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Ischemic preconditioning does not prevent placental dysfunction induced by fetal cardiac bypass

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Abstract

Background: Remote ischemic preconditioning (rIPC) has been applied to attenuate tissue injury. We tested the hypothesis that rIPC applied to fetal lambs undergoing cardiac bypass (CB) reduces fetal systemic inflammation and placental dysfunction.

Methods: Eighteen fetal lambs were divided into three groups: sham, CB control, and CB rIPC. CB rIPC fetuses had a hindlimb tourniquet applied to occlude blood flow for four cycles of a 5-min period, followed by a 2-min reperfusion period. Both study groups underwent 30 min of normothermic CB. Fetal inflammatory markers, gas exchange, and placental and fetal lung morphological changes were assessed.

Results: The CB rIPC group achieved higher bypass flow rates ($p < .001$). After CB start, both study groups developed significant decreases in PaO_2, mixed acidosis, and increased lactate levels ($p < .0004$). No significant differences in tissular edema were observed on fetal lungs and placenta ($p > .391$). Expression of Toll-like receptor 4 and intercellular adhesion molecule-1 in the placenta and fetal lungs did not differ among the three groups, as well as with vascular cell adhesion molecule-1 (VCAM-1) of fetal lungs ($p > .225$). Placental VCAM-1 expression was lower in the rIPC group ($p < .05$). Fetal interleukin-1 (IL-1) and thromboxane A2 (TXA2) levels were lower at 60 min post-CB in the CB rIPC group ($p < .05$). There were no significant differences in tumor necrosis factor-α, prostaglandin E2, IL-6, and IL-10 plasma levels of the three groups at 60-min post-bypass ($p > .133$).

Conclusion: Although rIPC allowed increased blood flow during fetal CB and decreased IL-1 and TXA2 levels and placental VCAM-1, it did not prevent placental dysfunction in fetal lambs undergoing CB.

KEYWORDS

fetal cardiac bypass, inflammation, ischemia–reperfusion, ischemic preconditioning, placenta

All the authors contributed equally to this study.

1 | INTRODUCTION

Systemic inflammatory response syndrome remains one of the major causes of cardiopulmonary bypass (CPB)-associated organ injury in the majority of neonates undergoing cardiovascular surgery. Despite significant advances, CPB is still complicated by multisystem injury, the mechanisms of which include ischemia–reperfusion (IR) injury and a detrimental systemic inflammatory response. In conditions such as Ebstein's anomaly, when neonatal surgery is indicated, the patient may be so compromised by the time of the operation that mortality and morbidity are unacceptably high. Fetal cardiac surgery may be a logical solution. This life-saving surgical intervention is only possible with fetal cardiac bypass (CB) support. Previous experimental studies have shown that placental dysfunction remains the major cause of fetal mortality during and after CB.[1–3] Remote ischemic preconditioning (rIPC) has been applied to patients undergoing cardiac surgery in an attempt to attenuate tissue injury related to the effects of inflammatory mediators.[4] However, the impact of applying this strategy to fetal CB has yet to be demonstrated to determine whether this noninvasive therapeutic intervention can offer fetal multiorgan protection. We assessed whether rIPC applied to fetal lambs undergoing CB reduced fetal systemic inflammation and placental dysfunction.

2 | METHODS

2.1 | Animals

This study was reviewed and approved by the Institutional Animal Research Committee. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals of the American National Academies. Eighteen fetal lambs, between 130 and 140 days of gestational age and of comparable weights ($p = .872$), were randomly divided into three groups: sham ($n = 6$; weight, 2.68 ± 0.20 kg), CB control ($n = 6$; weight, 2.98 ± 0.42 kg), and CB rIPC ($n = 6$; weight, 2.96 ± 0.39 kg). The two CB groups are referred to as the "study groups." Sham group fetuses were submitted to fetal sternotomy only. CB control group fetuses underwent fetal sternotomy and fetal CB. Near 3 h before fetal CB, CB rIPC group fetuses had one hindlimb exposed so a tourniquet could be applied to occlude blood flow for a 5-min period. This rIPC stimulus was repeated for four cycles, followed by a 2-min reperfusion period. This protocol was based on several clinical trials of different surgeries to promote rIPC.[5–7] The observational period was the same for all three groups. The total duration of the experiment was approximately 250 min. After the follow-up period (60 min post-CB), all fetuses were humanely euthanized, while under deep general anesthesia, with high-dose pentobarbital sodium (10 mg kg^{-1}). The fetuses were then delivered for fetal weight measurements and placental and lung biopsies. The womb and abdominal wall were closed with absorbable sutures. All ewes were extubated right after the surgical procedure and remained ambulatory, under the use of analgesic. The protocol to minimize suffering and distress included tramadol (3 mg kg^{-1} bid for 5 days), dipyrone (25 mg kg^{-1} bid for 5 days), and dexamethasone (4 mg q. day for 3 days). Two days after the procedure and complete recovery from surgery, all ewes were taken back to the animal farm with no otherwise adverse health events.

2.2 | Anesthesia

Maternal anesthesia was induced with intramuscular injections of ketamine (10 mg kg^{-1}) and midazolam (0.5 mg kg^{-1}). Venous and arterial lines were then placed in the ewe's jugular vein and auricular artery for delivery of intravenous fluids (Ringer's solution, 6 ml kg^{-1} h^{-1}) and monitoring of blood gases and blood pressure (Dixtal DX-2020), respectively. Induction of anesthesia was supplemented with single intravenous doses of propofol (2 mg kg^{-1}), ketamine (2 mg kg^{-1}), and fentanyl (2 μg kg^{-1}). The ewe was then placed in the supine position, intubated, and ventilated through a tracheal tube with 100% oxygen to maintain the arterial oxygen saturation at 100% and the arterial carbon dioxide tension at approximately 30 mmHg. Maternal anesthesia was maintained with 5% isoflurane in 100% oxygen associated with lumbosacral regional anesthesia with bupivacaine (30 mg) and morphine (6 mg). The epidural anesthesia can give an anesthetic block for up to 6 h (covering most of the surgical procedure), followed by analgesia promoted by morphine, which extends for up to 24 h. Fetal anesthesia was supplemented with intramuscular ketamine (50 mg kg^{-1}). Antibiotic therapy (cefazolin 1000 mg iv and gentamicin 40 mg im) was administered just before the operation and maintained during 2 postoperative days.

2.3 | Surgical procedure

All operations were performed through a midline infraumbilical laparotomy. First, each fetal lamb in the CB rIPC group had its hindlimb exposed to apply the intermittent ischemic stimulus. It was then repositioned inside the uterus, and the small hysterotomy was closed with an absorbable suture. Second, through a 10-cm incision in the uterine wall and fetal membranes, the fetal forelimb was delivered for placement of fetal axillary venous and artery lines. The venous line was used to infuse heparin and Ringer's solution (in the event of bleeding during cannulation). The arterial line was used for continuous measurements of fetal arterial blood pressure and blood gases and inflammatory markers sampling.

2.4 | Fetal CB

The fetal heart was exposed through a midline sternotomy. Heparin (1000 I.U.) was administered intravenously before cannulation for anticoagulation. The pulmonary trunk and right atrium were

cannulated with 8-Fr arterial and 12-Fr venous wire-reinforced straight tip cannulas (Maquet Cardiopulmonary AG), respectively. These cannulas were connected to a CB circuit that included a Rotaflow RF-32 centrifugal pump (Getinge AB) with no oxygenator, primed with 60 ml of warm Ringer's solution (40°C). The placenta remained as the sole oxygenator in the circuit. Both the CB control and CB rIPC groups underwent CB for 30 min, under normothermic conditions, aiming at an ideal flow rate of 200 ml kg^{-1} min^{-1}. The blood flow was precisely measured in the pump outlet by an ultrasonic transit-time flowmeter. Maximum fetal CB flow rate was achieved when the generation of extreme negative pressure at high rotation speeds did not change the higher flow rate. The animals were followed up for 60 min after CB termination.

2.5 | Blood sample protocol

Fetal arterial blood samples were collected for blood gas analysis (Nova Biomedical—Stat Profile Ultra) at baseline (i.e., right after axillary vessel cannulation), immediately before CB, 15 min into CB, and 30 and 60 min after bypass. Fetal blood samples for inflammatory mediator assays were collected at the end of the protocol, placed immediately into test tubes containing ethylenediaminetetraacetic acid on ice, and centrifuged at 3000 rpm for 15 min in a cooled centrifuge; the supernatants were frozen at −70°C.

2.6 | Histologic analysis

Biopsy samples from the lungs and placenta were fixed in 10% buffered formalin for 24 h and submitted to conventional histological processing techniques. Fixed tissue was embedded in paraffin, sectioned (4 µm thickness), and then stained with hematoxylin–eosin.

2.7 | Histomorphometric study of the lungs

Tissular edema was evaluated by morphometry as previously described.[8] The degree of pulmonary edema was assessed by determining the mean area fraction occupied by the interlobular septum. Random photographs were taken of five fields of each lung at a magnification of ×400. Morphometric measurement of the area occupied by the interlobular septum relative to the total lung area was performed using a point counting procedure, in which a test system containing 336 regularly spaced points was applied over each photograph. The number of hits (points incident) over the interlobular septum allowed the determination of the density of the interlobular septum in each photograph, as a percentage of the total area. The mean interlobular septal area fraction of each animal was calculated from the values for five fields. Considering that there was no intra-alveolar edema, it was assumed that the greater the dimensions of the interlobular septal area fraction relative to the entire parenchyma, the greater the degree of pulmonary edema, meaning that the interlobular septum had an increased water content.

2.8 | Histomorphometric study of the placenta

The area fraction of the cellular nuclei and interstitial vessels of the placenta was measured using the same test system used for the pulmonary edema assessment. For each animal, random photographs were taken from four fields of the placenta at a magnification of ×400. The mean area fraction occupied by the nuclei and interstitial vessels of each placenta was calculated from the values for four fields. It was assumed that the greater the value of the area fraction of the nuclei and interstitial vessels of the placenta, the lesser the degree of placental edema, meaning such structures (nuclei and vessels) were more close together.

2.9 | Immunohistochemical analysis

Immunostaining of 4-µm sections of lung and placental tissue with antibodies to intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), both with 1/200 dilution, and Toll-like receptor 4 (TLR 4), with 1/300 dilution, was performed according to manufacturer's protocol (ICAM-1 and TLR 4: Antibodies-online Inc.; VCAM-1: Biorbyt LLC). The Novocastra Novolink™ Polymer Kit (Post Primary block + Polymer) was used to detect any tissue-bound rabbit primary antibodies (Leica Biosystems NewCastle). All specimens were then counterstained with Harris hematoxylin (Merck) and coverslipped.

2.10 | Enzyme-linked immunosorbent assay (ELISA) of inflammatory markers

Blood samples were taken at the end of the protocol to assess inflammatory markers. ELISA was used to measure fetal plasma levels of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), IL-6, and IL-10 following the manufacturer's protocol (MyBioSource Inc.). ELISA was also used to measure prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) levels according to the manufacturer's protocol (Enzo Life Sciences).

2.11 | Statistical analysis

Values are expressed as mean ± SEM. Mixed-model procedure for repeated measures was used to compare CB flows. Kruskal-Wallis test was used to compare tissular edema, immunohistochemical and Inflammatory Markers parameters, followed by Dunn's Multiple Comparison Test. $p < .05$ was considered statistically significant. Statistical analysis was performed with the GraphPad Prism software version 6.07 (GraphPad Software Inc.).

3 | RESULTS

3.1 | Mortality

There was one fetal death in the CB control group, while under deep general anesthesia 13 min post-CB, because of placental dysfunction.

3.2 | Maternal and fetal hemodynamics

Lumbosacral regional anesthesia did not impair maternal hemodynamics throughout the protocol. The ewes maintained an adequate mean arterial pressure (60–70 mmHg) and heart rate (70–100 bpm) during the entire anesthetic procedure. No intervention on hemodynamics (high volumes of crystalloids, vasopressors, or inotropes) was needed. Fetal heart rates were higher in the CB rIPC group (134.98 ± 6.44 bpm) than in the CB group (111.90 ± 4.51 bpm) throughout the protocol ($p = .018$), as shown in Table 1. There was no significant difference in the mean systemic arterial pressures among the three groups ($p = .366$). Figure 1 shows the CB flow rates for both study groups during CB. The CB rIPC group achieved higher bypass flow rates than the CB control group ($p < .001$).

3.3 | Metabolic assessment

Table 2 shows fetal blood gas values and lactate concentrations of the three groups. Compared to the sham group, both study groups developed progressive acidosis, increased lactate levels, and decreased sodium bicarbonate levels during and after CB ($p < .0004$). However, no significant differences in pH and $PaCO_2$ were observed between the study groups ($p > .08$). Compared to the sham group, both study groups developed progressive hypoxemia after CB start ($p < .002$).

3.4 | Histomorphometric assessment of the lungs

Table 3 shows the interlobular septal area fractions of the three groups. No significant differences were observed among the three groups ($p = .589$).

3.5 | Histomorphometric assessment of the placenta

Concerning the area fraction of the cellular nuclei and interstitial vessels of the placenta, no significant differences were observed among the three groups ($p > .391$), as shown in Table 3.

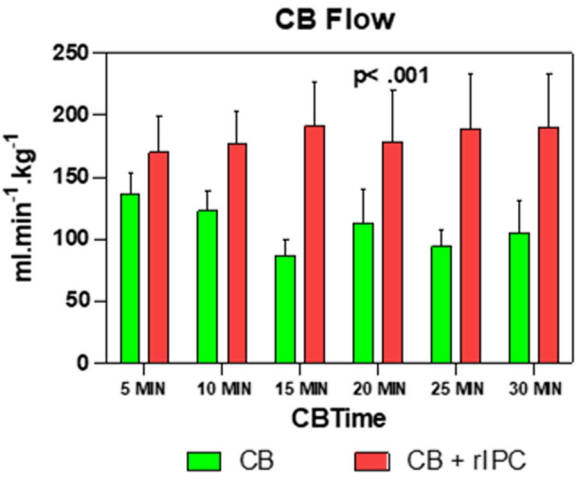

FIGURE 1 Fetal cardiac bypass (CB) flow rates in the CB control and CB rIPC groups. $n = 6$. Values (ml min^{-1} kg^{-1}) are represented as mean ± SEM. $p < .001$ between the two groups. CB, cardiac bypass; rIPC, remote ischemic preconditioning.

TABLE 1 Fetal heart rates and mean systemic arterial pressures in the sham, CB control, and CB rIPC groups

Group	Baseline	Pre-CB	10 min CB	20 min CB	30 min post-CB	60 min post-CB
HR (bpm)						
Sham	114 ± 5	128 ± 8	125 ± 5	125 ± 9	120 ± 6	119 ± 7
CB control	106 ± 6	114 ± 8	113 ± 10	120 ± 10	122 ± 4	114 ± 7
CB rIPC	133 ± 12	143 ± 8	143 ± 6	139 ± 7	144 ± 10	125 ± 16
MAP (mmHg)						
Sham	53.2 ± 4.5	52.5 ± 4.6	49.5 ± 4.5	48.5 ± 4.6	48.3 ± 5.4	45.8 ± 5.0
CB control	62.2 ± 2.8	44.7 ± 2.3	42.5 ± 6.6	36.8 ± 2.8	39.2 ± 2.6	36.4 ± 3.9
CB rIPC	53.7 ± 1.9	53.0 ± 3.9	42.8 ± 2.6	42.2 ± 2.4	44.2 ± 4.1	40.3 ± 5.2

Note: $n = 6$. Values are represented as mean ± SEM.

Abbreviations: CB, cardiac bypass; HR, heart rate; MAP, mean systemic arterial pressure; rIPC, remote ischemic preconditioning.

TABLE 2 Fetal placental gas exchange and metabolism in the sham, CB control, and CB rIPC groups

Group	Baseline	Pre-CB	15 min CB	30 min post-CB	60 min post-CB
Arterial pH					
Sham	7.19 ± 0.03	7.18 ± 0.02	7.18 ± 0.02	7.18 ± 0.02	7.18 ± 0.02
CB control	7.17 ± 0.02	7.14 ± 0.03	7.05 ± 0.06	6.97 ± 0.08	6.98 ± 0.09
CB rIPC	7.10 ± 0.04	7.07 ± 0.02	7.01 ± 0.03	6.97 ± 0.03	6.89 ± 0.06
PaO_2					
Sham	23.9 ± 1.5	28.1 ± 2.2	25.8 ± 1.7	25.3 ± 2.7	23.7 ± 2.4
CB control	28.2 ± 1.1	23.2 ± 3.0	18.1 ± 2.2	18.0 ± 2.0	17.4 ± 1.2
CB rIPC	25.1 ± 2.4	22.2 ± 2.3	17.2 ± 2.2	19.7 ± 2.2	18.6 ± 1.9
$PaCO_2$					
Sham	63.1 ± 4.1	60.2 ± 8.9	67.9 ± 2.9	67.5 ± 3.7	67.6 ± 2.7
CB control	52.3 ± 2.1	56.1 ± 1.5	64.8 ± 3.5	74.2 ± 5.9	74.3 ± 6.7
CB rIPC	46.6 ± 3.9	53.0 ± 3.4	61.1 ± 4.7	59.5 ± 3.3	75.5 ± 8.7
Bicarbonate					
Sham	19.1 ± 1.3	19.0 ± 0.8	19.8 ± 0.9	19.6 ± 1.0	20.0 ± 1.1
CB control	16.6 ± 0.9	15.6 ± 1.5	13.9 ± 1.7	12.1 ± 1.9	12.2 ± 2.3
CB rIPC	12.5 ± 0.7	12.5 ± 0.7	11.6 ± 0.7	10.2 ± 0.6	9.3 ± 0.9
Lactate					
Sham	2.8 ± 0.9	3.2 ± 0.7	3.1 ± 0.6	2.8 ± 0.5	2.6 ± 0.4
CB control	3.5 ± 1.1	4.3 ± 1.1	5.0 ± 1.1	6.3 ± 1.2	6.7 ± 1.5
CB rIPC	3.3 ± 1.1	3.9 ± 0.7	4.5 ± 0.5	5.8 ± 0.9	7.2 ± 1.5

Note: $n = 6$. Values are represented as mean ± SEM.

Abbreviations: CB, cardiac bypass; $PaCO_2$, arterial carbon dioxide saturation; PaO_2, arterial oxygen saturation; rIPC, remote ischemic preconditioning.

TABLE 3 Tissular edema assessment of fetal lungs and placenta in the sham, CB control, and CB rIPC groups at the end of the protocol

	Sham	CB control	CB rIPC	*p* value
Fetal lungs (%)	11.02 ± 2.79	11.08 ± 3.48	14.90 ± 2.67	.589
Placental nuclei (%)	0.21 ± 0.01	0.24 ± 0.03	0.23 ± 0.02	.616
Placental vessels (%)	0.15 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	.391

Note: $n = 6$. Values are represented as mean ± SEM. Fetal lungs: Mean area fraction (%) of the interlobular septums; placental nuclei: mean area fraction (%) occupied by placental cellular nuclei; placental vessels: mean area fraction (%) occupied by interstitial vessels of the placenta.

Abbreviations: CB, cardiac bypass; rIPC, remote ischemic preconditioning.

TABLE 4 Immunohistochemical assessment of fetal lungs in the sham, CB control, and CB rIPC groups at the end of the protocol

	Sham	CB control	CB rIPC	*p* value
ICAM-1 (%)	7.35 ± 1.32	5.15 ± 0.76	5.52 ± 1.91	.467
VCAM-1 (%)	10.36 ± 0.25	9.53 ± 0.39	9.61 ± 0.43	.240
TLR-4 (%)	9.77 ± 0.33	10.27 ± 0.17	10.50 ± 0.34	.225

Note: $n = 6$. Values are represented as mean ± SEM.

Abbreviations: CB, cardiac bypass; ICAM-1, intercellular adhesion molecule-1; rIPC, remote ischemic preconditioning; TLR-4, Toll-like receptor 4; VCAM-1, vascular cell adhesion molecule-1.

3.6 | Immunohistochemical assessment of fetal lungs

Table 4 shows the expression of the inflammatory mediators ICAM-1, VCAM-1, and TLR-4 in the fetal pulmonary parenchyma. No significant differences were observed among the three groups ($p > .225$).

3.7 | Immunohistochemical assessment of placenta

No significant differences in the expression of the inflammatory mediators ICAM-1 and TLR-4 in the placental interstitium were observed among the three groups ($p > .346$), as shown in Table 5.

Regarding the inflammatory marker VCAM-1, the CB rIPC group showed values similar to the sham group but lower than the CB control group ($p < .05$).

3.8 | Plasma inflammatory markers assessment

Table 6 shows plasma levels of TNF-α, PGE2, IL-6, and IL-10. There was not significant difference among the three groups at the end of the protocol. However, plasma levels of IL-1 and TXA2 were lower in the CB rIPC group than in the sham and CB control groups at 60 min post-CB (Figure 2; $p < .01$).

TABLE 5 Immunohistochemical assessment of placenta in the sham, CB control, and CB rIPC groups at the end of the protocol

	Sham	CB control	CB rIPC	*p* value
ICAM-1 (%)	3.82 ± 0.16	3.63 ± 0.19	3.48 ± 0.16	.392
VCAM-1 (%)	3.54 ± 0.09	3.97 ± 0.13	3.43 ± 0.13*	.013
TLR-4 (%)	5.29 ± 1.74	3.57 ± 0.16	3.29 ± 0.19	.346

Note: $n = 6$. Values are represented as mean ± SEM.

Abbreviations: CB, cardiac bypass; ICAM-1, intercellular adhesion molecule-1; rIPC, remote ischemic preconditioning; TLR-4, Toll-like receptor 4; VCAM-1, vascular cell adhesion molecule-1.

*$p < .05$ CB control × CB rIPC.

4 | DISCUSSION

Although rIPC has promoted better placental perfusion during CB, it did not attenuate placental dysfunction in a model of fetal CB, even if concentrations of some important proinflammatory mediators were minimized with the application of this noninvasive therapy. Although several investigators have documented significant benefits of miniaturized extracorporeal circuits[9,10] and the inhibition of vasoactive prostaglandin production[2,3] and proinflammatory cytokine synthesis[11–13] during fetal CB, inflammatory reactions and associated placental dysfunction still occur. The fundamental underlying mechanisms remain unknown. It is believed that fetoplacental unit dysfunction observed after fetal CB could be related to a systemic inflammatory phenomenon, partially associated with cytokine-mediated reactions.[14] In theory, rIPC could minimize the triggering of the inflammatory cascade and probably fetoplacental dysfunction. The first clinical application of rIPC in children who underwent total repair of congenital heart lesions was reported by the Toronto group in 2006.[4] They demonstrated that rIPC reduced levels of troponin I postoperatively, lowered the inotrope requirement, and modulated the systemic inflammatory response in that patient population. Nevertheless, rIPC remains controversial and has never been applied to fetal surgery. To test the hypothesis that rIPC reduces fetal systemic inflammation and placental dysfunction in a fetal model of CB, rIPC was applied to fetal lambs undergoing CB to investigate its effects on proinflammatory mediators and the consequences on fetal

TABLE 6 Plasma levels of TNF-α, PGE2, IL-6, and IL-10 in the sham, CB control, and CB rIPC groups at 60 min post-CB

	Sham	CB control	CB rIPC	*p* value
TNF-α (pg.ml^{-1})	16.07 ± 0.44	18.48 ± 2.85	48.37 ± 31.05	.339
PGE2 (pg.ml^{-1})	687.53 ± 105.49	454.51 ± 143.65	1588.00 ± 946.75	.133
IL-6 (pg.ml^{-1})	78.13 ± 0.00	93.89 ± 14.33	296.37 ± 182.83	.232
IL-10 (pg.ml^{-1})	76.16 ± 3.84	68.82 ± 11.18	64.11 ± 10.54	.443

Note: $n = 6$. Values (pg ml^{-1}) are represented as mean ± SEM.

Abbreviations: CB, cardiac bypass; IL, interleukin; PGE2, prostaglandin E2; rIPC, remote ischemic preconditioning; TNF-α, tumor necrosis factor-α.

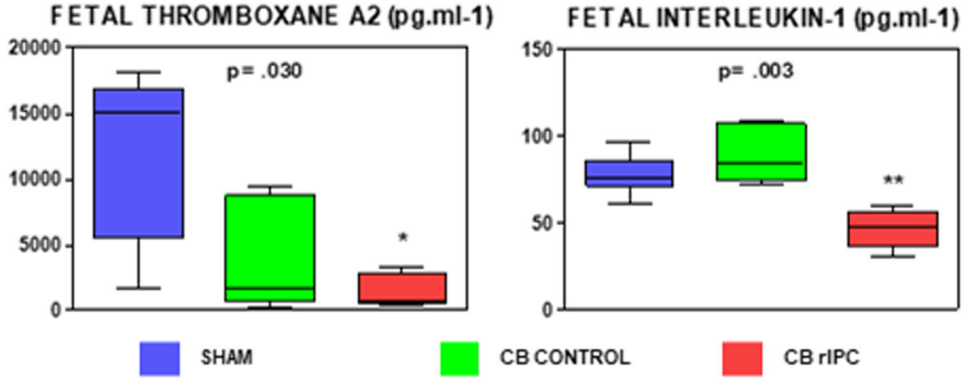

FIGURE 2 Fetal plasma levels of thromboxane A2 and interleukin-1 at the end of the protocol in the sham, CB control, and CB rIPC groups. $n = 6$; values: pg ml^{-1}. CB, cardiac bypass; rIPC, remote ischemic preconditioning. $p < .05$: *CB rIPC versus sham; **CB rIPC versus sham and CB control.

hemodynamics and placental function. All of the fetuses submitted to rIPC developed progressive worsening of placental gas exchange, similar to the fetuses in the CB control group. Corroborating our findings, Zhou et al.[13] have demonstrated in a goat model that pharmacologic inhibition of nuclear factor-κβ, a major regulator of the inflammatory response, did not alleviate fetal CB-induced placental dysfunction. Although it is speculative, one might argue that fetal venous blood exchanges gases with the maternal venous blood and that makes the fetal metabolism adapt to a low oxygen saturation. Maybe the length of the tissue ischemia was insufficient to trigger the anti-inflammatory response of rIPC.[15] The reduction in plasma TXA2 levels in the CB rIPC group is consistent with the greater observed bypass blood flow rates, having as a common denominator the predominance of nitric oxide (NO) in relation to reactive oxygen species. In fact, the CB circuit used in this protocol is directly influenced by the afterload. Our finding that rIPC resulted in higher CB blood flow rates in the preconditioned fetuses is probably associated with the systemic vasodilator effect of transient ischemia and consequent lower fetal systemic vascular resistance, a purported advantageous consequence of preconditioning.[16–18]

The possibility of minimizing the fetal CB inflammatory response with rIPC did not improve placental function, regardless of the reductions in the release of proinflammatory agents IL-1 and TXA2. Therefore, there is still much to clarify about mechanisms that promote placental dysfunction during and after fetal CB. The use of rIPC as a modulator of inflammation should progress to a new model, preferably in nonhuman primates with a single gestation to minimize bias introduced by multiple gestations.[19] Perhaps, the rIPC stimulus, with four cycles of a 5-min ischemic period, was insufficient to trigger protective humoral and neurogenic factors to suppress placental dysfunction. Therefore, the ischemic stimulus associated with rIPC during fetal CB should be more prolonged and performed in both the maternal and fetal organisms in future studies, with a poststimulation observation period of 4 h, during which the rIPC is fully expressed in its first window. Future studies, with varying times of ischemia are clearly required to assess stronger preconditioning stimuli as well as the robustness of the protective effects of rIPC.

4.1 | Study limitations

First, this study did not assess markers of triggers, mediators, or pathways to confirm the rIPC stimulus or trigger. Our protocol of rIPC was followed by 30 min of CB and 60 min of observation. As the first window of rIPC occurs within 4 h after intermittent transient ischemic stimulation, the data presented here do not necessarily reflect the highest expression of the early response to rIPC.[5] This is especially true with respect to the cytokines analyzed, which were evaluated approximately 3 h after rIPC stimulation. Second, this study was performed with fetuses of approximately 130–140 days of gestation (term: 148 days). However, this age does not correspond to the window currently accepted for effective fetal therapy in the clinical setting of 21–30 weeks of human gestation. Ideally, future studies of fetal CB should be performed in more preterm fetuses (80–110 gestational days) to simulate fetal lamb sizes and weights with those of human fetuses who are candidates for prenatal surgical intervention. Third, the anesthetic protocol used in this study could also have influenced the effects of the rIPC. rIPC induces its anti-inflammatory effect by neuronal and humoral mechanisms. The neuronal hypothesis considers that substances produced in the remote territory submitted to ischemia act locally in afferent neuronal pathways. This activates several efferent pathways, inducing the protective effect. Previous studies have pointed out that propofol could mitigate the effects of rIPC.[20] Although anesthetic induction used in this protocol included the administration of a small dose of propofol, this anesthetic agent is rapidly metabolized and has a short half-life, and thus, it probably did not affect the action of rIPC in the studied fetuses. Considering the duration of anesthesia of this experimental protocol (approximately 8–10 h), a balanced general anesthesia protocol was planned in our model, with the association between general anesthesia by isoflurane inhalation and epidural anesthesia and analgesia. The main objective was to allow stable maternal–fetal anesthesia and physiology, with deep analgesia for both and good quality recovery for the sheep (mother). Although the sympathetic block is one of the potential consequences of epidural anesthesia, no changes were observed in macrohemodynamics due to a significant sympathetic block.

5 | CONCLUSIONS

In summary, our data suggest that therapy with noninvasive rIPC does not prevent placental dysfunction or tissue hypoperfusion in fetal lambs undergoing CB, despite the decrease in placental VCAM-1 expression and IL-1 and TXA2 plasma levels and higher CB flow rates in fetuses submitted to rIPC. It is worth emphasizing that one should always consider the complex interactions among these proinflammatory agents. Experimental studies will be crucial in establishing the best strategy for applying rIPC to fetal CB before translation into clinical practice.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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