.³⁰

Carbon dioxide physiology

CO₂ is a highly soluble by-product of aerobic cellular metabolism that rapidly diffuses from tissues into the blood (Figure 1). Approximately 65-70% of CO₂ in blood reacts with water molecules within red blood cells to produce carbonic acid (H₂CO₃), a reaction catalyzed by carbonic anhydrases (CA).^{31,32} Carbonic acid quickly dissociates into hydrogen ions (H⁺) and bicarbonate ions (HCO₃⁻). H⁺ bind with deoxygenated hemoglobin and other proteins, whilst HCO₃⁻ diffuses out the cell (Figure 1(a)). This is known as the carbonic acid-bicarbonate buffer system (equation below) whereby the reaction direction depends on substrate and product levels.

Approximately 20-25% of CO₂ binds directly to hemoglobin in red blood cells (Figure 1(b)), while only 8-10% dissolves in plasma (Figure 1(c)). Due to its high solubility, postnatally CO₂ is efficiently eliminated from the pulmonary circulation during expiration.³³ In healthy non-pregnant adults, alterations in pulmonary ventilation (rate and/or tidal volume) maintain an arterial CO₂ pressure (PaCO₂) of 35-45mmHg. Meanwhile, H⁺ levels are controlled by binding to plasma proteins, renal H⁺ excretion and HCO₃⁻ reabsorption and H⁺/HCO₃⁻ re-association following pulmonary CO₂ elimination. Thus, pH is tightly regulated at 7.35-7.45, which is essential for cellular function.³³

Homeostatic response to hypercapnia and acidosis

In adults

Hypercapnia refers to high blood partial pressure of CO₂ (PaCO₂), typically resulting from imbalances in tissue CO₂ production and pulmonary elimination. If buffer systems are overwhelmed, hypercapnia results in excess free H⁺ in blood, lowering blood pH which is known as a respiratory acidosis. Conversely, metabolic acidosis results from a build-up of nonvolatile acids (e.g. lactic acid during anaerobic metabolism) or excessive loss of bicarbonate. Regardless of the cause of acidosis, the brain and carotid artery chemoreceptors detect increased H⁺ and stimulate breathing, promoting respiratory CO₂ elimination.³³ In chronic acidosis, renal HCO₃⁻ reabsorption and H⁺ excretion increase to minimize pH disturbances.³³

In the maternal-fetal unit

In the fetus, CO₂ regulation is limited by immature fetal systems and depends on transfer into the maternal compartment.³⁴⁻³⁶ Carbonic anhydrases (CA), a key catalyst

in buffering excess CO₂, are present in the fetus, yet blood CA levels and functional activity are low compared to adults.^{37,38} However, CA activity has been detected in multiple fetal tissues, which may mitigate the adverse effects of tissue hypercapnia and acidosis.³⁹⁻⁴⁴ In fetal skin, gastric mucosa, liver, pancreas and kidneys, CA expression is similar to that in adults.⁴⁰⁻⁴² Lung CA levels are greatest during fetal life and may play a role in fetal lung liquid secretion.^{39,42} Conversely, fetal brain CA activity increases during gestation, but remains significantly lower than adults, even at term.⁴²⁻⁴⁴ CA activity in placental tissues is hypothesized to facilitate CO₂ transfer across the placenta into the maternal circulation.⁴⁵⁻⁴⁹ However, low placenta CA levels reported in some studies may saturate this mechanism and limit fetal CO₂ elimination rates.⁴⁵⁻⁴⁸ In animals the capacity for fetal renal bicarbonate excretion and reabsorption increases throughout gestation, suggesting that the fetal kidneys are not a major contributor for acid-base regulation in early- and mid-gestation.^{36,50} Further, as bicarbonate is poorly transported across the placenta and fetal buffer systems are limited and rapidly exhausted, the main mechanism for fetal CO₂ elimination is believed to be diffusion across the placenta.^{34-36,51} CO₂ diffusion out of the fetal compartment is facilitated by relative maternal hypocapnia induced by physiological adaptations in pregnancy, which increase the partial pressure gradient.⁵²⁻⁵⁴ Increasing progesterone levels during pregnancy act as a respiratory stimulant, increasing alveolar minute ventilation by 30-50%. Consequently, maternal PaCO₂ decreases from 35-40mmHg to 26-32mmHg during pregnancy.⁵²⁻⁵⁴ A compensatory increase in renal bicarbonate excretion minimizes maternal alkalosis, maintaining a maternal pH of 7.40-7.47 compared to 7.35-7.45 in the non-pregnant state.⁵²⁻⁵⁴

Physiological effects of hypercapnia

While fetal hypercapnia and acidosis during hypoxia have a well-established association with neonatal morbidity and poor neurological outcomes, the consequences of fetal acidosis *in the absence of hypoxia* are largely unknown.⁵⁵ The effects in preterm neonates and adults are, however, well documented (Table 1) and discussed below.

Cerebrovascular

Hypercapnic acidosis causes vasodilation of cerebral vessels thereby increasing cerebral blood flow and intracranial pressure.³³ In adults, hypercapnia decreases cerebral oxygen metabolism, alters cerebral neurotransmission and increases markers of neuronal injury.⁵⁶⁻⁵⁸ Studies of permissive hypercapnia in ventilated premature and very low birth weight infants show progressively impaired cerebral blood flow auto-regulation at PaCO₂ levels above 45mmHg and an increasing risk of intraventricular hemorrhage with increasing PaCO₂.^{59,60} Conversely, a more recent large randomized control trial using permissive hypercapnia in extremely low birth weight infants (PHELBI study) did not confirm an increased risk of intraventricular hemorrhage.⁶¹ Nonetheless, in addition to demonstrating no benefit of permissive hypercapnia, the authors also questioned its safety as necrotizing enterocolitis was more common in hypercapnic infants and was thought to result from impaired intestinal cell function caused by the acidosis.⁶¹

Cardiovascular

Hypercapnic acidosis acts locally by directly suppressing myocardial contractility, decreasing heart rate and promoting vasodilation.⁶² However, to maintain cardiac output, hypercapnia also stimulates central sympathetic activity leading to tachycardia,

increased cardiac contractility and promoting peripheral vasoconstriction.^{63,64} In adults, hypercapnia increases cardiac output, heart rate and risk of arrhythmia,⁶⁵ but peripheral vasodilation limits increases in mean arterial pressure.⁶³ With worsening hypercapnia and a pH below 7.2, the central response is impaired resulting in cardiac depression and bradycardia.^{33,64}

Immunological

In vitro and adult clinical studies show that hypercapnia impairs immune cell function and reduces the systemic inflammatory response.⁶⁶⁻⁷⁰

Carbon dioxide insufflation for endoscopic surgery

High solubility, non-combustibility, low cost and wide availability of CO₂ make it the gas of choice for abdominal insufflation in laparoscopic surgery.⁷¹ High gas solubility diminishes the risk of gas embolism which, although a very rare event, has limited treatment options and high mortality.⁷¹ However, CO₂ rapidly absorbs into body tissues causing hypercapnia and acidosis.^{63,72}

In infants, there are some concerns with CO₂ insufflation for endoscopic procedures, including an age-dependent reduced capacity for CO₂ elimination and increased susceptibility to cardiovascular depression and hypotension in response to hypercapnia, compared to older children and adults.⁷³⁻⁷⁵ Retrospective clinical studies report significant hypercapnia, acidosis and decreased cerebral oxygen saturation during thoracoscopy with CO₂ insufflation to repair congenital diaphragmatic hernia and esophageal atresia in neonates.^{76,77} However, long-term neurodevelopmental outcomes are lacking and an overarching scarcity of high quality prospective evidence in this field limits data interpretation.^{75,78}

Nonetheless, laparoscopic surgery in adults and children is considered safe, especially when abdominal insufflation pressures do not exceed 12mmHg and pulmonary ventilation is increased to enhance CO₂ elimination.^{79,80} In pregnancy, laparoscopy is an increasingly common alternative to open abdominal surgery for intra-abdominal pathology whenever possible.⁸¹

Animal models of PACI

Four animal studies investigated the effects of PACI on fetal acid-base status, all using pregnant sheep (Table 2).¹⁰⁻¹³ All four studies observed fetal hypercapnia and acidosis after CO₂ insufflation. However, these studies did not include a control group and instead assessed fetal acid-base changes from baseline. This approach does not account for potential effects of fetal surgery and prolonged anesthesia on fetal acid-base status. Additionally, some studies used older anesthetic agents (3% halothane) that have known fetal acid-base effects.⁸²

One study compared the effects of uterine distension using CO₂, helium and water and found that only CO₂ amniotic insufflation resulted in fetal hypercapnia and acidosis.¹¹ Distension with water led to mild fetal hyponatremia and hypochloremia, whereas helium insufflation did not affect fetal acid-base status or electrolytes. These findings suggest fetal acid-base disturbances were associated with the CO₂ diffusion and fetal uptake, rather than uterine distention. Additionally, fetoscopic neck dissection and fetal tracheal clipping during CO₂ insufflation did not worsen fetal acidemia compared to insufflation-only.¹³

Whilst fetal hypercapnia and acidosis occurred in all four animal studies, the route of CO₂ absorption into the fetal bloodstream, whether via fetal skin, placental or fetal vessels or another pathway, was not investigated hence remains unclear.

Insufflation duration

In all four studies, fetal acidemia worsened with longer insufflation duration.¹⁰⁻¹³ Gratacos *et al* had the longest insufflation period (60-minutes) resulting in the largest increase in fetal PaCO₂ (from 55 to 88mmHg) and decrease in pH (from 7.24 to 7.06).¹⁰⁻¹³ Importantly, the duration of PACI currently used in published human fetoscopic surgeries averages 183 to 250 minutes, significantly exceeding that tested in animal models.^{7,9}

Insufflation pressures

Altering PACI pressures could have multiple impacts on fetal and maternal wellbeing. Both the insufflation pressure required for adequate uterine distension and the subsequent fetal and maternal effects are potentially influenced by whether the uterus is exteriorized during PACI. Insufflation pressures used for PACI in sheep studies were lower when the uterus was exteriorized during CO₂ insufflation (3-4mmHg vs. 10-15mmHg, Table 1).¹⁰⁻¹³ Uterine exteriorization may reduce pressures required for necessary uterine distension by eliminating additional resistance created by a closed abdominal wall and the intraabdominal contents.

Increasing insufflation pressures increases the partial pressure for CO₂, thereby increasing tissue CO₂ absorption and thus the severity of resulting hypercapnia and acidosis. Indeed, increasing abdominal CO₂ insufflation pressures worsens hypercapnia in adult laparoscopic surgery and increases fetal hypercapnia in pregnant ewes.⁸³⁻⁸⁷

Another consideration is the mechanical effect of increasing uterine distension. Sheep studies of intrauterine distension with a 'loosely closed' abdomen using saline or medical air showed that intrauterine pressures greater than 20mmHg and 15mmHg

respectively significantly reduced uterine and umbilical artery flow leading to fetal hypoxia.⁸⁸⁻⁹⁰ Additionally, where the laparotomy incision is closed, increasing uterine insufflation pressures may increase intra-abdominal pressures and thereby decrease venous return and cardiac output.⁹¹⁻⁹³ Increased pressure on the diaphragm also reduces respiratory system compliance, increases airway pressures, reduces inspiratory reserve volumes and decreases functional residual capacity, making the patient more vulnerable to hypoxia and reducing the capacity for pulmonary CO₂ elimination.^{63,80,94} However, in spontaneously breathing baboons, it has been shown that intra-abdominal insufflation pressures up to 77mmHg were rarely lethal, despite significant respiratory distress and hemodynamic compromise.⁹⁵ In the pregnant patient, the gravid uterus exacerbates increased intra-abdominal pressure.^{52,53} Further, pregnancy is associated with a 50% increased cardiac output, with a 45-50% increased circulating blood volume and moderate increases in maternal heart rate.^{53,96-98} This hyperdynamic cardiovascular state increases vulnerability to cardiopulmonary compromise and reduces compensatory capacity.

PACI pressures reported in clinical series of fetoscopic MMC repair are in a broad range (9 – 30mmHg), with lower maximum pressures reported when the uterus is exteriorized for the procedure (12±2mmHg).^{7,9} A safe pressure range for PACI, has not been identified yet, but is an important consideration because of potential adverse effects for both mother and fetus.

Maternal ventilation during PACI

During abdominal CO₂ insufflation, increasing minute ventilation increases respiratory CO₂ elimination, thereby mitigating the associated acidosis in adult humans undergoing laparoscopy⁸⁰ and preventing fetal acid-base disturbances in pregnant

sheep.⁸⁷ As such, altering maternal ventilation targets during PACI may increase the fetal-maternal PaCO₂ gradient and enhance fetal CO₂ elimination.

Maternal ventilation targets varied significantly between animal PACI studies.¹⁰⁻¹³ When ventilation settings were fixed at a level producing maternal PaCO₂ of 35 – 40mmHg prior to insufflation, PACI resulted in increased maternal PaCO₂ and, to a greater extent, fetal PaCO₂.^{12,13} Furthermore, fetal PaCO₂ increases during PACI even when maternal PaCO₂ is maintained with ventilation adjustments.¹⁰ It is suggested that further lowering maternal PaCO₂ levels (hypocapnia) with maternal hyperventilation could theoretically enhance fetal CO₂ elimination. On the other hand, hyperventilation and hypocapnia (PaCO₂ 20mmHg) in pregnant ewes is associated with reduced uteroplacental blood flow and decreased fetal oxygenation.⁹⁹ Interestingly, uterine artery flow remains diminished during maternal hyperventilation when CO₂ is added to inspired air to establish maternal normocapnia and hypercapnia.⁹⁹ Thus the increase in mean airway pressure during hyperventilation may impair uterine artery flow by reducing venous return. Additionally, fetal oxygenation is only impaired during maternal hypocapnic periods, which could be explained by a left shift in the maternal oxygen-hemoglobin dissociation curve.⁹⁹ This would increase maternal hemoglobin oxygen affinity and impair oxygen offloading to the fetus.^{100,101}

The effect of maternal hyperventilation and hypocapnia during PACI was addressed in two animal studies with conflicting outcomes. Saiki *et al* found fetal hypercapnia and acidosis was completely reversed by maternal hyperventilation, whilst Gratacos *et al* found fetal acid-base changes could be reduced but not prevented.^{12,13} Saiki *et al* reported significant decreases in fetal PaO₂ during maternal hyperventilation, whilst Gratacos *et al* reported no change.^{12,13} Multiple differences between the studies may have contributed to conflicting outcomes. Saiki *et al* had a shorter insufflation period

(30 vs. 60-minutes) and slightly lower maternal PaCO₂ targets (25 – 28 vs. 28 – 30mmHg) compared to Gratacos *et al.*^{12,13} Saiki *et al* reported increased maternal hemodynamics (systolic blood pressure 124 – 142 vs. 98mmHg, heart rate 128 – 157 vs. 79bpm), which may have increased placental blood flow and facilitated fetal elimination of CO₂.^{12,13}

Neurological effects of PACI

The effects of PACI on the developing fetal brain at insufflation pressures and durations used clinically remain unclear. Kohl *et al* exposed fetal lambs to PACI and followed them to term, observing no obvious abnormalities in neonatal movements or feeding patterns and no gross histological evidence of neonatal brain hemorrhage, infarction, inflammation, or abnormal cortical maturation on H&E staining.¹ However, compared to clinical fetoscopic surgery, this study used shorter PACI durations (45 – 80 vs. 140 – 450-minutes) and lower maximum insufflation pressures (7 – 15 vs. 9 – 30mmHg).^{1,7-9} Additionally, subtle neurological impairment that is clinically significant in human newborns may not be evident on gross observation of newborn lambs. Likewise, subtle markers of neonatal brain injury may require more rigorous histological analysis to identify. Although reassuring at low pressure for a short duration, this single study is not sufficient to demonstrate fetal neurological safety of pressures and duration used clinically.

Uterine membrane effects of PACI

Whilst abdominal CO₂ insufflation for laparoscopy is known to cause peritoneal tissue damage,¹⁰²⁻¹⁰⁴ little is known about the effects of PACI on uterine membranes.

Replacing amniotic fluid with a cold, dry gas introduces potential risks of uterine and fetal membrane dehydration and inflammation, such as described in peritoneal membranes during insufflation with cold dry CO₂.¹⁰²⁻¹⁰⁴ Theoretically, these effects could contribute to an apparently higher incidence of premature labor following fetoscopic MMC repair, and may, in this way, indirectly have consequences for fetal wellbeing.⁵⁻⁸ Importantly, using heated-humidified CO₂ for PACI may mitigate such effects, as has been observed for abdominal CO₂ insufflation. Indeed, using heated-humidified CO₂ reduces peritoneal damage, adhesion formation, intraoperative hypothermia and postoperative pain observed using unheated gas.¹⁰³⁻¹⁰⁵ The potential benefits of using heated-humidified CO₂ clinically are also suggested by promising preliminary results observed in a recent case series.⁵ However, other procedural factors such as lower insufflation pressures, uterus exteriorization and primary skin closure of MMC defect are also unique to this series.

Other candidate gases for insufflation

Using alternative gases for uterine distension has been suggested to avoid fetal hypercapnia and acidosis associated with CO₂ absorption. Medical air and oxygen are highly combustible, whilst low solubility of nitrogen increases risk of gas embolism.⁷¹ Nitrous oxide and helium are considered safe alternatives because of their non-combustible properties. Indeed, sheep studies do not demonstrate fetal hypercapnic acidosis during uterine distension with helium or abdominal insufflation with either helium or nitrous oxide.^{11,86,87} However, uterine insufflation may have mechanical effects on fetal-placental blood flow, as described previously, that would presumably occur independent of the distension media used.⁸⁵⁻⁹⁰

Limitations of the ovine model

Pregnant sheep are commonly used for pregnancy related research, largely due to the possibility for invasive placental and fetal physiological monitoring.¹⁰⁶ Its relatively large size and uterine quiescence permits fetal and placental manipulation and instrumentation without high risk of preterm labor.¹⁰⁶⁻¹⁰⁸ Pregnant ewes have a relatively long gestation and deliver a precocious offspring, allowing assessment of the effects of PACI at clinically relevant times in gestation. The fetal sheep brain is well-studied and considered a reliable model of gross human fetal brain injury owing to sufficient anatomical and developmental similarities.¹⁰⁹⁻¹¹¹

However, the ovine model has limitations when translating the effects of PACI to human pregnancies. The sheep uterus is thought to be more compliant than the human uterus, thereby increasing its susceptibility to overdistension and potentially overestimating the observed effects of PACI.¹¹² The relative quiescence of the sheep uterus precludes its use for assessing preterm birth risk in response to PACI.¹⁰⁶⁻¹⁰⁸ Additionally, The sheep placenta consists of multiple fetal cotyledons paired with maternal caruncles interspersed throughout the uterine wall (Figure 2 (b, c)). Fetal cotyledons are joined by inter-cotyledonary fetal vessels that travel between the fetal membranes (Figure 2 (b, c)). In contrast, cotyledons comprising the human placenta join adjacently to form a single discoid structure (Figure 2 (a)). Thus, it is possible that inter-cotyledonary fetal vessels in sheep provide a greater surface area for CO₂ absorption or that 'stretching' of these vessels by uterine distension may impair placental gaseous exchange. Furthermore, the placental unit in sheep is synepitheliochorial, with additional maternal tissue layers separating the maternal and fetal vasculature compared to the human hemochorial placenta.^{112,113} Nonetheless,

numerous studies demonstrate the feasibility of using the ovine model to investigate human placental physiology, including gaseous diffusion.¹⁰⁷

Finally, potential subtle yet clinically significant human fetal brain injury and neurological impairment may not be well assessed using an ovine model. Whilst primates are an alternative animal model more closely reflecting the human uterine membrane and placental structure,^{106,114} this species is financial and ethically a limited resource in animal research.^{106,114,115}

Conclusion

In the last decades, fetoscopic myelomeningocele repair has been suggested as an alternative to open fetal surgery.⁵⁻⁹ The complexity of this operation still requires amniodistention using a gas, and CO₂ is clinically the gas of choice so far. However, there is concern about its use.¹¹⁶ In sheep, PACI causes fetal hypercapnia and acidosis and the fetal consequences of this acid-base disturbance are not well understood.¹⁰⁻¹³ Clinical outcomes following the use of PACI are difficult to interpret, especially because there is a large variability between current clinical techniques, including regarding insufflation pressures, duration, gas temperature and humidity. On the other hand, the available literature report no major adverse effects that can be clearly related to iatrogenic fetal acidosis during fetoscopy. Nevertheless, we believe that there are still very relevant research questions concerning the use of PACI in humans to guide future studies, such as examining the effects on fetal brain oxygen delivery and metabolism, fetal brain injury, placental blood flow and the uterine membranes.

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Table 1. Summary of System Responses to Hypercapnia

System	Effects of Hypercapnia
Cerebral	<p>↑Cerebral Blood Flow³³</p> <p>↑Intracranial Pressure³³</p> <p>↓Cerebral metabolic rate of oxygen consumption⁵⁶</p> <p>↓NMDA and GABA brain receptor activity⁵⁷</p> <p>Neuronal injury⁵⁸</p>
Cardiovascular	<p>↑Cardiac Output (↓ if severe – pH <7.2)</p> <p>↑Heart Rate (↓ if severe – pH <7.2)</p> <p>↑Myocardial oxygen demand</p> <p>↓Systemic Vascular Resistance</p> <p>Small ↑Mean Arterial Pressure^{33,64}</p> <p>Cardiac arrhythmias⁶⁵</p>
Respiratory	↑Minute ventilation ³³
Endocrine	↑Cortisol ¹¹⁷
Immunological	<p>↓ Immune cell function⁶⁶⁻⁷⁰</p> <p>↓ Systemic inflammatory response⁶⁶⁻⁷⁰</p>

Table 2. Studies Monitoring Fetal Acid Base Status During PACI

	Luks, 1994 ¹⁰	Pelletier, 1995 ¹¹	Saiki, 1997 ¹²	Gratacos, 2001 ¹³
Gestation† (days)	95-105	120	105	92-100
Uterus exteriorized	Yes	No, laparotomy incision open	No, laparotomy incision closed	Yes
Insufflation Pressure (mmHg)	3-4	15	10	4-5
Insufflation Duration (minutes)	30	30	30	60
Maternal Ventilation goals	Maintain end tidal CO ₂ of 30mmHg (~PaCO ₂ 38mmHg) throughout insufflation	NS	Obtain PaCO ₂ 35- 40 prior to insufflation. Settings fixed and PaCO ₂ allowed to rise during insufflation.	Obtain PaCO ₂ 38- 40 prior to insufflation. Settings fixed and PaCO ₂ allowed to rise during insufflation
Fetal Acid Base‡				
PaCO ₂	57.6±1.6 → 87±7 ^c	64±4 → 83±6 ^c	51±3 → 72±5 ^c	55±3 → 88±3 ^c
pH	7.22±0.03 → 7.11±0.08 ^c	7.26±0.03 → 7.16±0.04§	7.35±0.01 → 7.24±0.03 ^c	7.24±0.01 → 7.06±0.03 ^c
PaO ₂	“Stable”	20±2 → 24±1§	24±2 → 28±3	19±1 → 22±2
Maternal Acid Base‡				
PaCO ₂	38¶ throughout insufflation	40±4 → 47±5	~38 → ~47	40±1 → 49±1 ^c
pH		7.32±0.03 → 7.29±0.03	7.33-7.38 → 7.28- 7.32	7.38±0.02 → 7.33±0.02 ^c

PaO ₂		161±32 → 159±28	333±53 → 264±42	222±22 → 226±24
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† Sheep term 147 days

‡ Acid base values are based on averages ± standard deviation

§ Statistically significant change

¶ Approximate values read off a graph

(Studies referenced in this table are numbered as appears in Main Text)

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Figure 1

A diagrammatic representation of the how CO₂ produced by active cells is buffered and transported in the plasma. Three pathways (a, b, c) are shown with approximate percentages for each. Pathway (a) represents the combination of CO₂ and water, catalyzed by carbonic anhydrase within red blood cells, to produce carbonic acid (H₂CO₃). Carbonic acid quickly dissociates into hydrogen ions, which bind with deoxygenated hemoglobin, and bicarbonate ions, which diffuse out the cell. Pathways (b) and (c) represent CO₂ binding to hemoglobin directly and dissolving plasma respectively. (Schematic by Ben Amberg using Powerpoint Image Bank, Servier Medical Art, Servier).

Figure 2
The discoid hemochorial placenta in the humans is diagrammatically represented (a) with maternal blood from uterine spiral arteries bathing fetal chorionic villi containing fetal vessels. The sheep cotyledonary synepitheliochorial structure is compared (b) with multiple placentomes throughout the uterus. Each placentome is composed of a maternal caruncle (*) in the uterine wall containing maternal vessels paired with a fetal cotyledon (#) containing fetal vessels. Fetal cotyledons are joined by inter-cotyledonary fetal vessels that travel through the uterine membranes and coalesce to form the umbilical vessels. The internal aspect of the sheep uterus at postmortem is depicted photographically (c). (Schematic drawings by Myrthe Boymans (www.myrtheboymans.nl) for, and copyright by, UZ Leuven, Belgium).