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## Autonomic response to fetal acidosis using an experimental sheep model

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14 **Keywords: fetal sheep, acidosis, heart rate variability, autonomic nervous system, labor**  
15 **monitoring.**

16

17 **Short title:** Sheep fetal autonomic nervous system response to acidosis

18

19 **ABSTRACT**

20 **Background:** The autonomic nervous system has a major role in fetal adaptation to hypoxia.  
21 Its activity might be assessed using heart rate variability and heart rate deceleration analyses.

22 **Objective:** To evaluate the ability of different heart rate variability and morphological  
23 deceleration analyses to predict fetal acidosis during labor in an experimental fetal sheep  
24 model.

25 **Study Design:** Repeated 1-minute total umbilical cord occlusions were performed at mild (1  
26 minute every 5 minutes), moderate (1 minute every 3 minutes), and severe (1 minute every 2  
27 minutes) umbilical cord occlusion periodicities until arterial pH reached 7.10. Hemodynamic,  
28 blood gas analysis, morphological analysis of decelerations (magnitude, slope, and area of  
29 decelerations), and heart rate variability parameters were recorded throughout the experiment.  
30 Heart rate variability analysis included temporal analysis (root mean square of successive  
31 differences between adjacent R-R intervals, standard deviation of normal to normal R-R  
32 intervals, short term variability), spectral analysis (low frequencies, high frequencies,  
33 normalized high frequencies), and a new index developed by our team, the Fetal Stress Index.  
34 We defined and compared three pH groups: >7.20, 7.10–7.20, and <7.10.

35 **Results:** Eleven experiments were performed. Repetitive umbilical cord occlusions resulted  
36 in progressive fetal acidosis. Fetal Stress Index was correlated with pH and lactate ( $p < 0.05$ )  
37 and increased with acidosis. There were no significant correlations between pH, lactate, and  
38 other indices (spectral analysis, temporal analysis, or morphological analysis of  
39 decelerations).

40 **Conclusion:** This protocol allowed us to identify the progressive onset of fetal acidosis in an  
41 experimental model close to labor. Fetal Stress Index is a heart rate variability method that  
42 varies with acidosis and indicates an increase in parasympathetic nervous system activity in  
43 response to fetal acidosis.

44

## 45 INTRODUCTION

46 During labor, a decrease in maternofetal gas exchange can lead to fetal acidosis, which  
47 can cause severe neonatal complications, including a risk of cerebral palsy. Almost 10% of  
48 cerebral palsy is induced by peripartum asphyxia (1). Currently, fetal wellbeing is assessed  
49 using fetal heart rate (FHR) analysis, subject to high intra- and inter-observer variability, and  
50 imperfectly reflecting fetal oxygenation (2,3). There is a need for development of new tools for  
51 evaluation of fetal wellbeing and analysis of autonomic nervous system (ANS) activity may  
52 help optimize diagnosis.

53 Indeed, the ANS plays a major role in fetal homeostasis (4,5). Heart rate is under ANS  
54 influence (6) through chemoreceptors and baroreceptors, and vary constantly according to  
55 fetal homeostasis (7). Heart rate variability (HRV) is therefore an efficient way to study ANS  
56 activity. Many HRV analysis techniques have been described. Time domain analysis includes  
57 indices such as standard deviation of normal to normal R-R intervals (SDNN), root mean  
58 square of successive differences between adjacent R-R intervals (RMSSD), or short-term  
59 variability (STV), the latter being specially developed for fetal HRV analysis (8). Frequency  
60 (or spectral) analysis consists of a time frequency transformation, which distinguishes: very  
61 low frequencies reflecting thermoregulation and endocrine activity, low frequencies (LF)  
62 reflecting sympathetic and parasympathetic activities associated with the baroreflex, and high  
63 frequencies (HF) specifically linked to parasympathetic activity (9). Variations in these HRV  
64 indices have been studied in acidosis situations with inconsistent results (4,10).

65 Traditional FHR analysis relies on deceleration classification, associated with  
66 variability and baseline rate (11). Cahill et al. even focused on deceleration cumulative effects  
67 on acidosis occurrence (12). HRV analysis combined with deceleration analysis may provide  
68 a complementary method toward quantifying fetal status.

69           Our team has developed a new index, the Fetal Stress Index (FSI), based on an  
70 original HRV analysis method (13). In previous experimental studies, we demonstrated that  
71 the FSI reflects parasympathetic fluctuation (14,15) and that the FSI is correlated with  
72 acidosis in a fetal sheep model of continuous umbilical cord occlusion (UCO), and in a  
73 repeated 1 minute total UCO every 2.5 minutes model (11,16). Nonetheless, that protocol was  
74 distinct from the labor physiology and was focused on only our marker. Other HRV markers  
75 and aspect of deceleration were not simultaneously evaluated.

76           Therefore, our objective was to evaluate different HRV analysis methods and  
77 morphology of decelerations' ability to reflect fetal acid-basis status in an experimental model  
78 of cord occlusions reproducing the different stages of labor.

79

## 80 **METHODS**

### 81 **Ethics**

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

### 85 **Surgical preparation**

86 Near-term pregnant sheep (race 'Ile de France', Tours'INRA, Orfrasière animal  
87 physiology experimental Unit, Val de Loire Center) with a gestational age of  $124 \pm 1$  days  
88 (term = 145 days) underwent our previously described surgical procedure (11,12,16).

89 Briefly, under general anesthesia, after maternal laparotomy and hysterotomy,  
90 catheters (4Fr diameter, Arrow©, USA) were placed in the fetal right axillary artery and vein  
91 and in the left axillary artery. Four electrocardiogram (ECG) electrodes (Mywire 10©,  
92 Maquet, Rastatt, Germany) were placed on the fetal intercostal muscles near the heart to  
93 record fetal ECG. An inflatable silicone occluder (OC16; In Vivo Metric, Healdsburg, CA)  
94 was placed around the umbilical cord.

### 95 **HRV analysis**

96 ECG analysis for fetal RR series computation was conducted offline using an  
97 automatic R-wave detection algorithm. HRV time domain analyses included RMSSD, SDNN,  
98 and STV, defined as the mean difference between successive 3.75 seconds R-R interval  
99 epochs. RMSSD and STV are related to parasympathetic nervous system activity whereas  
100 SDNN evaluates ANS global activity (8).

101 Spectral HRV analysis included the LF component, from 0.04 to 0.15 Hz, related to  
102 both sympathetic and parasympathetic activity, and also associated with baroreflex activity.  
103 We also studied the HF component, superior to 0.15 Hz, related only to the parasympathetic  
104 nervous system. The HF normalized value traduced the imbalance between parasympathetic

105 and sympathetic, and was also computed as  $HF_{nu} = HF/(HF+LF)$ . We have previously  
106 described the algorithm used to compute the FSI (15,17–19). Continuous computation of the  
107 HRV indexes is assumed by sliding the moving window with a 1-second moving period.  
108 Indices are then at an average over 3 minutes.

### 109 **Morphologic analysis of FHR deceleration**

110 Morphologic deceleration analysis included duration (defined by the time needed to  
111 recover FHR measured before the deceleration, or, if not reached, the time to recover a stable  
112 FHR baseline), magnitude (from beginning to nadir), and slope (amplitude/duration). As  
113 described by Cahill et al., the deceleration's total area was also evaluated as ( $\frac{1}{2} \times$  duration  $\times$   
114 magnitude) (12).

### 115 **Experimental procedure**

116 Experiments began four days post-surgery. A 60-minute period without any stimulus  
117 was recorded to ensure fetal stability (normal gas blood and hemodynamic parameters).  
118 Injection of saline solution inside the occluder allowed complete cord occlusion. The protocol  
119 was divided into three phases, each lasting one hour, as described by Prout et al. (20). During  
120 the first hour, a mild UCO series was performed consisting of 1-minute cord occlusions  
121 repeated every 5 minutes. During the second hour, a moderate UCO series was performed  
122 consisting of 1-minute cord occlusions repeated every 3 minutes. During the third hour, a  
123 severe UCO series was performed consisting of 1-minute cord occlusions repeated every 2  
124 minutes. For each stage, 5-minute periods with no UCO were observed at 20, 40, and 60  
125 minutes for blood samples and hemodynamic and HRV indexes analyses. Morphologic  
126 deceleration analysis was performed on the last deceleration before the 5-minute period.

### 127 **Statistical analysis**

128 Qualitative values were described in term of frequency and percentage. Gaussian  
129 numerical values were described in term of mean and standard error. Normality of numerical

130 values were checked graphically and tested using the Shapiro–Wilk test. Three pH groups  
131 were defined: >7.20 (group A), 7.10–7.20 (group B), and <7.10 (group C). Associations  
132 between indices and pH groups were studied using a mixed linear model, including pH group  
133 and time as fixed effects, and fetal sheep as a random effect. Linear contrasts were identified  
134 based on post-hoc trends to determine whether the relations between markers and pH groups  
135 were linear. Correlations between different markers and pH, and lactate and base excess were  
136 evaluated at each time point using a mixed linear model with marker and time as fixed effects,  
137 and fetal sheep and number of manipulations for each lamb as random effects. The normality  
138 of residuals for each model was checked graphically. Bivariate tests required an alpha level of  
139 5% to be considered statistically significant. When a correlation was significant, a ROC  
140 analysis was performed to study the ability of the FSI to detect  $\text{pH} \leq 7.20$  and  $\text{pH} \leq 7.10$ .  
141 Statistical analyses were conducted using SAS software (SAS Institute 9.4, Cary, USA).

142



143 **RESULTS**

144           Eleven healthy fetal sheep underwent surgery. The protocol was interrupted due to pH  
145 <7.10 after one hour of the mild stage occlusion for three fetal sheep, during the moderate  
146 stage for another three, and during the intensive stage for another three sheep. Only two fetal  
147 sheep underwent the complete three-period UCO protocol.

148           Table 1 shows variation in recorded parameters according to pH. Mean heart rate was  
149 174.4 bpm ( $\pm 8.9$ ) in group A vs 193 bpm ( $\pm 3.4$ ) in group C ( $p=0.06$ ). No significant  
150 correlation between heart rate and blood gas status was observed (pH, lactate or base excess)  
151 (Table 2). MAP (mean arterial pressure) was significantly increased in group C ( $62.3 \pm 3.4$   
152 mmHg) compared with group B ( $57.5 \pm 3.2$  mmHg), which was also higher compared with  
153 group A ( $53.2 \pm 3.2$  mmHg) ( $p=0.048$ ) (Table 1). MAP was also correlated with pH ( $\beta \pm$   
154 SEM  $-0.003 \pm 0.001$ ,  $p=0.006$ ), lactate ( $\beta \pm$  SEM =  $0.079 \pm 0.026$ ,  $p=0.004$ ), and base excess  
155 ( $\beta \pm$  SEM =  $-0.110 \pm 0.051$ ,  $p=0.036$ ).

156           The FSI increased progressively from  $61.4 (\pm 3.8)$  to  $65.3 (\pm 3.7)$  until reaching  $77.1$   
157 ( $\pm 3.9$ ) in the group C ( $p<0.001$ ) (Table 1). The FSI was correlated with pH and lactate  
158 ( $p<0.001$  and  $0.015$ ) (Table 2). The AUC (area under curve) for FSI to distinguish  $\text{pH} \geq 7.20$   
159 vs  $\text{pH} < 7.20$  was at  $0.711$ , whereas AUC was  $0.903$  when separating  $\text{pH} > 7.10$  vs  $\text{pH} \leq 7.10$   
160 (Fig. 1). RMSSD, SDNN, STV, LF, HF, and HFnu did not differ significantly between pH  
161 groups (Table 1) and were not significantly correlated with blood gas variations (Table 2).  
162 SDNN increased progressively from  $14.7 (\pm 3)$  in the group A, to  $15.3 (\pm 2.9)$  in group B until  
163  $20.7 (\pm 3.1)$ , and in the group with group C; however, this increase was not significant  
164 ( $p=0.083$ ) (Table 1). SDNN was not significantly correlated with lactate or base excess (Table  
165 2).

166           Concerning morphological deceleration analysis, a moderate increase in FHR  
167 magnitude of decelerations was observed between group A ( $2.88 \text{ bpm/s} \pm 0.54$ ) and group B

168 (4.12 bpm/s  $\pm$  0.52) but this difference was not statistically significant (p=0.068) (Table 1).

169 No significant correlation between deceleration slopes or total area deceleration with gas

170 blood parameters was observed (Table 2).

171

## 172 **DISCUSSION**

173           The aim of fetal monitoring during labor is to detect the fetus at risk of acidosis. ANS  
174 analysis may be a promising area of research due to its major role in maintaining fetal  
175 homeostasis. Different indices for evaluating ANS activity exist, though their correlations  
176 with fetal acidosis are still being evaluated. In the current study, we have demonstrated that  
177 the FSI, which reflects parasympathetic fluctuations, increased with the onset of acidosis,  
178 whereas heart rate, SDNN, and deceleration slopes tended to increase when acidosis  
179 appeared. RMSSD, STV, LF, HF, and HFnu, and total area of decelerations were not  
180 significantly correlated with acidosis in our experimental model.

181           Different ANS analysis methods have been proposed. HRV time domain analysis  
182 relies on indices such as RMSSD, SDNN, or STV, which are well known by obstetricians  
183 (21–24). RMSSD reflects short-term HRV variations and vagal responses to stimulus (25).  
184 Frasch et al. described RMSSD increases during cord occlusion, each lasting 4 minutes and  
185 repeated every 30 minutes (9). Frasch et al. also emphasized that RMSSD increase was  
186 associated with cerebral adaptive mechanisms seen in cord occlusions (26). The idea that  
187 RMSSD may reflect cerebral inflammation was strengthened by Yamaguchi et al. In Humans,  
188 data are only available postnatally, after the anoxic event, with a relationship shown between  
189 RMSSD decrease and severe brain lesions or EEG grades (28,29). In our study, acidosis level  
190 remained moderate, only occurring at the end of the protocol. Cerebral consequences may,  
191 thus, just begin, without neuronal death. SDNN reflects global ANS tonus (25); in our study,  
192 we noticed a trend toward progressive increase in SDNN while pH decreased, showing a  
193 global ANS activation between each deceleration. As for RMSSD, in severe, long occlusions  
194 associated with ischemia and cerebral lesions, SDNN is first abolished, then highly increased  
195 compared with controls, before decreasing at six hours (27). In Humans, data only exist for  
196 the postnatal period, with a decrease in SDNN linked to EEG severity (28).

197 STV is used in growth restricted fetuses and interest in it during labor has been  
198 discussed (30). A meta-analysis has shown a moderate capacity for STV to predict acidosis  
199 with a sensitivity of 0.57 (95% CI: 0.45–0.68) and a specificity of 0.81 (95% CI: 0.69–0.89)  
200 (21). Our model showed a progressive increase in STV when pH decreased, though this was  
201 not statistically significant. This is consistent with others' findings on STV during labor (19).  
202 Lu et al. noticed an STV elevation when lactate increased during labor (24); in acidosis cases  
203 (lactate >4.8 mmol/L), median STV 30 minutes prior to fetal blood sample was 7.10  
204 milliseconds compared with 6.09 milliseconds in the pre-acidosis (4.2–4.8 mmol/L) and 5.23  
205 milliseconds in the normal (<4.2 mmol/L) groups ( $p < .05$ ).

206 Spectral analysis is another way to evaluate HRV. Chung et al. performed spectral  
207 analysis during the last two hours before birth and observed a diminution of every index in  
208 fetuses with acidosis. Siira et al. noticed that in the last hour of labor, LF and HF increased in  
209 every fetus ( $N=334$ ) and decreased near birth in fetuses with pH under 7.05 (31). Van Laar et  
210 al. found an increase in LFnu and a decrease in HFnu during the last 30 minutes of labor  
211 when pH <7.05 at birth (32). Such studies are of interest as they show different modification  
212 of the HRV index in cases with acidosis, though with inconsistent results. This may be due to  
213 different timings of analysis. Indeed, Warmerdam et al. (33) focused on analyses based on  
214 externally recorded contractions in order to gain better ECG signals and avoid mathematical  
215 artefacts linked to decelerations. In our study, we did not find any statistically significant  
216 associations between LF, HF, or HFnu, although these indices varied between pH groups.

217 Morphologic deceleration analysis might be another valuable tool to evaluate fetal  
218 physiologic response to acidosis, as proposed in the FIGO guidelines (34). Cahill et al. also  
219 evaluated the total area of deceleration during the last two hours of labor in 8580 term fetuses  
220 (12) to show this as a discriminative indicator for suspecting fetal acidosis with a pH <7.10  
221 (AUC 0.76, IC95% [0.72–0.80]). In our experimental study, we did not find a significant

222 correlation between morphologic deceleration analysis and acidosis. This could be explained  
223 by the fact that our protocol stopped after reaching pH <7.10.

224 Our HRV method, already used in adult and newborn monitoring (35,36), has been  
225 adapted to propose a physiological tool reflecting the fetal parasympathetic activity. In  
226 previous experimental studies, we have demonstrated that the FSI is associated with lower  
227 inter-individual variability and a higher effect size than commonly used HRV analysis  
228 methods, suggesting that it may be better to detect parasympathetic variations (13). In the  
229 present study, the FSI was the only index of HRV significantly associated with variations in  
230 pH and lactate. These results confirm the important implications of the parasympathetic  
231 nervous system for fetal adaptation to hypoxia (37), as previously described by Lear et al.  
232 (38). Using such an index may allow a more physiologic approach to fetal monitoring.

233 This experimental study had some limits. Although we used an animal model  
234 resembling human gestation, reproducibility of our physiologic findings and their applications  
235 to human fetuses must be carefully established. It is still premature to develop in current  
236 clinical practice at this time. However, with the improvement of fetal ECG recording systems  
237 and thus of R-R signal quality, we can imagine a clinical application in the automated analysis  
238 of the fetal heart rate (baseline, characteristics of decelerations) and the heart rate variability,  
239 reflecting fluctuations of the autonomous nervous system. Therefore, it is important to  
240 continue this experimental project to better understand physiological mechanisms, while  
241 starting preliminary studies in humans. Fetal hypoxia was obtained through umbilical cord  
242 compression as described in previous studies from others teams. It reflects a part of the  
243 mechanism of hypoxia during contraction also caused by compression of uteroplacental  
244 vessels. Finally, others indices approached statistical significance and it will be interesting to  
245 evaluate in a larger study.

246

247

248 **CONCLUSIONS**

249 HRV is an interesting tool to detect fetal acidosis during labor, as it reflects ANS  
250 modulations and, thus, allows a more physiologic approach to fetal monitoring. The new  
251 index developed by our team, the FSI, is the only discriminative HRV index to detect  
252 acidosis. These findings strongly suggest that changes in the parasympathetic nervous system  
253 may reflect progressive fetal asphyxia. It will be of interest to replicate these findings using an  
254 animal model to assess the risk of anoxic cerebral lesions.

255

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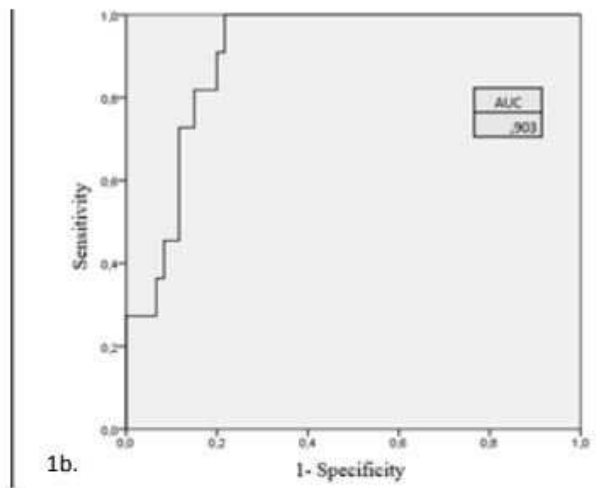
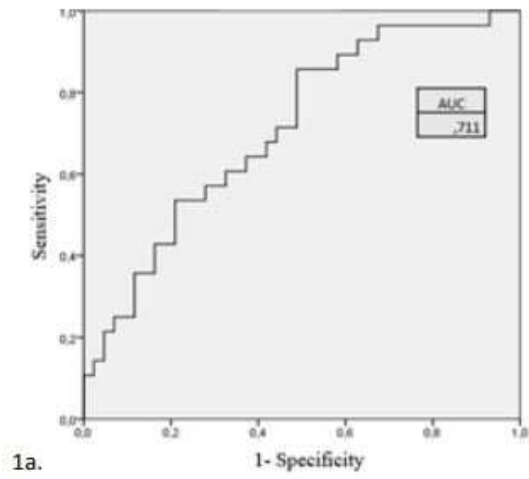
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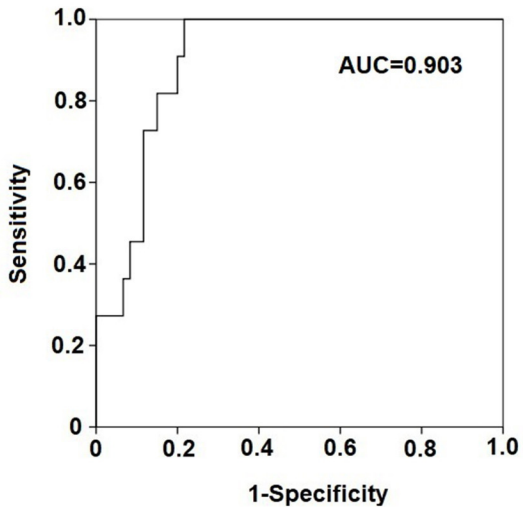
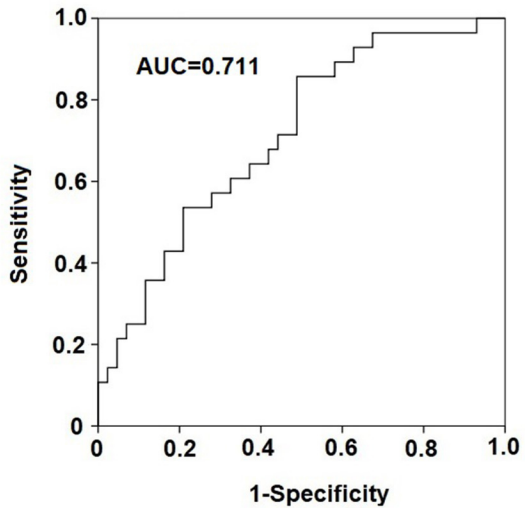
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**Fig. 1: ROC curves for FSI accuracy to predict acidosis.**

1a: pH <7.20 vs pH  $\geq$  7.20

1b: pH <7.10 vs pH  $\geq$  7.10



	pH group			<i>P</i>
	<7.10	7.10–7.20	>7.20	
n	11	17	44	
FHR (bpm)	193.1 ± 9.2	173.5 ± 8.5	174.4 ± 8.9	0.066
MAP (mmHg)	62.3 ± 3.4	57.5 ± 3.2	53.2 ± 3.2	0.016
RMSSD	21.1 ± 4.2	13.1 ± 4.1	14.8 ± 1.1	0.13
SDNN	20.7 ± 3.1	15.3 ± 2.9	14.7 ± 3.0	0.083
STV	5.03 ± 0.83	4.49 ± 0.77	3.80 ± 0.78	0.21
LF	0.37 ± 0.10	0.09 ± 0.10	0.18 ± 0.10	0.13
HF	0.23 ± 0.08	0.05 ± 0.07	0.12 ± 0.01	0.25
HFnu	0.31 ± 0.04	0.31 ± 0.03	0.33 ± 0.03	0.76
FSI	77.1 ± 3.9	65.3 ± 3.7	61.4 ± 3.8	<0.001
Amplitude (bpm)	92.4 ± 10.6	108.1 ± 8.6	89.8 ± 9.2	0.83
Slope (bpm/sec)	4.20 ± 0.62	4.12 ± 0.52	2.88 ± 0.54	0.068
TAD (bpm/sec)	123171 ± 6362	121889 ± 5740	113334 ± 6153	0.14

Values showed as mean ± standard error

**Table 1:** Comparison between markers and pH groups at each time point (from stability to last occlusion).

FHR = fetal heart rate, MAP = mean arterial pressure, RMSSD = root mean square of successive differences, SDNN = standard deviation of normal to normal R-R intervals, STV = short-term variation, LF = low frequencies, HF = high frequencies, HFnu = normalized high frequencies, FSI = fetal stress index, TAD = total area of deceleration

**Table 2: Correlation between hemodynamic, HRV and characteristics of deceleration with pH, lactate and base excess at each time point (from stability to last occlusion)**

	pH		Lactate		Base excess	
	$\beta \pm \text{SEM}$	P	$\beta \pm \text{SEM}$	P	$\beta \pm \text{SEM}$	P
FHR	$-0.0007 \pm 0.0004$	0.063	$0.009 \pm 0.011$	0.39	$-0.0147 \pm 0.019$	0.45
MAP	$-0.003 \pm 0.001$	<b>0.006</b>	$0.079 \pm 0.026$	<b>0.004</b>	$-0.110 \pm 0.051$	<b>0.036</b>
RMSSD	$-0.001 \pm 0.001$	0.21	$0.036 \pm 0.026$	0.17	$-0.054 \pm 0.041$	0.19
SDNN	$-0.001 \pm 0.001$	0.35	$0.041 \pm 0.032$	0.20	$-0.004 \pm 0.060$	0.95
STV	$-0.001 \pm 0.004$	0.87	$0.14 \pm 0.11$	0.21	$0.29 \pm 0.21$	0.18
LF	$-0.021 \pm 0.032$	0.51	$0.798 \pm 0.882$	0.37	$0.321 \pm 1.756$	0.86
HF	$-0.024 \pm 0.042$	0.57	$0.983 \pm 1.132$	0.39	$-0.099 \pm 2.290$	0.97
HFnu	$0.02 \pm 0.10$	0.83	$0.086 \pm 2.873$	0.98	$-4.57 \pm 5.15$	0.38
FSI	$-0.003 \pm 0.001$	<b>&lt;0.001</b>	$0.056 \pm 0.022$	<b>0.015</b>	$-0.065 \pm 0.037$	0.087
Amplitude	$-0.0002 \pm 0.0004$	0.64	$0.005 \pm 0.008$	0.52	$0.016 \pm 0.019$	0.42
Slope	$-0.010 \pm 0.006$	0.070	$0.104 \pm 0.119$	0.39	$-0.127 \pm 0.311$	0.69
TAD*	$-0.007 \pm 0.006$	0.28	$0.19 \pm 0.16$	0.24	$0.34 \pm 0.25$	0.19

\*for the increase of 10000 units of TAD.

FHR = fetal heart rate, MAP = mean arterial pressure, RMSSD = root mean square of successive differences, SDNN = standard deviation of normal to normal R-R intervals, STV = short-term variation, LF = low frequencies, HF = high frequencies, HFnu = normalized high frequencies, FSI = fetal stress index, TAD = total area of deceleration