CEOG Clinical and Experimental Obstetrics & Gynecology

Original Research

Fetal acidosis in a sheep model: can we perform a second day of protocol in order to reduce the numbers of animals needed?

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Summary

Objective: To determine if a model of acidosis caused by occlusion of the umbilical cord could be repeated in the same sheep in ethicaly order to reduce the number of animals needed. *Method:* To obtain fetal acidosis, 1 min of total umbilical cord occlusion (UCO) every 2.5 min was performed on fetal sheep (n=7) until pH reached 7.10. Chronic instrumentation regularly recorded arterial blood gas and hemodynamic parameters. The occlusions were repeated to compare the data between the 2 days for each animal. *Results:* At the end of the UCO period, fetal acidosis was obtained with a pH 7.14 on day 1 (D1) and 7.06 on day 2 (D2). At baseline, fetuses were more hypotensive at D2 (41 mmHg vs 44 mmHg on D1, $p < 0.05$). During the UCO period, there was no significant difference in blood gas and hemodynamic data between D1 and D2. *Conclusion:* To reduce the number of animals needed, performing a second experiment on the same animal appears possible even if results must be interpreted cautiously when severe acidosis is reached.

Key words: Fetal sheep, Animal experimentation, Animal ethics, 3R, Umbilical cord occlusion, Fetal acidosis

Introduction

Normal fetal oxygenation depends on maternal, fetal, and placental factors. Alteration of maternal–fetal exchanges can lead to fetal acidosis. In utero, fetal preacidosis is defined as a pH <7.25 and lactates >4.2 mmol/L, and fetal acidosis is defined as a pH <7.20 and lactates >4.8 mmol/L [1]. Severe acidosis can lead to serious neonatal complications with risk of cerebral palsy [2].

The monitoring of fetal well-being during labor is essential and is based on fetal heart rate (FHR) [3]. The analysis is subjective and imperfect to fully assess the state of oxygenation of the fetus or neonatal risk [4]. To improve fetal monitoring during labor, our team is devel-oping a new tool to predict the risk of acidosis [5-7].

To validate this tool, we used an experimental model of fetal acidosis obtained by quick and repeated umbilical cord occlusion (UCO) in fetal sheep as described previously [8-10].

In these studies, a single manipulation in 24 hours was performed per animal before euthanasia.

There have been no studies to determine whether manipulations could be repeated to reduce the number of animals required and comply with the 3R ethical principle of reduction [11]. The present study aimed to determine whether a model of acidosis effected by occlusion of the umbilical cord could be repeated in the same sheep to reduce the number of animals needed and comply with the 3R ethical principles (i.e., replacement, reduction, and refinement).

Materials and Methods

This study is a complementary analysis of a protocol which aimed was to evaluate our new index reflecting parasympathetic fluctuation in case of fetal acidosis [12].

Ethics

The anesthesia, surgery, and experimentation protocols were consistent with the recommendations of the Ministry of Higher Education and Research, and the study was approved by our institutional Animal Experimentation Ethics Committee (CEEA #2016121312148878).

Surgical preparation

Near-term pregnant Charmoise sheep (INRA, Bressonville, France) with a gestational age of 123 2 days (term around 145 days) underwent the surgical procedure. The anesthetic and surgical technique protocols followed those previously described by our team or in previous studies. Sheep were fasted for 24 h before general anesthesia and surgery. Before surgery [8-9], sheep were placed in a supine position, anesthetized with an intravenous injection of xylazine (Sedaxylan1; CEVA SantéAnimale, Libourne, France), intubated and anesthesia maintained with 2% isoflurane (Aerranel; Baxter, Guyancourt, France).

Published: 15 February 2020

Clin. Exp. Obstet. Gynecol. - ISSN: 0390-6663
XLVII, n. 1, 2020
doi: 10.31083/j.ceog.2020.01.5120

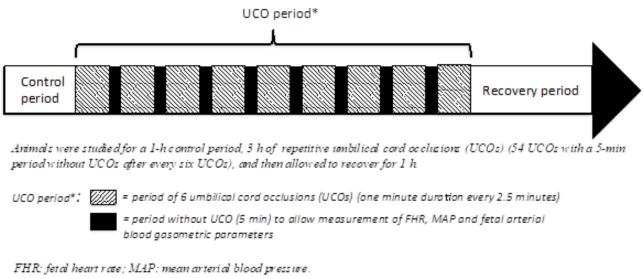

Figure 1. — Study periods, umbilical cord occlusions (UCO) rhythm, and fetal parameters studied (FHR, MAP).

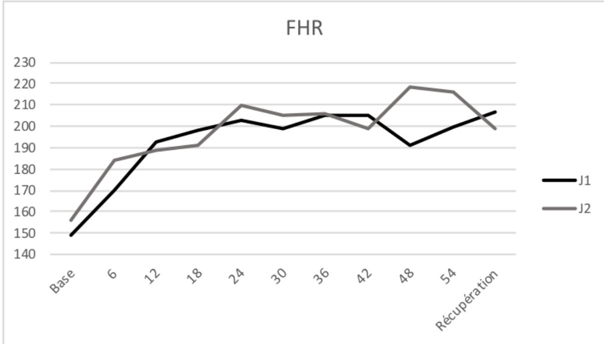

Figure 2. — Evolution of the fetal heart rate (FHR) according to the number of occlusions during day 1 (D1) and day 2 (D2).

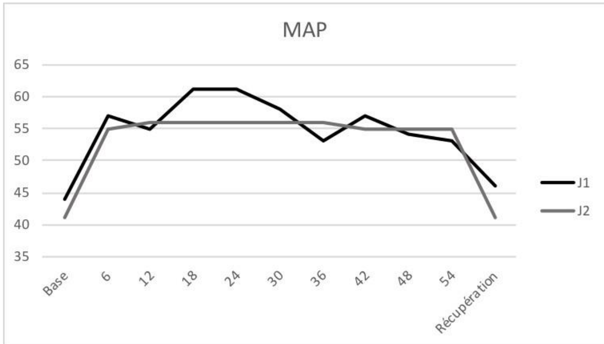

Figure 3. — Evolution of the mean arterial pressure (MAP) according to the number of occlusions during day 1 (D1) and day 2 (D2).

After maternal laparotomy and hysterotomy, catheters (4 Fr diameter; Arrow1, USA) were placed in the fetal right axillary artery and vein and in the left axillary artery. Four electrocardiogram (ECG) electrodes (Mywire 101; Maquet, Rastatt, Germany) were placed on the fetal intercostal muscles near the heart to record fetal ECG. A 5 Fr-diameter catheter, Arrow1, was placed into the amniotic cavity to measure baseline intra-amniotic pressure (IAP) and to replace amniotic fluid lost during surgery with 500 mL saline containing antibiotics (amoxicillin–clavulanic acid).

An inflatable silicone occluder (OC16; In Vivo Metric, Healdsburg, CA, USA) was placed around the umbilical cord and the volume of saline solution required to achieve a complete occlusion was determined.

All leads were exteriorized through the maternal flank. After surgery, ewes were given free access to food and drink. Postoperative analgesia was provided by a maternal intramuscular injection of 0.3 mL/10 kg buprenorphine (Buprenodale; Dechra Veterinary Products, Montigny-le-Bretonneux, France) at 24 and 48 hours after surgery.

Data acquisition

The fetal arterial and intra-amniotic catheters were connected to pressure sensors (Pressure Monitoring Kit 1; Baxter). Blood pressure sensors and ECG electrodes were connected to a multiparametric anesthesia monitor (Merlin; Hewlett Packard, Palo Alto, CA, USA).

Mean arterial pressure (MAP) was measured from the blood pressure phasic signal and corrected for the IAP value (calculated MAP = observed MAP – observed IAP). ECG and blood pressure signals were recorded through a data acquisition board (Physiotrace; Estaris Monitoring, Lille, France) [13].

Experimental procedure

The experiments began after the sheep had rested for at least 48 h after surgery. Before the first UCO, a 60 min period (stability period) was recorded to ensure that the animals were healthy (normal gas blood and hemodynamic parameters). The hemodynamic (MAP, FHR), arterial blood gas (pH, lactate, pO_2, pCO_2) parameters were recorded during the stability period to obtain baseline values.

After this baseline control period, 54 repetitive UCOs of 1 min duration were performed every 2.5 min as described by Bennet et al. [8] UCO was induced by complete inflation of the occluder cuff with a volume of saline solution de-fined at the time of surgery. After each six UCOs, a 5 min period without UCOs was performed to measure FHR, MAP, and fetal arterial blood gas parameters (Figure 1). UCOs were stopped when arterial pH reached 7.10 or after the 54th UCO. Variables were then recorded at 1 h and 2 h after the end of the occlusion period. A second experiment was repeated on the same animal 24 hours later on day 2 (D2).

Statistical analysis

Hemodynamic and arterial blood gas parameters are expressed as median with interquartile range. To validate the efficiency of the acidosis model, variables were compared to their basal values for D1 and D2.

By contrast, the values compared between D1 and D2 were measured at each time for each sheep. All comparisons were made using a nonparametric Wilcoxon test. A $p < 0.05$ was considered significant. All tests were performed using IBM SPSS Statistics for Windows (v. 20.0; IBM Corp, Armonk, NY, USA).

Table 1. — *Difference between J1 and J2 on the fetal arterial blood gas parameters according to the number of occlusions.*

		Ground State			**Number of repeated total cord occlusions of 1 min each 2'30 min**							**Recovery period**
			6	**12**	**18**	**24**	**30**	**36**	**42**	**48**	**54**	**1h**
n												
	D1	7	7	7	7	7	7	6	5	5	5	7
	D2	7	7	7	7	7	7	6	5	5	4	6
pH												
	D1	7,38 (7,37-7,40)	7,28* (7,27-7,30)	7,19* (7,15-7,26)	7,18* (7,11-7,25)	7,16* (7,07-7,22)	7,14* (7,03-7,20)	7,15* (7,07-7,21)	7,16* (7,11-7,20)	7,17* (7,10-7,20)	7,14* (7,06-7,19)	7,27* (7,21-7,27)
	D2	7,38 (7,38-7,40)	7,24* (7,21-7,29)	7,21* (7,15-7,24)	7,18* (7,10-7,23)	7,19* (7,09-7,22)	7,15* (7,05-7,19)	7,15* (7,05-7,20)	7,14* (7,04-7,18)	7,06* (7,01-7,18)	7,06 (7,02-7,17)	7,24* (7,16-7,31)
P02 mmHg												
	D1	16 (13-24)	15 (12-18)	15 (15-19)	15 (13-19)	17 (13-20)	17 (15-18)	17 (16-23)	17 (15-20)	18 (15-21)	17 (14-21)	17 (15-21)
	D2	18 (17-21)	16* (15-17)	17 (13-19)	16 (13-17)	17 (13-19)	17 (13-20)	18 (16-27)	18 (15-37)	16 (14-20)	17 (16-19)	17 (15-19)
PC02 mmHg												
	D1	39 (36-45)	49* (40-52)	49 (35-51)	52* (40-53)	47* (39-52)	46* (45-51)	47* (44-52)	49* (46-53)	44* (43-48)	45* (44-56)	40 (34-46)
	D2	42 (39-43)	49* (42-56)	50* (41-55)	50 (47-53)	49* (43-51)	51* (44-54)	49 (40-53)	51 (35-52)	52* (50-53)	53 (47-61)	44 (42-46)
Lact mmol/l												
	D1	1,05 (0,92-1,51)	3,85* (3,01-5,50)	5,44* (4,00-8,15)	8,44* (5,56-10,83)	9,14* (7,39-12,25)	11* (7,19-14,06)	10,9* (9,21-15,44)	11,5* (9,59-13,61)	11,42* (9,17-13,09)	12,74* (10,78-14,28)	11* (8,51-13,01)
	D2	1,13 (0,8-1,43)	3,85* (3,76-5,37)	6,57* (4,51-8,04)	8,02* (5,12-9,61)	8,48* (6,63-11,35)	10,9* (7,80-12,97)	11* (9,71-12,48)	12,1* (9,58-15,05)	11,9* (10,54-15,41)	14,2 (12,15-15,68)	10,8* (8,56-12,49)

Results presented in median, and (1st quartile-3rd quartile) * = $p<0.05$ versus ground state # = $p<0.05$ versus day 1.

Results

Seven sheep underwent surgery and experiments were conducted twice. Hemodynamic parameters were normal at ground state on D1 and D2. As soon as the UCO started, we observed tachycardia and arterial hypertension.

The highest heart rate was at the 36th UCO on D1 (205 bpm vs 149 bpm at ground state, $p < 0.05$) and faster, at the 24th UCO on D2 (210 bpm vs 156 bpm at ground state, $p < 0.05$). Fetal tachycardia persisted during the recovery period for both groups. The maximum increase in MAP level was observed at the 18th UCO (61 mmHg vs 44 mmHg at ground base, $p < 0.05$) on D1 and faster, at the 12th UCO (56 mmHg vs 41 mmHg at ground base) on D2, with a MAP-level gradual decline to baseline at the end of the occlusions for both groups. When we compared the hemodynamic parameters between the 2 days of manipulation, fetuses were significantly more hypotensive on D2, at baseline (41 mmHg vs 44 mmHg $p<0,05$) and during the recovery period (41 mmHg vs 46 mmHg, $p < 0.05$).

There was no significant difference between D1 and D2 for FHR at baseline, during the UCO period and during the recovery period. Likewise, there was no significant difference between D1 and D2 in the MAP during the UCO period (Figures 2 and 3). All fetuses had normal arterial blood gas parameters (pH, lactate level) at baseline on D1 and D2. During the UCO period, we progressively reached a significative ($p < 0.05$) fetal acidosis with pH <7.20 at the 12th UCO at D1 and at the 18th UCO on D2.

Lactate levels gradually increased until the fetal acidosis reached 4.8 mmol/L at the 12th UCO on D1 and D2. When we compared the blood gas parameters between the 2 days of manipulation, there was no significant difference during any of the experiments (Table 1).

Discussion

To improve fetal monitoring during labor, our team is developing a new tool to predict the risk of acidosis.

This tool has been studied in an experimental model of acidosis effected by repeated UCO in fetal sheep [5, 14,15]. This model has been described in the literature but no study has determined the outcome of a second day of experimentation to reduce number of animals. In this study, we obtained a moderate and progressive fetal acidosis, as seen during human labor, on the second day of experimentation.

Several models of fetal acidosis using brief and repeated UCO have been described in the literature with variations in the duration, type (complete or partial), and repeat rate of UCO [16]. Bennet et al. [8] studied a protocol with 1 min UCO each 2.5 min. This procedure lead to severe acidosis (minimal pH 6.92±0.04) with an important metabolic component (maximal lactate = 14.8±1.2 mmol/L).

Thus, we used the various published protocols, in particular that of Bennet et al. to create our model of acidosis with 1 min UCO each 2.5 min. In D1 of our study, we obtained an acidosis with a pH ranging from 7.38 at baseline to 7.14 and lactates from 1.1 mmol/L to 12.7 mmol/L after 3 hours of UCO, in agreement with the results obtained by Bennet et al. We observed a decrease in FHR and an increase of MAP during UCO.

These results are consistent with those reported in the literature. The 3R rule [11] described by Russell and Burch in 1959 is the foundation of the guiding ethical principles applied to animal research: i.e., to “reduce” the number of animals used, “refine” the methodology used to optimize the experiment to improve the welfare of animals in experimentation, and “replace” animal models by using in vitro models if possible.

We could reduce the number of animals needed by performing two manipulations per animal. There was no significant difference between the two experiments on the same animal on the variables recorded (i.e., FHR, MAP, pH, lactates) during UCO, which suggests that the data from a second experiment could be used.

However, at baseline, fetuses at D2 were significantly more hypotensive. Even if this result was not statistically significant, we notice that at the end of the occlusion period, after 42 UCO, the fetuses at D2 had a lowest pH (pH 7.17 at D1 vs pH 7.06 at D2). Performing a second experiment on the same animal appears possible, but the results must be interpreted cautiously when severe acidosis is reached. The study has certain limitations. We observed the animals for only 48 h of rest after surgery and fetal pO_2 at the start of the experiments was low (16 mmHg [13 mmHg to 24 mmHg]), which could have reflected the effects of the surgical procedure or particularities of this species, or both. This pO_2 could be considered to indicate moderate hypoxia [17]; therefore, it will be important to ensure a longer period of recovery between surgery and experimentation in future studies.

Another limitation of the study is the observation of non-significant differences, which were probably because of the reduced number of animals, especially on D2 at the end of manipulation.

Conclusion

To reduce the number of animals needed, a second experiment conducted with the same animal appears possible. However, when severe acidosis is reached, the results must be interpreted cautiously.

Acknowledgement

We would like to thank authors who contributed to this paper. We would also like to express our gratitude to the DHURE (Hospital and University Department of Experimental Research) and people who take ethicaly care about animals during all experimentation.

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