How does fetal inflammatory response syndrome change fetal response to hypoxia? An experimental study in a fetal sheep model

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ORIGINAL RESEARCH

How does fetal inflammatory response syndrome change fetal response to hypoxia? An experimental study in a fetal sheep model

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Abstract

Introduction: Fetal inflammatory response syndrome associated with acidosis during labor is a high-risk situation for the fetus. This study evaluated hemodynamic, gasometric, and heart rate variability changes during acute fetal inflammatory response syndrome associated with hypoxia, compared with isolated hypoxia.

Material and Methods: Acute fetal inflammatory response syndrome was obtained via an intravenously injection of lipopolysaccharide derived from *Escherichia coli*. Hypoxia was induced by repeated umbilical cord occlusions during three phases: mild, moderate, and severe umbilical cord occlusions. Two groups were created with chronically instrumented near-term fetal sheep: one group with isolated hypoxia, the other with hypoxia and fetal inflammatory response syndrome. Hemodynamic, gas parameters, and fetal heart rate variability were compared between the groups.

Results: The hypoxia and fetal inflammatory response syndrome group had a higher mortality rate ($n=4/9$) compared with the hypoxia group ($n=0/9$). Gasometric state was altered earlier in case of lipopolysaccharide injection (pH = 7.22 (7.12–7.24) vs 7.28 (7.23–7.34) $p=0.01$; lactate = 10.3 mmol/L (9.4–11.0) vs 6.0 mmol/L (4.1–8.2) $p<0.001$ after mild occlusions). After mild occlusions, the hypoxia and fetal inflammatory response syndrome group had higher values on seven heart rate variability parameters compared with the hypoxia group. After moderate occlusions, two parameters remained significantly higher.

Conclusions: During fetal inflammatory response syndrome, fetal adaptation to hypoxia is impaired. In case of fetal infection, acidosis during labor is likely to become severe more rapidly, requiring closer fetal monitoring during labor.

Abbreviations: ANS, autonomic nervous system; FIRS, fetal inflammatory response syndrome; FSI, fetal stress index; HF, high frequencies; HRV, heart rate variability; LF, low frequencies; LPS, lipopolysaccharide; LTV, long-term variability; PS, parasympathetic system; PCA, principal component analysis; RMSSD, root mean square of successive differences between adjacent R-R intervals; SDNN, standard deviation of normal-to-normal R-R intervals; SS, sympathetic system; STV, short-term variability; UCO, umbilical cord occlusion.

KEYWORDS
fetus, FIRS, heart rate variability, hypoxia, inflammation, lipopolysaccharide, monitoring, occlusion, sheep

1 | INTRODUCTION

During labor, a decrease in maternofetal gas exchange can lead to fetal acidosis, which can cause severe neonatal complications, including the risk of cerebral palsy.[1] Fetal infection associated with fetal acidosis is a high-risk situation for the fetus,[1] with an increased risk of severe neonatal complications, including high risk of cerebral palsy.[1] Indeed, intrauterine infection/inflammation (III) can induce a fetal inflammatory response syndrome (FIRS), a systemic inflammation in which fetal plasma interleukin-6 is elevated.[2] During FIRS, increased interleukin-6 and other proinflammatory cytokines are associated with morbidity including the development of periventricular leukomalacia, cerebral palsy, neonatal sepsis, and mortality.[3–8] Concurrent fetal infection and hypoxia during labor creates particular risk for neurological damage.[1]

Hemodynamic and gasometric changes, in relation to these neurological complications, have been studied extensively in cases of isolated fetal infections and isolated hypoxia, especially in ewes.[1,9–14] In contrast, hemodynamic and gasometric changes in cases of hypoxia associated with fetal infection have received little attention despite the likehood that these changes are at least probably partly responsible for neurological complications. Thus, the primary aim herein was to study how FIRS changes hemodynamic and gasometric status during worsening hypoxia.

Fetal heart rate variability (HRV) assesses the variations in time interval between two heartbeats (RR interval), reflecting autonomic nervous system (ANS) activity.[15] Several HRV indices, based on either time or spectral analysis of the RR intervals identified with fetal electrocardiography (ECG), have been developed.[15,16] These indices change during isolated fetal hypoxia or isolated infection and therefore could be used to detect this situation in isolated cases.[6,9–11,17–19] The secondary study's aim was to determine how FIRS changes HRV status during worsening hypoxia.

Key message

In case of fetal infection, acidosis during labor is likely to be more rapidly severe. Fetal inflammatory response syndrome changes heart rate variability parameters in hypoxic situation.

2 | MATERIAL AND METHODS

2.1 | Surgical preparation

Near-term pregnant sheep (race "Ile de France," Tours'INRA, Orfrasière Animal Physiology Experimental Unit, Val de Loire Center) of gestational age 124 days (term = 145 days) underwent our previously described surgical procedure.[1–3] Briefly, sheep were fasted for 24 h before general anesthesia and surgery. They were then placed supine, anesthetized with an intravenous injection of xylazine (Sedaxylan®; CEVA Santé Animale, Libourne, France), intubated, and maintained with 2% isoflurane (Aerrane®; Baxter, Guyancourt, France). After maternal laparotomy and hysterotomy, the left forelimb of the fetus was delivered. A skin incision in the axillary fossa was made after subcutaneous injection of lidocaine (xylocaine; 1%, 4 mL) providing access to the axillary vessels and thorax. Catheters (umbilical catheters 4Fr diameter, Vygon, France, Ecouen) were placed in the fetal left axillary artery and vein. Arterial catheter was advanced 5–6 cm from the axillary artery to the aortic arch. Venous catheter was advanced 5 cm from the axillary vein to the superior vena cava. Two electrocardiogram electrodes (Mywire 101; Maquet, Rastatt, Germany) were placed on the fetal intercostal muscles to record fetal electrocardiogram. Axillary fossa incision was sutured, and the anterior limb was reinserted into the uterus. Same procedure was realized on the right side with an arterial catheter and two electrocardiogram electrodes. A catheter (umbilical catheters 5Fr diameter, Arrow®) was placed in the amniotic cavity. This amniotic catheter was used to replace amniotic fluid lost during surgery with 500 mL saline-containing antibiotics (amoxicillin and clavulanic acid) and to measure baseline intra-amniotic pressure. An inflatable silicone occluder (OC16; In Vivo Metric, Healdsburg, CA, USA) was placed around the umbilical cord. All leads were exteriorized through the right maternal flank. Hysterotomy and laparotomy were closed. After surgery, ewes were awakened with free access to food and drink. Postoperative analgesia was provided by maternal intramuscular injection of 0.3 mL/10 kg buprenorphine (Buprenodale®; Dechra Veterinary Products, Montigny-le-Bretonneux, France) at 24 and 48 h after surgery.

2.2 | Data acquisition

Blood pressure sensors and electrocardiogram electrodes were connected to a multiparametric anesthesia monitor (Merlin; Hewlett Packard, Palo Alto, CA, USA). Fetal arterial and intra-amniotic catheters were connected to pressure sensors (Pressure Monitoring Kit®; Baxter). Mean arterial pressure (MAP) was measured from blood pressure phasic signals and corrected for intra-amniotic pressure value (calculated MAP = observed MAP − observed intra-amniotic pressure). Blood pressure signals and electrocardiogram were recorded through a Physiotrace™ data acquisition board (Estaris Monitoring, Lille, France). Fetal heart rate (FHR) and heart

rate variability (HRV) parameters were calculated over a period of 3 min. Blood pressure values were taken just before the onset of the following occlusions.

2.3 | Experimental procedure

The experiments began after the sheep had rested for 4 days after surgery. Two groups were created: Hypoxia group had already been created for a previous study.[20] Hypoxia + FIRS group was secondary created for this study by the same team and according to the same procedure. Hypoxia was induced by repetitive umbilical cord occlusions (UCO). UCO were performed by injecting an isotonic solution into the occluder to obtain a total occlusion for 1 min. The protocol was divided into three periods of 1 h each, as described previously by Prout et al.[21] During the mild phase, UCO were repeated every 5 min (mild UCO). During the second phase (moderate UCO), the rhythm was one UCO every 3 min. Finally, the last period (severe UCO) consisted of one UCO every 2 min (Figure 1). The protocol was stopped before the end-of-phase intense UCO, if pH reached 6.95 or below.

FIRS was obtained by one intravenous injection of lipopolysaccharide (LPS) derived from *Escherichia coli*, serotype 0111:B4 (Sigma-Aldrich, Merck, Darmstadt, Germany).

Before procedure, a 1-h stability period was recorded to ensure that the animals were healthy (normal gas blood and normal hemodynamic parameters). Hemodynamic (MAP, fetal heart rate [FHR]), gasometric (pH, lactate, pO_2, and pCO_2), and HRV measures were recorded at the end of the stability period to obtain baseline values. In the group with hypoxia and infection (UCO + LPS group), LPS was injected after the stability period and 2 h before UCO. We have previously shown that 2 h after LPS injection is sufficient to achieve acute FIRS without decompensation.[10] FHR and arterial blood pressure were monitored continuously during the stability period, after LPS injection, and during UCO. HRV analyses are known to be very sensitive to rapid and transient changes in heart rate. To avoid any risk of artifacts due to such changes, after each six UCOs, a 5-min period with no UCO was allowed. MAP and fetal arterial blood gasometric parameters were reported at the end of each 5-min period without UCO.

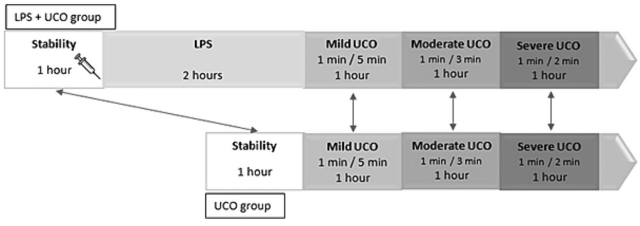

FIGURE 1 Experimental procedure. Hypoxia was induced by repetitive umbilical cord occlusions (UCO). During mild UCO, UCO were repeated every 5 min. During moderate UCO, UCO were repeated every 3 min. During severe UCO, UCO were repeated every 2 min. In hypoxia + FIRS group, LPS was injected after the stability period and 2 h before UCO. LPS, lipopolysaccharides; UCO, umbilical cord occlusion.

Euthanasia was administered at the end of the experimental procedure, or in case of fetal death. Euthanasia was carried out by maternal intravenous injection of 6 mL/50 kg T61 (1 mL contains embutramide 200 mg + mebezonium 26.92 mg + tetracaine 4.39 mg, MSD, France).

2.4 | HRV analysis

ECG analysis to compute fetal R-R series was conducted offline using an automatic R-wave detection algorithm. R-R series artifact were removed using a specific filtering algorithm.[22] HRV indices were computed through a program developed in MATLAB (version R2017B, MathWorks, Inc., Natick, MA, USA). Continuous computation of the HRV indexes is assumed by sliding the moving window with a 1-second moving period. Indices are then at an average of over 3 min.

HRV time domain analyses included: standard deviation of normal-to-normal R-R intervals (SDNN); root mean square of successive differences (RMSSD) between adjacent R-R intervals; short-term variability (STV), defined as the mean difference between successive 3.75-s R-R interval epochs; and long-term variability (LTV), defined by the difference between the highest and lowest values within the 16 epochs of an analyzed minute.[23]

Spectral HRV analysis included: the low-frequency (LF) component, from 0.04 to 0.15 Hz, which is related to both sympathetic and parasympathetic activity, and associated with baroreflex activity. The high-frequency (HF) component is >0.15 Hz, which is related to the parasympathetic nervous system alone. The LF/HF ratio represents parasympathetic–sympathetic imbalance.[16]

Fetal stress index (FSI) was developed by our team based on an original HRV analysis method that combines spectral and time domain analyses. In previous experimental studies, we demonstrated that FSI reflects parasympathetic fluctuation.[9,18] The algorithm used to compute the FSI has been described previously.[18]

2.5 | Fetal arterial blood samples

Arterial blood gas parameters were measured with the i-STAT 1 blood analyzer (i-STAT 1 System; Abbott Point of Care, Inc., Princeton, NJ, USA) using CG4+ cartridges. Due to normal body temperature at 39°, we apply temperature correction to blood gas values.

2.6 | Statistical analyses

Variables are reported as medians and interquartile ranges. Comparisons between UCO and UCO + LPS groups during three periods (stability phase, mild occlusion phase, and moderate occlusion phase) were performed using the Mann–Whitney *U*-test. A *p*-value <0.05 was considered to be significant. The variables for which

values were not statistically different between the two groups in stability phase were kept for multivariate analyses presented in supplementary analyses. Because of a high number of missing values due to deaths, data during severe occlusion have not been included in analyses. Data were analyzed using R version 4.3.2.

3 | RESULTS

3.1 | Cohort characteristics

In UCO group, 14 pregnant sheep underwent the surgical procedure. Two fetuses died in utero at postoperative day 1. One delivery occurred on postoperative day 3. One experimental procedure was stopped because of rupture of the occlusion balloon. One fetus was later excluded because of severe chronic neural injury that occurred before the protocol (laminar necrosis, slit-like cavities in the deep cortical area, gliosis, and macrophage infiltration).[11] Finally, nine experimental fetuses were analyzed.

In UCO + LPS group, 11 pregnant sheep underwent the surgical procedure. Two fetuses died during the first 3 days after surgery. Nine fetuses underwent the experimental procedure and were analyzed.

In UCO group, five fetuses reached endpoint before the end of the experiment: one at the end of moderate UCO and the other four during severe UCO. No fetal death occurred. In UCO + LPS group, one fetus reached endpoint before the end of the experiment at the end of moderate UCO. Four fetuses died at the end of moderate UCO or at the beginning of severe UCO. Because of small remaining sample size ($n = 4$), we did not realize statistical analysis in severe UCO.

Median fetal body weight was 3335 g (3135; 3643). Four of 18 fetuses were singletons. Gestational age at the experimental procedure was 128 +/−1 days for all fetuses (term = 145 days).

Comparison of hemodynamic and blood gas measures between UCO + LPS group and UCO group is shown in Table 1.

3.2 | Hemodynamic variations

At baseline, FHR and MAP were normal in both groups with no significant difference (Table 1). After mild UCO and moderate UCO, FHR was not significantly different between the two groups. After mild UCO, MAP was not significantly different between the two groups. After moderate UCO, MAP was significantly higher in LPS + UCO group in comparison to UCO group (62 mmhg [51–65] vs 49 mmhg [48–55], $p = 0.02$).

3.3 | Blood sample parameters

At baseline, pH, pO_2, and lactate were normal in both groups with no significant difference (Table 1). After mild UCO, pH was significantly lower (7.22 [7.12–7.24] vs 7.28 [7.23–7.34]; $p = 0.02$) and lactate was significantly higher (10.3 mmol/L [9.4–11.0] vs 6.0 mmol/L [4.1–8.2]; $p < 0.01$) in UCO + LPS group in comparison to UCO group. After moderate UCO, there was no significant difference in pH and lactate between LPS + UCO and UCO groups.

TABLE 1 Hemodynamic and blood gas measures in UCO group and UCO + LPS group.

Period	Stability			A = Mild UCO			B = Moderate UCO		
Group	UCO ($N = 9$)	LPS + UCO ($N = 9$)	p	UCO ($N = 9$)	LPS + UCO ($N = 9$)	p	UCO ($N = 9$)	LPS + UCO ($N = 9$)	p
Hemodynamic markers									
HR bpm	181 (169–188)	172 (165–182)	0.57	178 (155–186)	175 (170–188)	0.82	189 (162–193)	224 (180–238)	0.09
MAP mmHg	48 (44–49)	47 (42–48)	0.56	58 (47–61)	51 (44–56)	0.31	62 (51–65)	49 (48–55)	**0.02**
Chemical arterial blood markers									
pH	7.39 (7.37–7.40)	7.41 (7.39–7.42)	0.21	7.28 (7.23–7.34)	7.22 (7.12–7.24)	**0.02**	7.10 (7.07–7.25)	7.05 (6.99–7.18)	0.29
PCO_2 mmHg	45.3 (43.4–48.2)	52.1 (50.4–53.3)	**0.01**	50.6 (48.8–55.5)	63.3 (59.9–69.7)	**<0.001**	54.0 (52.9–61.7)	61.6 (60.2–74.8)	**0.02**
PO_2 mmHg	12 (11–17)	20 (14–20)	0.26	16 (13–16)	18 (17–20)	**0.01**	15 (14–17)	18 (17–19)	**0.05**
BE	3 (2–4)	7 (6–7)	**<0.01**	−3 (−4 to −1)	−4 (−7 to −2)	0.31	−11 (−15 to −3)	−10 (−15 to −7)	0.82
Lactate mmol/L	2.2 (1.7–3.0)	2.7 (2.1–3.0)	0.34	6.0 (4.1–8.2)	10.3 (9.4–11.0)	**<0.001**	12.7 (8.8–15.0)	14.8 (12.4–17.7)	0.16

Note: Variables are reported as medians and interquartile ranges. Statistical analysis: Comparisons between UCO + LPS group and UCO groups were performed using the Mann–Whitney U-test. Because of small size ($N = 4$), we did not realize statistical analysis after severe UCO. $p < 0.05$ is significant (given in bold).

Abbreviations: BE, des base excess; HR, heart rate; LPS, lipopolysaccharides; MAP, mean arterial pressure; UCO, Umbilical cord occlusions.

At baseline, pCo_2 was slightly but significantly higher in LPS + UCO group (52.1 mmhg [50.4–53.3] vs 45.3 mmhg [43.4–48.2]; $p = 0.01$). The difference remained after mild and moderate UCO. At baseline, base excess was significantly higher in LPS + UCO group (7.0 mEq/L [6.0–7.0] vs 3.0 mEq/L [2.0–4.0]; $p < 0.01$). The difference did not remain after mild and moderate UCO.

3.4 | Principal components analysis of hemodynamic and gasometric data

Principal components analysis, presented in supplementary information, that the main variability present in the dataset is related to phase (Figure S1). This figure illustrates the fact that gasometric and hemodynamic changes are more rapid in the UCO + LPS group than in the UCO group when looking at the first principal component. Consequently, positions of the projected points regarding the *x*-axis highlight that the gasometric and hemodynamic alteration was as altered with mild UCO + LPS as moderate UCO without LPS.

3.5 | HRV analysis

Comparisons of HRV indices between UCO + LPS group and UCO group are shown in Table 2.

3.5.1 | Time domain analysis

At baseline, there was no significant difference in SDNN, RMSSD, LTV, and STV between the two groups. After mild UCO, five indices were significantly higher in UCO + LPS group in comparison to UCO group: (SDNN = 68.9 ms [50.8–79.3] vs 19.7 ms [16.1–20.8]; $p < 0.001$), RMSSD = (27.4 ms [24.6–38.0] vs 13.7 ms [8.1–17.6]; $p < 0.001$), LTV = (80.0 ms [66.7–83.1] vs 36.8 ms [32.1–44.3]; $p = 0.01$), and STV = 7.0 ms [5.8–8.7] vs 3.5 ms [3.1–5.6]; $p = 0.01$). After mild UCO, SDNN and RMSSD remained significantly higher (SDNN = 55.5 ms [40.3–67.7] vs 18.7 ms [16.9–20.1]; $p < 0.001$; RMSSD = (27.9 ms [20.9–32.4] vs 12.7 ms [11.9–14.4]; $p < 0.001$) in UCO + LPS group in comparison to UCO group.

3.5.2 | Spectral HRV analysis

At baseline, there was no significant difference in LF, HF, and HF.nu between the two groups. After mild UCO, LF was significantly higher (0.30 dB [0.19–0.40] vs 0.19 dB [0.04–0.21]; $p = 0.02$) and HF was significantly higher (0.18 dB [0.10–0.22] vs 0.07 dB [0.02–0.10]; $p = 0.03$) in UCO + LPS group in comparison to UCO group. After moderate UCO, there was no significant difference in LF and HF. There was no significant difference in HF.nu after mild and moderate UCO.

TABLE 2 Heart rate variability measures in UCO group and UCO + LPS group.

Period	Stability			A = Mild UCO			B = Moderate UCO		
Group	**UCO N = 9**	**LPS + UCO N = 9**	***p***	**UCO N = 9**	**LPS + UCO N = 9**	***p***	**UCO N = 9**	**LPS + UCO N = 9**	***p***
Time domain analysis									
SDNN ms	17.5 (16.7–19.9)	21.7 (14.8–25.4)	0.60	19.7 (16.1–20.8)	68.9 (50.8–79.3)	**<0.001**	18.7 (16.9–20.1)	55.5 (40.3–67.7)	**<0.001**
RMSSD ms	10.7 13.6–15.7)	15.4 (12.6–21.3)	0.22	13.7 (8.1–17.6)	27.4 (24.6–38.0)	**<0.001**	12.7 (11.9–14.4)	27.9 (20.9–32.4)	**<0.001**
LTV ms	39.4 (27.1–41.1)	44.0 (34.6–57.5)	0.44	36.8 (32.1–44.3)	80.0 (66.7–83.1)	**0.01**	50.7 (45.9–56.2)	58.0 (36.1–80.5)	0.73
STV ms	3.7 (2.4–4.5)	5.5 (3.1–6.0)	0.34	3.5 (3.1–5.6)	7.0 (5.8–8.7)	**0.01**	4.6 (4.2–5.0)	5.4 (4.4–7.4)	0.43
Spectral HRV analysis									
LF dB	0.08 (0.06–0.11)	0.13 (0.10–0.35)	0.06	0.19 (0.04–0.21)	0.30 (0.19–0.40)	**0.02**	0.13 (0.12–0.19)	0.16 (0.28–0.35)	0.16
HF dB	0.05 (0.03–0.07)	0.11 (0.06–0.21)	0.08	0.08 (0.02–0.10)	0.18 (0.10–0.22)	**0.03**	0.06 (0.05–0.12)	0.14 (0.08–0.18)	0.16
HF.nu	0.30 (0.29–0.40)	0.41 (0.37–0.44)	0.16	0.31 (0.25–0.36)	0.36 (0.33–0.38)	0.60	0.33 (0.30–0.36)	0.37 (0.29–0.39)	0.39
Spectral and time domain analysis									
FSI	59.3 (58.2–62.4)	68.3 (63.0–70.3)	0.08	53.7 (45.8–69.4)	61.8 (50.8–62.1)	0.73	53.3 (50.3–65.8)	63.4 (57.6–70.7)	0.14

Note: Variables are reported as medians and interquartile ranges. Statistical analysis: Comparisons between UCO + LPS group and UCO groups were performed using the Mann–Whitney *U*-test. $p < 0.05$ is significant (given in bold). Because of small size ($N = 4$), we did not realize statistical analysis after severe UCO.

Abbreviations: FSI, fetal stress index; HF, high frequencies; LF, low frequencies; LTV, long-term variability; LPS, lipopolysaccharides; RMSSD, root mean square of successive differences, R-R intervals; SDNN, standard deviation of normal-to-normal; STV, short-term variability; UCO, umbilical cord occlusions.

3.5.3 | Spectral and time domain analysis

There was no significant difference in FSI, at baseline, after mild UCO, and after moderate UCO.

4 | DISCUSSION

The rate of mortality rate was high in the LPS + UCO group ($n = 4/9$ vs $n = 0/9$ in UCO group). In this near-term fetal sheep experimentation, pH was lower and lactate was higher during mild UCO in the UCO + LPS group in comparison to the UCO group. The gasometric and hemodynamic states were altered earlier in the UCO + LPS group; Indeed, during mild UCO, the UCO+LPS group responded similarly to the UCO group during moderate UCO HRV also differed significantly between the groups during both mild and moderate UCO.

Our acute FIRS model via LPS IV injection was previously by both Durosier et al and our team.[10,24] Using this model, interleukin-6 increases from one hour after injection, pH decreases from one hour after injection, lactate increases from two hours after LPS injection, and heart rate increases from five hours after LPS injection. The progressive hypoxia model, described by Prout et al. and used by our team[11,21] has been shown to induce a progressive decrease in pH dropping to around 7.0 during severe UCO.[10] Simultaneous fetal infection and hypoxia during labor conveys particular risk for the fetus.[1,5,25] The high mortality rate in the LPS + UCO group herein is concistent with this. The morbidity and mortality shown by this model confirms the need for early detection during intrapartum period.

To our knowledge, administration of LPS associated with UCO in fetal sheep has only been combined in two studies.[12,26] Nitsos et al. investigated the impact of chronic intrauterine inflammation on the physiological and neurodevelopmental consequences of intermittent UCO in fetal sheep. Several differences between that study and ours make it difficult to comapre results: their fetuses were preterm (80 days), LPS was infused intra-amniotically and for weeks, and UCO protocol was different (series of 5 UCO, each lasting 2 min at 30-min intervals). Xu et al. investigated the ovine fetal and placental inflammatory response to umbilical cord occlusions with worsening acidosis. As in our study, fetuses were near term. However, their UCO protocol differs (all UCO were 1 min in duration and occurred every 2.5 min with worsening decelerations of 30, 60, and 90 bpm), as did their LPS protocol (hourly intra-amniotic injections of 2 mg/h beginning 1 hour prior to the first UCO and continuing throughout the UCO). Hemodynamic and gasometric changes were smaller in that stydy than herein (respectively, pH after moderate UCO = 7.19 +/− 0.05 vs 7.05 (6.99–7.18)). No fetal deaths were reported. Nevertheless, the FIRS induced by our protocol is already moderate, since isolated LPS injection induces a moderate decrease in pH (nadir = 7.32 (7.30;7.35)) without arterial hypotension or cardiovascular failure.[10] Our results thus show that during acute (even moderate) FIRS, acidosis during labor is likely to become severe more rapidly. This poorer adaptation to hypoxia can be partly explained by an increased placental resistance. During FIRS, several mechanisms can cause increased placental resistance[27]: First, secretion of endothelin 1, a vasoconstrictive peptide; second, the formation of placental edema caused by increase in permeability; and third, increased flow to the heart, brain, and adrenals at the expense of placental flow.[27]

To our knowledge, this was the fist study of HRV changes in concomitant hypoxia and FIRS in any fetal animals.

We previously studied HRV changes after LPS injection compared to control group.[10] Among HRV index analyzed in this study, only SDNN and LTV changed after LPS injection; both were significantly higher from H2 to H4 after LPS injection. We also studied HRV after gradual UCO. The same HRV indices as those used here were analyzed and compared between the three phases: mild, moderate, and severe occlusion. In those analyses, only FSI changed significantly: It was significantly higher during severe occlusion in comparison to mild and moderate occlusion.[11] We performed a complementary analysis to study the changes in these HRV markers compared to the stability period during mild and moderate occlusions. Only SDNN increased significantly during these two periods compared with the stability period.

SDNN increased after LPS injection, it is unsurprising that SDNN was also higher, after mild and moderate UCO in the LPS + UCO group compared with the UCO group. RMSSD, LF, HF, and STV were all significantly higher, after mild UCO, in the LPS + UCO group compared to the UCO group, while none changed significantly after isolated LPS injection or isolated UCO. Thus a primary finding herein is that changes in HRV indices after LPS injection associated with UCO are therefore not the sum of the changes after LPS or isolated UCO.

After moderate UCO, only RMSSD and SDNN remain significantly higher in the LPS + UCO group compared with the UCO group. These differences between mild and moderate UCO might be explained by the finding that pH and lactates differed significantly only during mild UCO. During moderate UCO, decreased pH and increased lactates were mainly related to occlusions, so the mild impact induced by the LPS injection may not have contribuated meaningfully to the significant between-group differences. Thus, some of the differences in HRV could be explained by gasometric differences. Additionally, given the small sample sized, it is possible that the lack of significance differences after moderate UCO may be due to low statistical power.

HRV is partially linked to the autonomic nervous system (ANS). It is therefore classic to make a link between change in HRV and activation/inhibition of sympathetic and parasympathetic nervous system.[15] RMSSD and HF are also associated with parasympathetic nervous systems and SDNN and LF is associated with global activity of ANS.[15] Greater LPS+UCO group increases in HRV indices after mild occlusion could therefore be linked to that group's differential ANS activation. However, the physiological interpretation of HRV markers must be

made with caution as no HRV index strictly reflect direct sympathetic or parasympathetic changes[28] and there is a substantial overlap between these systems.[29] The differential increase in LPS + UCO group SDNN and RMSSD during moderate UCO, with no differences for the other indices, is also difficult to explain by ANS activity.

As shown by high UCO + LPS group fetal mortality, simultaneous acute FIRS and hypoxia during labor is particularly high-risk. During FIRS, fetal adaptation to hypoxia is impaired and acidosis during labor is likely to become severe more rapidly. While intraamniotic infection alone is not an indication for immediate delivery,[30] extreme vigilance is required and rapid delivery should be considered if fetal infection associated hypoxia is suspected during labor.

To our knowledge, it is the first time a model of FIRS with worsening hypoxia shows a difference in mortality in comparison to isolated hypoxia in near-term fetal sheep. HRV changes were studied herein for the first time in a model of acute fetal infection associated with hypoxia. HRV was significantly different between UCO and UCO + LPS groups during mild and moderate UCO. Use of HRV could therefore be interesting to detect this high-risk situation. Other studies are necessary to precise the exact methods of detection.

Our study has some limitations. The two groups were created by the same team, in the same conditions, and in the same seasons. Nevertheless, the non-randomized creation of the two groups is a first limitation. This may explain the differences in pCO_2 and base excess in the two groups. However, there were no other significant hemodynamic, gasometric, and HRV differences. In addition, the multivariate analyses (PCA) presented in Figures S1 and S2 only relied on variables for which values were not different in stability phase and confirmed the results of univariate analyses. The second study limitation is the small number of individuals to perform the statistical analyses. In univariate analyses, this leads to a lack of power. Therefore, we preferred to perform multivariate exploratory analyses to strengthen results from univariate analyses rather than controlling the family-wise error rate by performing, for example, Bonferroni adjustment to correct for multiple testing. The advantage of PCA is to get rid of multiple testing problems while also analyzing the variability between all individuals. The third study limitation is that although we used an animal model of human gestation, generalizability of findings and their applications to human fetuses must be carefully established. Fourth, we did not record fetal sex for comparison between the two groups. Finally, we focused specifically on acute FIRS. The model does not therefore reflect the entire range of fetal infections, which may be subacute or chronic with differing intensity. Furthermore, we focused only on the fetal system. Concomitant infections of the placenta, amniotic fluid, or maternal system could alter these findings

5 | CONCLUSION

Simultaneous acute FIRS and hypoxia during labor is a particularly high-risk fetal context. During acute FIRS, fetal adaptation to hypoxia changes, and acidosis during labor, is likely to become severe more rapidly, requiring closer fetal monitoring.

AUTHOR CONTRIBUTIONS

Geoffroy Chevalier wrote the manuscript and conceived and designed the work. Louise Ghesquière, Laurent Storme, and Charles Garabedian conceived and designed the work. Yasmine Ould Hamoud, Kevin Le Duc, and Louis Galan acquired the data. Valeria De Stephano, Anne Wojtanowski, Julien De Jonckheere, and Guillemette Marot analyzed and interpreted the data. All authors substantively revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

All the authors report no conflict of interest.

ETHICS STATEMENT

Anesthesia, surgery, and experiment protocols were consistent with the recommendations of the French Ministry of Higher Education and Research, and the study was approved by the Animal Experimentation Ethics Committee on June 30, 2020 (APAFIS#25894-201907071726306v9).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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