



Physiological effects of partial amniotic carbon dioxide insufflation with cold, dry *vs* heated, humidified gas in a sheep model

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ABSTRACT

Objective Partial amniotic carbon dioxide (CO₂) insufflation (PACI) is used to improve visualization and facilitate complex fetoscopic surgery. However, there are concerns about fetal hypercapnic acidosis and postoperative fetal membrane inflammation. We assessed whether using heated and humidified, rather than cold and dry, CO₂ might reduce the impact of PACI on the fetus and fetal membranes in sheep.

Methods Twelve fetal lambs of 105 days' gestational age (term = 145 days) were exteriorized partially, via a midline laparotomy and hysterotomy, and arterial catheters and flow probes were inserted surgically. The 10 surviving fetuses were returned to the uterus, which was then closed and insufflated with cold, dry (22°C at 0–5% humidity, n = 5) or heated, humidified (40°C at 100% humidity, n = 5) CO₂ at 15 mmHg for 180 min. Fetal membranes were collected immediately after insufflation for histological analysis. Physiological data and membrane leukocyte counts, suggestive of membrane inflammation, were compared between the two groups.

Results After 180 min of insufflation, fetal survival was 0% in the group which underwent PACI with cold, dry CO₂, and 60% (n = 3) in the group which received heated, humidified gas. While all insufflated fetuses became progressively hypercapnic (PaCO₂ > 68 mmHg), this was considerably less pronounced in those in which heated, humidified gas was used: after 120 min

of insufflation, compared with those receiving cold, dry gas (n = 3), fetuses undergoing heated, humidified PACI (n = 5) had lower arterial partial pressure of CO₂ (mean ± standard error of the mean, 82.7 ± 9.1 mmHg for heated, humidified CO₂ vs 170.5 ± 28.5 for cold, dry CO₂ during PACI, P < 0.01), lower lactate levels (1.4 ± 0.4 vs 8.5 ± 0.9 mmol/L, P < 0.01) and higher pH (pH, 7.10 ± 0.04 vs 6.75 ± 0.04, P < 0.01). There was also a non-significant trend for fetal carotid artery pressure to be higher following PACI with heated, humidified compared with cold, dry CO₂ (30.5 ± 1.3 vs 8.7 ± 5.5 mmHg, P = 0.22). Additionally, the median (interquartile range) number of leukocytes in the chorion was significantly lower in the group undergoing PACI with heated, humidified CO₂ compared with the group receiving cold, dry CO₂ (0.7 × 10⁻⁵ (0.5 × 10⁻⁵) vs 3.2 × 10⁻⁵ (1.8 × 10⁻⁵) cells per square micron, P = 0.02).

Conclusions PACI with cold, dry CO₂ causes hypercapnia, acidosis, hypotension and fetal membrane inflammation in fetal sheep, raising potential concerns for its use in humans. It seems that using heated, humidified CO₂ for insufflation partially mitigates these effects and this may be a suitable alternative for reducing the risk of fetal acid–base disturbances during, and fetal membrane inflammation following, complex fetoscopic surgery. © 2018 The Authors. *Ultrasound in Obstetrics & Gynecology* published by John Wiley & Sons Ltd on behalf of the International Society of Ultrasound in Obstetrics and Gynecology.

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INTRODUCTION

Open fetal surgery is considered the gold standard for antenatal repair of myelomeningocele, following the results of the Management of Myelomeningocele Study¹. However, significant maternal morbidity associated with open fetal surgery and implications for future pregnancies has driven the development of less invasive, fetoscopic approaches^{1–3}. Unfortunately, the feasibility of complex fetoscopic surgery is hampered by limited space, poor visibility within the cloudy amniotic fluid and surgical instruments that do not work in a fluid environment². Partial drainage of the amniotic fluid and gaseous distention of the uterus with carbon dioxide (CO₂) overcomes these challenges². CO₂ gas seems a logical choice, given its low risk of causing gas embolism, extensive use for endoscopic surgery in adults and infants and the fact that it has virtually no known adverse effects⁴.

However, potential adverse effects of partial amniotic CO₂ insufflation (PACI) on fetal development have been suggested, and its use remains controversial. These safety concerns are based on observations in animal experiments demonstrating fetal hypercapnic acidosis^{5–8}. Yet, human clinical case series have reported no adverse effects of PACI during surgery and immediately after delivery^{2,9–12}. Invasive fetal monitoring during human fetal surgery is not easy and human data are scarce. In a recent case series¹³ ($n = 3$), umbilical venous blood gasses were sampled before and after fetoscopic myelomeningocele repair using PACI. Reassuringly, this study observed only very mild changes in fetal acid–base balance in two of the cases. Interestingly, these changes were absent in the third case, which used heated, humidified CO₂ for insufflation. There are no animal studies investigating the physiological effects on the fetus of PACI with heated, humidified gas. Additionally, lower rates of postoperative preterm prelabor rupture of membranes (PPROM) have been observed at fetoscopic centers which use heated, humidified CO₂ for PACI, compared with others that use cold, dry CO₂ (23% *vs* 75–85%)^{2,9,10}, although procedural differences may explain this in part.

We hypothesized that the use of cold, dry CO₂ for PACI triggers an inflammatory process in the choriodecidua and precipitates PPRM by dehydrating the membranes^{14,15}. Our aim in this study, therefore, was to investigate in sheep the effects of using heated and humidified, rather than dry and cold, CO₂ for PACI, with clinically relevant insufflation pressures and durations, on the fetal acid–base balance and cardiovascular function, and on fetal membrane inflammation.

METHODS

Surgery

The experimental method was approved by the Monash Medical Centre Animal Ethics Committee and followed a series of experiments in our laboratory to optimize the surgical protocol. Twelve pregnant Merino-Border

Leicester ewes between 105 and 108 days' gestation were anesthetized with an intravenous bolus of sodium thiopentone (Pentothal, Boehringer Ingelheim, Warriewood, NSW, Australia). Ewes were ventilated via an endotracheal tube and general anesthesia was maintained with 2–2.5% inhaled isoflurane (Isoflow, Abbot Pty Ltd, North Chicago, IL, USA) in air/oxygen. End-tidal CO₂ was measured using a SurgiVet vital signs machine (SurgiVet® Advisor®, Smiths Medial, Dublin, OH, USA). A temperature probe was placed in the ewe's esophagus and a polyvinyl catheter (internal diameter, 2.6 mm; Dural Plastics, Sydney, NSW, Australia) was inserted into the carotid artery. Arterial blood gasses were sampled at 10-min intervals to adjust the ewe's ventilation rate and tidal volume to maintain the maternal partial pressure of CO₂ (PaCO₂) between 35 and 45 mmHg.

The fetus was partially exteriorized via a midline laparotomy and hysterotomy, and we inserted surgically a carotid artery and jugular vein catheter (internal diameter, 0.86 mm), umbilical vein flow probe (Transonic Systems, Ithaca, NY, USA) and esophageal temperature probe. Insufflation tubing and an amniotic drainage catheter were inserted through the uterine wall into the amniotic sac. All catheters and flow probes were exteriorized and the hysterotomy incision closed in three layers to maintain an airtight seal of the uterus for insufflation. A flow probe was also placed around a large branch of the uterine artery supplying the pregnant horn. The uterus was then returned to the maternal abdomen and the laparotomy incision closed to allow insufflation of the uterus within the abdominal cavity as performed clinically in percutaneous approaches^{2,10,11}.

Maternal (temperature, carotid artery pressure, heart rate and uterine artery flow) and fetal (temperature, carotid artery pressure, umbilical vein flow and heart rate) parameters were recorded continuously using the LabChart and PowerLab data acquisition system (ADInstruments, Bella Vista, NSW, Australia).

Two fetuses did not survive the initial surgery and were excluded, leaving 10 fetuses for insufflation.

Partial amniotic CO₂ insufflation

After surgery, the ewe and fetus were allowed to stabilize for 10 min before baseline physiological recordings were taken and blood gasses sampled (time = 0). The amniotic fluid was drained and ewes were allocated arbitrarily to PACI with cold, dry (22 °C at 0–5% humidity, $n = 5$) or heated, humidified (40 °C at 100% humidity, $n = 5$) CO₂. The intra-amniotic pressure was increased to 15 mmHg with an insufflator (40 L High-Performance Insufflator, Stryker South Pacific, St Leonards, NSW, Australia) over a 5-min period, and this pressure was maintained for 180 min. The insufflation pressure and duration were selected based on averages obtained from human case series involving insufflation of the uterus within the abdomen^{2,10,11}. In ewes receiving heated, humidified CO₂, the gas was passed through a laparoscopic humidification

system (MR860, Fisher and Paykel Healthcare, Auckland, New Zealand) before it entered the uterus.

Fetal blood gas analysis was performed every 10 min for the first 30 min and every 30 min thereafter. The uterus was desufflated after 180 min and monitoring continued for a further 20 min. Both ewe and fetus were then euthanized using intravenous pentobarbitone (Lethobarb, Virbac Pty Ltd, Peakhurst, Australia) and postmortem examination was conducted to collect sections of fetal membrane.

Fetal membrane collection and histology

From each ewe, two arbitrarily selected regions of insufflated fetal membrane some distance from the hysterotomy incision were dissected away, immersion-fixed in 10% formalin and embedded in paraffin for histological analysis. Two 5- μ m tissue sections were cut from each region, immunostained for CD45-positive cells (leukocytes) and counterstained with Mayer's hematoxylin, as described previously¹⁶. The number of CD45-positive cells in the amnion and in the chorion were counted by a single observer (B.J.A.), who was blinded to the insufflation group. Counts were performed using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA), in five arbitrarily selected, non-overlapping fields of view at 40 \times magnification (BX-41 Laboratory Microscope, Olympus America Inc., Center Valley, PA, USA). Each cell count was adjusted for membrane thickness and the average in the amnion and chorion of each ewe calculated.

Data and statistical analysis

Fetal carotid arterial pressure was adjusted for amniotic pressure and umbilical vein blood flow was adjusted for fetal weight. Maternal uterine artery blood flow is

expressed as percentage change from baseline. Blood gas and physiological data were normally distributed. These are presented as mean \pm standard error of mean (SEM) and compared at matched timepoints using mixed analysis of variance (ANOVA) with Holm-Šidák *post-hoc* analysis. Cell counts were not normally distributed. These are presented as median (interquartile range (IQR)) and compared using the Mann-Whitney *U*-test. Statistics were processed and graphed using GraphPad Prism (Prism V7, GraphPad Software Inc., La Jolla, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Five ewes were insufflated with cold, dry CO₂ and five with heated, humidified CO₂. Blood gas and physiological recordings at baseline and after 120 min of insufflation are given for the fetuses in Table 1 and for the ewes in Table 2. 120 min was chosen for comparison because of poor survival in the cold, dry CO₂ group beyond this point.

Fetal effects

All five fetuses in the group receiving cold, dry CO₂ developed severe metabolic disturbances and died before the end of the 180-min insufflation period, two before and three after 120 (range, 63–139) min. Only two of five died (between 120 and 180 min) in the group receiving heated, humidified CO₂. One of the heated, humidified CO₂ group died suddenly following a maternal anesthetic complication, while the other became progressively hypercapnic and acidotic in the same way as the cold, dry CO₂ animals. This second fetus had a much greater reduction from baseline in uterine artery blood flow compared with the other fetuses in the group,

Table 1 Fetal blood gas and hemodynamic parameters at baseline and after 120 min of partial amniotic carbon dioxide (CO₂) insufflation with cold, dry or with heated, humidified CO₂

Parameter	Baseline			After 120 min insufflation		
	Cold, dry CO ₂	Heated, humidified CO ₂	P*	Cold, dry CO ₂	Heated, humidified CO ₂	P*
<i>n</i>	5	5	—	3	5	—
Gestational age (days)	105.8 \pm 0.6	105 \pm 0.3	0.28	—	—	—
Carotid artery blood gas						
pH†	7.21 \pm 0.04	7.24 \pm 0.02	0.99	6.75 \pm 0.04	7.10 \pm 0.04	< 0.01
PaCO ₂ (mmHg)†	63.4 \pm 4.4	67.4 \pm 2.5	0.99	170.5 \pm 28.5	82.7 \pm 9.1	< 0.01
PaO ₂ (mmHg)†	22.4 \pm 2.2	26.6 \pm 0.6	0.02	12.0 \pm 0.05	31.6 \pm 1.2	< 0.01
SaO ₂ (%)	52.5 \pm 2.7	61.0 \pm 8.3	0.98	9.5 \pm 2.0	59.9 \pm 4.0	< 0.01
Bicarbonate (mmol/L)	22.4 \pm 1.2	26.6 \pm 0.6	0.07	24.6 \pm 3.8	22.4 \pm 1.0	0.96
Lactate (mmol/L)	3.9 \pm 0.8	2.8 \pm 0.3	0.90	8.5 \pm 0.9	1.4 \pm 0.4	< 0.01
Physiology						
Carotid artery pressure (mmHg)	36.1 \pm 2.5	33.0 \pm 1.5	> 0.99	8.7 \pm 5.5	30.5 \pm 1.3	0.22
Heart rate (bpm)	155 \pm 11	144 \pm 6	> 0.99	65 \pm 7.1	161.2 \pm 3.7	< 0.01
Umbilical vein flow (mL/kg/min)	138.5 \pm 8.7	127.6 \pm 26	> 0.99	7.4 \pm 0.7	124.6 \pm 26	0.25
Temperature (°C)	39.7 \pm 0.3	39.8 \pm 0.2	> 0.99	39.6 \pm 0.2	40.2 \pm 0.3	> 0.99

Data are presented as mean \pm standard error of the mean. Baseline values recorded immediately before insufflation (time = 0 min). *Mixed ANOVA with Holm-Šidák *post-hoc* analysis; $P < 0.05$ considered statistically significant. †Presented as body temperature-corrected values. PaCO₂, arterial partial pressure of carbon dioxide; PaO₂, arterial partial pressure of oxygen; SaO₂, arterial hemoglobin oxygen saturation.

Table 2 Maternal blood gas and hemodynamic parameters at baseline and after 120 min of partial amniotic carbon dioxide (CO₂) insufflation with cold, dry or with heated, humidified CO₂

Parameter	Baseline			After 120 min insufflation		
	Cold, dry CO ₂	Heated, humidified CO ₂	P*	Cold, dry CO ₂	Heated, humidified CO ₂	P*
<i>n</i>	5	5		3	5	—
Carotid artery blood gas						
pH†	7.40 ± 0.02	7.42 ± 0.04	> 0.99	7.37 ± 0.03	7.38 ± 0.03	> 0.99
PaCO ₂ (mmHg)†	42.2 ± 1.3	42.2 ± 1.9	> 0.99	43.6 ± 1.7	41.2 ± 2.1	> 0.99
PaO ₂ (mmHg)†	199.8 ± 45.7	190.0 ± 15.3	> 0.99	114.4 ± 47	157.5 ± 30	> 0.99
SaO ₂ (%)	95.0 ± 3.5	98.1 ± 0.7	> 0.99	90.6 ± 7.1	96.3 ± 1.4	0.97
Bicarbonate (mmol/L)	24.1 ± 1.3	23.6 ± 1.5	> 0.99	22.3 ± 1.7	22.6 ± 1.2	> 0.99
Lactate (mmol/L)	1.4 ± 0.2	1.1 ± 0.3	> 0.99	0.97 ± 0.1	1.1 ± 0.3	> 0.99
Physiology						
Uterine artery flow (% change from baseline)	0	0	> 0.99	-34.7 ± 24	-22.4 ± 12	> 0.99
Carotid artery pressure (mmHg)	66.1 ± 1.1	72.2 ± 6.2	> 0.99	48.8 ± 1.0	70.4 ± 9.3	0.62
Heart rate (bpm)	93.6 ± 9.0	102.1 ± 8.6	> 0.67	95.5 ± 7.8	107.3 ± 3.9	0.14
Amniotic pressure (mmHg)	2.6 ± 2.0	3.0 ± 1.9	> 0.99	15.6 ± 0.6	15.5 ± 0.4	> 0.99
Temperature (°C)	38.7 ± 0.2	38.7 ± 0.2	> 0.99	38.2 ± 0.3	38.9 ± 0.3	> 0.99
Ventilation rate (breaths/min)	11.5 ± 1.0	11.4 ± 1.2	> 0.99	14.0 ± 1.2	13.0 ± 1.1	> 0.99

Data are presented as mean ± standard error of the mean. Baseline values recorded immediately before insufflation (time = 0 min).

*Comparison at each timepoint using mixed ANOVA with Holm-Šidák *post-hoc* analysis; $P < 0.05$ considered statistically significant.

†Presented as body temperature-corrected values. PaCO₂, arterial partial pressure of carbon dioxide; PaO₂, arterial partial pressure of oxygen; SaO₂, arterial hemoglobin oxygen saturation.

which seemingly limited its ability to tolerate acid–base disturbances (data not shown).

While all insufflated fetuses became progressively hypercapnic (PaCO₂ > 68 mmHg), after 120 min, the PaCO₂ was significantly higher in fetuses insufflated with cold, dry CO₂ compared with those receiving heated, humidified CO₂ (mean ± SEM, 170.5 ± 28.5 *vs* 82.7 ± 9.1 mmHg, $P < 0.01$) (Table 1, Figure 1a). Additionally, fetal lactate levels were higher (8.5 ± 0.9 *vs* 1.4 ± 0.4 mmol/L, $P < 0.01$) (Figure 1b) and arterial pH was lower (pH 6.75 ± 0.04 *vs* 7.10 ± 0.04, $P < 0.01$) (Figure 1c) in those undergoing PACI with cold, dry CO₂ at this timepoint. Serum bicarbonate was similar and remained stable in both groups (Figure 1d). After 120 min, there was a non-significant trend for fetal carotid artery pressure (Figure 1e) and umbilical vein flow (Figure 1f) to be higher in fetuses insufflated with heated, humidified compared with cold, dry CO₂. Fetal esophageal temperatures remained stable and were similar between groups.

Maternal effects

There was no significant difference in the ventilation rates (Table 2, Figure 2a) required to maintain maternal PaCO₂ at 35–45 mmHg (Figure 2b). Maternal arterial pH (Figure 2c), PaO₂, SaO₂, temperature and carotid artery pressure were not significantly different between experimental groups at any timepoint. In both groups, uterine artery flow decreased by 30–40% immediately after PACI commenced (Figure 2d).

Fetal membrane effects

More CD45-positive cells (leukocytes) were present in chorionic membranes exposed to cold, dry CO₂

during PACI than in those exposed to heated, humidified CO₂ (median (IQR), 3.2×10^{-5} (1.8×10^{-5}) *vs* 0.7×10^{-5} (0.5×10^{-5}) cells per square micron, $P = 0.02$) (Figure 3a). A similar trend was observed in the amniotic membranes, although the difference did not reach significance (2.4×10^{-5} (1.9×10^{-5}) *vs* 0.2×10^{-5} (0.58×10^{-5}) cells per square micron, $P = 0.12$) (Figure 3b). Representative images of fetal membranes insufflated with cold, dry CO₂ and with heated, humidified CO₂ during PACI are shown in Figures 3c and 3d, respectively.

DISCUSSION

In this study, we assessed, using clinically relevant insufflation parameters, the effects on fetal lambs and membranes of PACI with cold, dry and with heated, humidified CO₂. Our results show that the disturbance to fetal physiology and membrane inflammation caused by PACI can be partially mitigated by using heated, humidified rather than cold, dry CO₂. Our findings with cold, dry CO₂ are consistent with those of previous sheep models of PACI, which showed a similar degree of fetal hypercapnia and acidosis over 30–60 min of insufflation^{5–8}. Additionally, our study shows that prolonged exposure (up to 120 min) to cold, dry CO₂ during PACI causes progressive acid–base disturbances that have a significant effect on fetal physiology in sheep.

There are several mechanisms which might help to explain why fetuses insufflated with heated, humidified CO₂ had significantly lower PaCO₂ after 120 min than had those exposed to cold, dry CO₂ during PACI. Heating CO₂ gas from 22 °C to 40 °C reduces its solubility by nearly 40%. Additionally, humidification would have reduced the intra-amniotic partial pressure of CO₂ (from 775 to ≈720 mmHg), due to the contribution of water

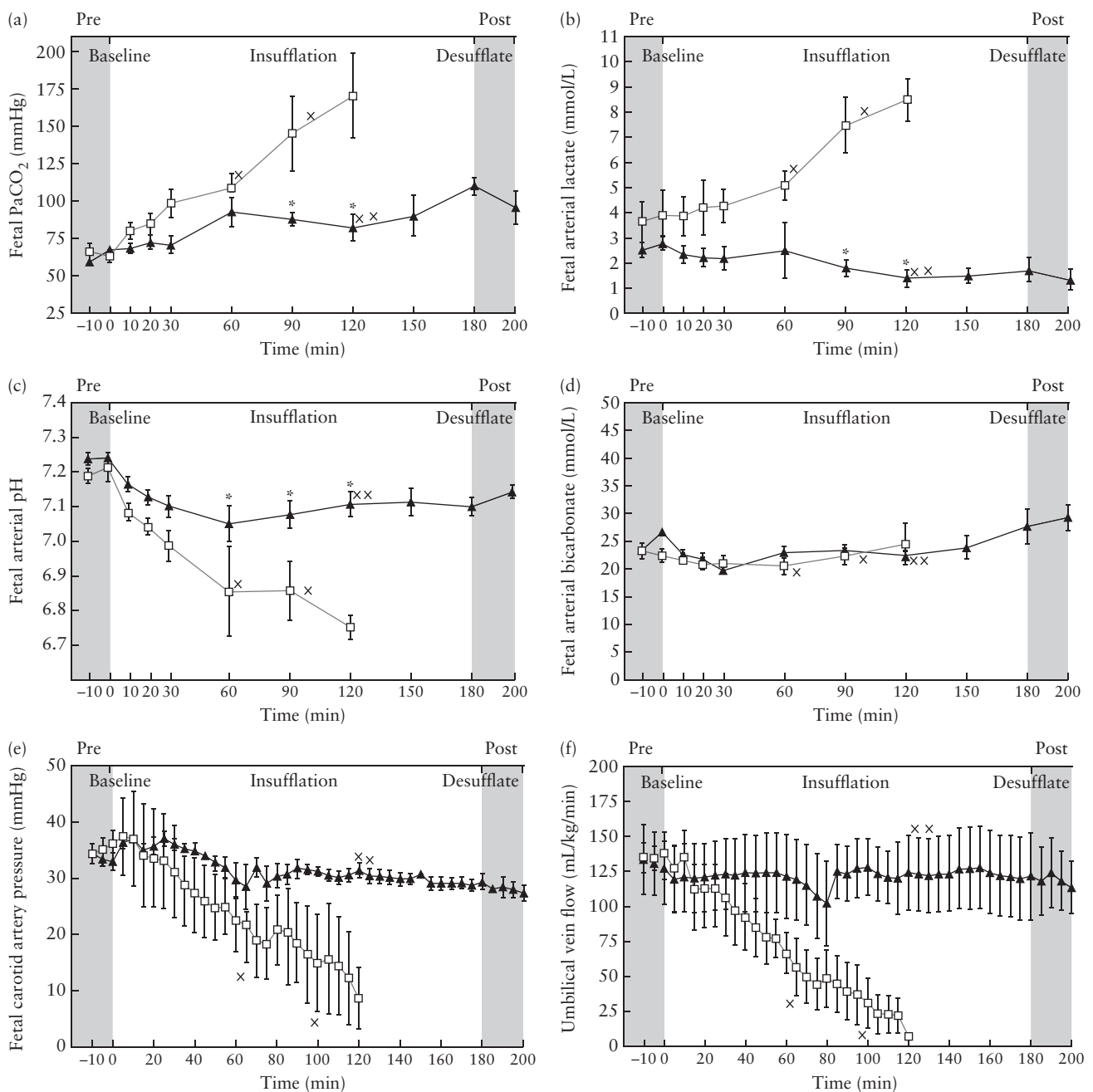


Figure 1 Fetal blood gas and hemodynamic parameters during partial amniotic carbon dioxide (CO₂) insufflation (PACI) with cold, dry CO₂ (□, *n* = 5) or with heated, humidified CO₂ (▲, *n* = 5). Blood gasses were sampled every 10 min for first 30 min and every 30 min thereafter during 180-min period of insufflation, as well as 10 min before and 20 min after this period. Physiological data were recorded every 5 min. Arterial partial pressure of CO₂ (PaCO₂) (a) and lactate (b) were significantly higher and pH (c) significantly lower with cold, dry CO₂ compared with heated, humidified CO₂. Fetal arterial bicarbonate (d) remained stable. Fetal carotid artery pressure (e) and umbilical vein flow (f) decreased progressively during PACI with cold, dry CO₂ and remained stable with heated, humidified CO₂. Data are presented as mean ± standard error of the mean. x indicates removal of case from analysis due to fetal death, at 63 and 98 min in cold, dry CO₂ group and at 120 and 130 min in heated, humidified CO₂ group. **P* < 0.05 vs PACI with cold, dry CO₂.

vapor pressure^{17–19}. Collectively, these may have reduced the absorption of CO₂ in fetuses exposed to heated, humidified gas.

It is also possible that the low temperature of cold, dry gas during PACI may have reduced clearance of CO₂ from the fetal compartment. Reducing the temperature of umbilical vessels from body temperature to 22 °C may cause vessel vasoconstriction and reduce umbilical

blood flow²⁰. Indeed, we observed reductions in umbilical venous flow in fetuses undergoing PACI with cold, dry gas, and the associated reduction in placental perfusion likely reduced CO₂ clearance, adding to the fetal hypercapnia. Heating the gas may have avoided these changes, potentially explaining the improved placental CO₂ clearance and therefore the lower fetal PaCO₂ observed in the group in which this was done.

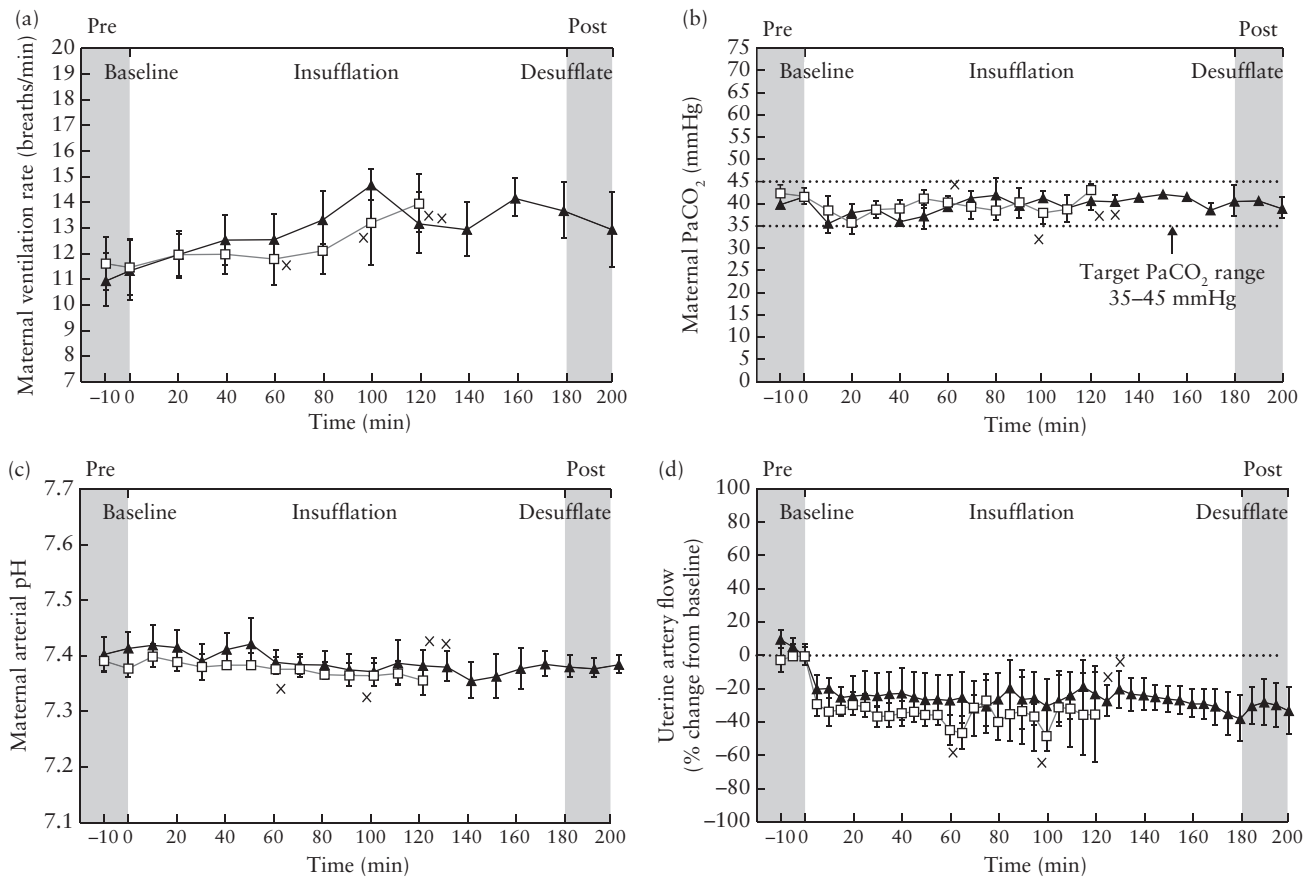


Figure 2 Maternal blood gas and hemodynamic parameters during partial amniotic carbon dioxide (CO₂) insufflation (PACI) with cold, dry CO₂ (□, *n* = 5) or with heated, humidified CO₂ (▲, *n* = 5). Maternal ventilation rate was recorded every 20 min, blood gasses sampled every 10 min and physiological data recorded every 5 min. Maternal ventilation rate (a) required to maintain arterial partial pressure of CO₂ (PaCO₂) between 35 and 45 mmHg (b) was similar in ewes receiving cold, dry CO₂ and those receiving heated, humidified CO₂. Maternal arterial pH (c) also remained stable. Maternal uterine artery blood flow (d) reduced to a similar degree in both groups during PACI. Data are presented as mean ± standard error of the mean. x indicates removal of case from analysis due to fetal death, at 63 and 98 min in cold, dry CO₂ group and at 120 and 130 min in heated, humidified CO₂ group. There were no significant differences in PACI with cold, dry CO₂ vs heated, humidified CO₂.

Finally, fetal carbonic anhydrase is most biologically active between 35 and 45 °C and has < 50% activity at temperatures below 25 °C²¹. Carbonic anhydrase catalyzes the reversible reaction that reforms CO₂ (along with water) from bicarbonate and hydrogen ions in fetal umbilical vessels, allowing it to diffuse into the maternal blood at the placenta²². PACI with cold, dry CO₂ may cool the blood moving through the small placental blood vessels and reduce the ability of the fetus to eliminate CO₂ via the mother. PACI with heated, humidified CO₂ may optimize the activity of carbonic anhydrase and improve fetal CO₂ elimination. While fetal and maternal temperatures in our experiment were similar between the two groups, our results were recorded from esophageal temperature probes and not at the placental surface where CO₂ exchange takes place. Future experiments should investigate how heated, humidified CO₂ affects placental clearance of CO₂ and the activity of placental carbonic anhydrase.

Severe hypercapnia and acidosis reduce cardiac contractility directly^{23,24}. We observed progressive reduction in carotid artery pressure in fetuses exposed to cold, dry

CO₂ during PACI, all of which ultimately died. Clearly, this physiological disturbance was more severe than that observed in human fetuses, which generally remain stable and survive fetoscopic surgery. Several researchers have suggested that this difference is due to mechanical reductions in uterine blood flow that occur during overdistension of the compliant sheep uterus^{11,12,25}. Indeed, we observed a 30–40% reduction in uterine artery blood flow during both PACI with cold, dry gas and that with heated, humidified gas. Importantly, however, changes in uterine artery blood flow were not different between the group receiving cold, dry gas and that receiving heated, humidified gas, indicating that this reduction in uteroplacental perfusion alone could not explain the metabolic differences observed between the two groups.

The non-significant rise in fetal PaO₂ during PACI with heated, humidified CO₂ is consistent with findings of previous sheep studies of PACI and likely reflects a rightward shift in the fetal oxyhemoglobin dissociation curve during mild hypercapnic acidosis^{7,8}. It is unclear why the fetal PaO₂ was slightly lower at baseline in the group undergoing PACI with cold, dry CO₂; however,

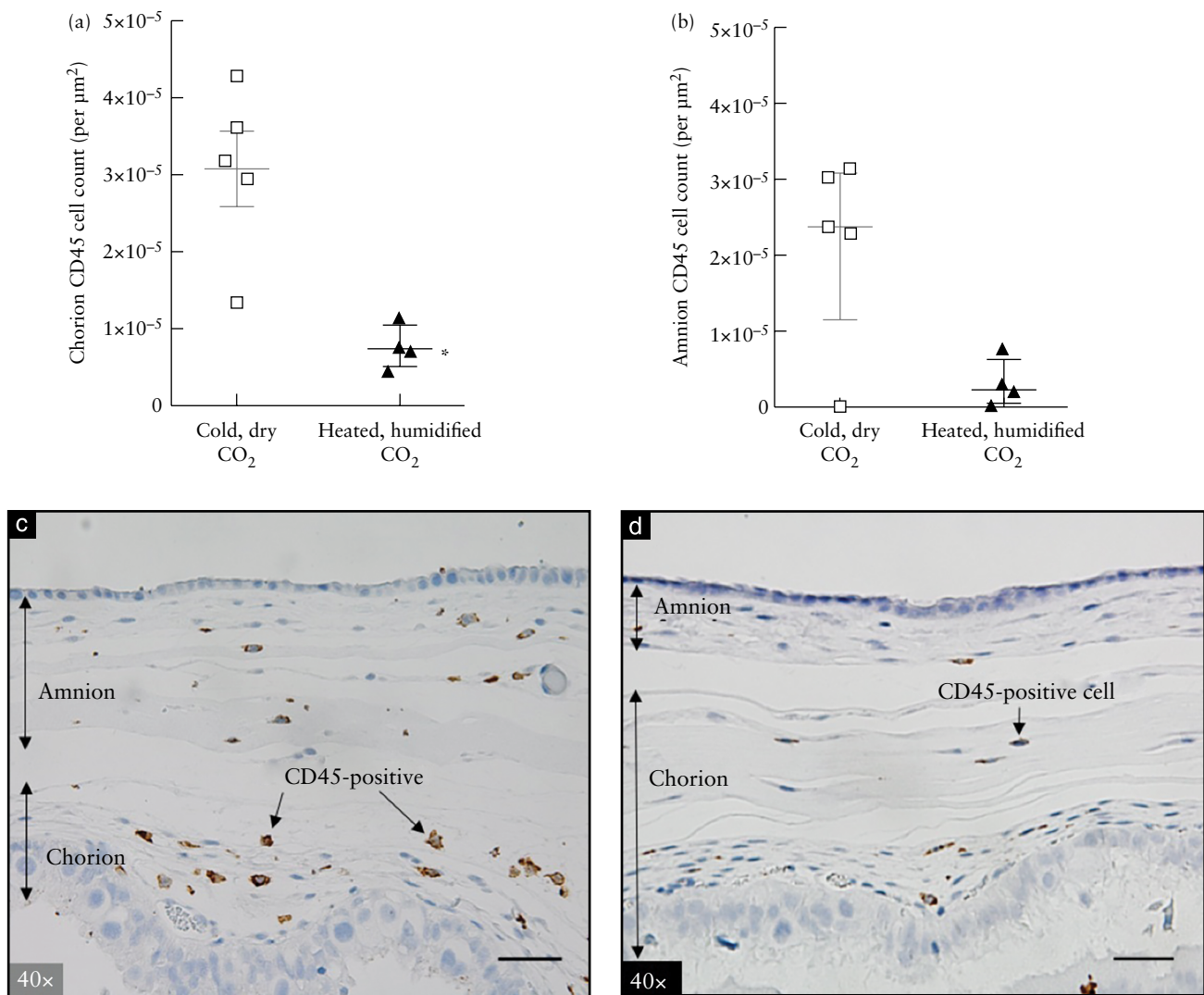


Figure 3 CD45-positive cell counts (leukocytes, indicative of inflammation) of insufflated fetal membranes. Significantly more CD45-positive cells were seen in chorion (a) from fetuses undergoing partial amniotic carbon dioxide (CO₂) insufflation (PACI) with cold, dry CO₂ (□) compared with those insufflated with heated, humidified CO₂ (▲). A similar trend was observed for amnion (b) but this did not reach significance. Data are presented as median (interquartile range). **P* < 0.05 *vs* PACI with cold, dry CO₂. (c,d) Representative images of immunostained tissue sections from fetal membranes that were insufflated during PACI with cold, dry CO₂ (c) or with heated, humidified CO₂ (d). Scale bars represent 50 μm.

the progressive reduction in fetal PaO₂ in this group was likely due to reduced fetal perfusion of the placenta as severe hypercapnic acidosis progressed and cardiovascular function declined.

The etiology of PPRM or iatrogenic membrane rupture is not entirely understood, although fetal membrane rupture has been hypothesized to be preceded by a progressive weakening of membrane integrity¹⁴. This weakening is believed to be caused by inflammation in the choriodecidua and recruitment of leukocytes (CD45-positive cells). We found significantly more immune cells present in chorionic membranes exposed to cold, dry CO₂ during PACI compared with those exposed to heated, humidified CO₂. This suggests that PACI induced an inflammatory response that was partially mitigated by using heated, humidified gas. We speculate that cold, dry CO₂ mediates fetal membrane inflammation

by cooling and dehydrating the surface of the amnion. These changes have been seen in the human peritoneum following abdominal insufflation with CO₂ and can be reduced by heating and humidifying the gas^{26,27}. While the peritoneum and fetal membranes are different anatomically, both membranes are exposed to only liquids normally and likely have little capacity to cope with dehydration. As such, it is not surprising that the fetal membranes became inflamed in response to dehydration and that this could be reduced markedly by humidifying the CO₂ to 100%. Although these changes have not been correlated directly with an increased risk of membrane rupture, this may be one of the reasons that relatively low rates of postoperative PPRM were observed in recent clinical series using heated, humidified CO₂ for PACI⁹. Future animal studies of PACI should build on our findings and examine membrane markers of cellular

injury, apoptosis and collagen degradation known to correlate with membrane rupture²⁸.

Our sheep model of PACI has limitations in predicting the effects of PACI in humans. The cotyledonary arrangement of the sheep placenta increases the surface area for fetal CO₂ absorption. Also, the potentially more compliant sheep uterus predisposes to reduction in uterine blood flow during insufflation. These differences may explain the greater fetal acid–base disturbances in sheep compared with humans, as confirmed by three human cases in which only modest acid–base changes were observed in the fetal umbilical vein¹³. It should be noted, however, that our arterial blood gasses were sampled during insufflation, which would be more reflective of the fetal status intraoperatively compared with sampling in human cases after desufflation, when intraoperative changes may have resolved. Future animal studies should compare the effects of PACI with cold, dry and with heated, humidified CO₂ on the developing fetal brain.

In summary, we examined the effects of PACI with cold, dry and with heated, humidified CO₂ on fetal sheep physiology and fetal membranes. Our results support observations in humans suggesting that heating and humidifying the gas used during PACI can mitigate fetal acid–base disturbances caused by cold, dry insufflation and reduce fetal membrane inflammation, which may play a role in reducing postoperative PPRM. Further studies are required to identify the mechanisms by which heating and humidifying the gas conveys these benefits.

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