Fetal heart rate variability with hypoxemia in an instrumented sheep model

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Abstract

Objective

We examined the effect of hypoxemia on fetal heart rate variability using the instrumented fetal sheep model.

Methods

In this prospective study, 19 pregnant sheep were instrumented under general anesthesia at a mean gestational age of 127 days. After a 5-day recovery, hypoxaemia was induced by attaching the mother to a re-breathing circuit. Hypoxemia was further extended till 120 minutes, following which it was reversed till matenal and fetal pO_2 returned back to baseline. The heart rate recordings at baseline, hypoxemia of 30 and 120 minutes, and recovery were analysed to calculate short term variation (STV) in 16 epochs of 3.75sec each, every minute. Phase rectified signal averaging (window length L= 10, time T= 2 and Scale S=T) was used to calculate acceleration capacity (AC) and deceleration capacity (DC).

Results

At baseline, mean (SD) fetal pO_2 was 2.90±0.38 kPa. Acute hypoxaemia was associated with a significant reduction in mean pO_2 at 30 (1.60±0.37 kPa) and 120 (1.50±0.16 kPa) minutes. Mean (SD) fetal pO_2 at recovery was 2.80±0.32 kPa. The median STV, AC and DC were 1.307 msec (IQR: 0.515 to 2.508), 1.295 (IQR: 0.990 to 2.685) BPM and 1.197 (IQR: 0.850 to 1.836) BPM respectively, at baseline. With 30-minute hypoxaemia, the values were 1.323 (IQR 0.753 to 2.744) msecs, 1.696 (IQR: 1.310 to 3.013) BPM & 1.584 (IQR 1.217 to 4.132) BPM. With 120-minute hypoxaemia, the values were 1.760 (IQR: 0.928 – 4.656) msecs, 3.098 (IQR: 1.530 – 5.163) BPM & 3.054 (IQR: 1.508 – 4.522) BPM. At recovery they changed to 0.962 (IQR: 0.703 – 1.154) msecs, 1.228 (IQR: 1.071 – 2.234) BPM & 1.086 (IQR: 0.873 – 1.568) BPM respectively. Hypoxemia for 30 and 120 minutes were associated with a significant increase in the DC compared to baseline ($p = 0.014$ & 0.017 respectively). The changes in STV and AC were not significant.

Conclusion

Acute hypoxaemia is associated with a significant increase in the deceleration capacity of the fetal heart rate.

Introduction

There are several tests available for antenatal assessment of fetal wellbeing, and the analysis of the fetal heart rate and rhythm is one of them. Fetal hypoxemia is reported to be associated with initial increase followed by a reduction in heart rate variability in fetuses of pregnant ewe subjected to placental embolisation[1]. On the other hand, in a pregnant sheep model where hypoxaemia was induced by repeated intermittent umbilical cord compression, Green et al[2] reported that high variability episodes of the fetal heart are less commonly observed with fetal hypoxemia, and only after four days. A commercial system that uses a computer to analyse antenatal fetal heart tracing is available[3], and is used clinically for decision making to decide the time of delivery in early onset fetal growth restriction[4]. This commercial system acquires fetal heart rate using a trans-abdominal Doppler transducer. Variation between the interval of successive heart beats (short term variation, STV) is calculated in 16 epochs of 3.75 seconds each, every minute, and expressed as milliseconds[3]. Phase-rectified signal averaging (PRSA), is a relatively new technique, which synchronizes the phase of all periodic components of a noisy, non-stationary signal irrespective of their frequencies or characteristic time scales. It has been used for computerized assessment of the fetal heart rate variability antenatally[5-7] as well as intrapartum[8].

In this study, using an instrumented pregnant fetal sheep model, we explored the effect of acute hypoxemia induced by maternal hypo-oxygenation on the fetal heart rate and its variability.

Material and methods

The study protocol was reviewed and approved by the National Animal Experiment Board of Finland (ESAVI/1007/04.10.07/2014). The animal care and experimental procedures were conducted according to the national legislation and the EU Directive 2010/63/EU.

Intrumentation

Data from 19 instrumented pregnant sheep were used for this report. Surgery was performed at 119–128 gestational days (term 145 days, 0.88 of term). The details of the surgical technique have been described before[9]. Ewes were fasted overnight and pre-medicated with intramuscular ketamine (2 mg/kg) and midazolam (0.2 mg/kg). The maternal external jugular vein was cannulated and Ringer's lactate solution was infused at a rate of 200 ml/h. General anaesthesia was induced with intravenous Propofol (4–7 mg/kg) and maintained with Isoflurane (1.5–2.5%) in an oxygen–air mixture delivered via an endotracheal tube. Intravenous boluses of fentanyl (0.05–0.15 mg) were administered as required.

A laparotomy was performed under general anesthesia and endotracheal intubation. The fetal head and neck were delivered through a small uterine incision. Catheters were introduced in the internal jugular vein and the carotid artery to allow access to arterial venous circulations and to collect blood samples. A catheter was anchored to the fetal skin to measure amniotic fluid pressure. After the replacement of amniotic fluid by 0.9% warm saline and closure of the surgical wounds, all catheters and probes were tunnelled subcutaneously and exteriorized through a small skin incision in the ewe's flank. Postoperative analgesia was provided with a fentanyl patch 50 mg/h attached to the ewe's tail, with additional intramuscular injections of fentanyl 1.5 to 2 mg/kg twice daily. Throughout the recovery period of 4-5 days, the ewes received daily intravenous infusions of one liter of Ringer's lactate solution with Ampicillin 1 g, and the fetuses were given intravenous injections of benzyl penicillin 1 000 000 IU. After a 4-day recovery at 123–132 gestational days, general anaesthesia was induced again as described above. A 16-gauge polyurethane catheter was inserted into the maternal descending aorta through a femoral artery. Thereafter, the ewe was placed supine with a left lateral tilt and allowed to stabilize for 30 min before the baseline measurements. Thereafter, maternal hypoxemia was induced by replacing inhaled oxygen with medical air in the re-breathing circuit to further decrease fetal pO_2. Maternal oxy-hemoglobin saturation was kept at the

level of 75–80% for 20 min before the data collection for the hypoxemia phase was started and was maintained at this level during the data collection. Hypoxaemia was continued and another set of data were collected at 120 minutes of hypoxaemia. Thereafter, maternal hypoxaemia was reversed and the last set of data were collected. At the end of the experiment, the animals were euthanized with an intravenous overdose (1.0 mg/kg) of pentobarbital sodium. Fetal weight was recorded.

Fetal heart rate analysis

Fetal blood pressure and amniotic fluid pressure were measused continuously using pressure transducers (model DT-XX, Ohmeda, Hatfield, UK) at a sampling rate of 100 Hz using a polygraph (model UIM100A, Biopac Systems, Santa Barbara, CA) and computerized data acquisition software (Acqknowledge version 4.0 for Windows, Biopac Systems). Fetal blood pressure was referenced to amniotic fluid pressure. Fetal heart rate (FHR) was obtained from the arterial pressure by measuring the peak to peak intervals in the pressure waveforms (Figure 1). Self-developed program in Matlab (2017b, Mathworks, Nattick, MA, USA). was used for all analyses of the traces.

Disturbances in the traces at the times of collection of fetal blood samples were manually selected and excluded. The heart rate recordings at baseline and hypoxaemia were analysed to calculate short term variation (STV) in 16 epochs of 3.75 sec each, every minute after exclusion of accelerations and decelerations. The PRSA methods has been described in detail elsewhere[6, 7, 10, 11]. The PRSA algorithm for the time series of the FHR was created using Matlab (MATLAB 2018b, The MathWorks, Inc., MA). The first step was to filter the atrial pressure wave and de-trend the signal. Next step was to find peaks in the pressure wave-form to identify the RR intervals. Next step was to create epochs from the RR intervals in 3.75 second segments. Then we identified acceleration and deceleration in the signal as anchor points. When all anchor points were identified by the algorithm, a window is selected surrounding each anchor point. All data from the windows are aligned at every anchor point in one common window. From this common window the acceleration part (AC) and deceleration part (DC) can be calculated. The calculation of AC and DC depend of three parameters: L, T and S and need to be specified. L is the length of the window surrounding

each anchor point. We have chosen L=50 in this study of the time series. T sets the low-pass filter value and is used when detecting the anchor points in the signal. The S value changes the settings of the oscillations when calculating the acceleration & deceleration capacity. In our study we used S=T. (Please see appendix for details of calculating STV, AC & DC).

PRSA (Phase rectified signal averaging, window length L= 10, T= 2 and Scale S=T) was used to calculate average acceleration capacity (AC) and average deceleration capacity (DC).

The animals in this experiment were under the effect of anaesthesia. Therefore, anaesthetics may play a role in modifying the fetal heart rate and its variability. In order to assess the effect of anaesthesia, we studied the heart rate and its variability of two animals before and after anaethetic was administered.

Statistical analysis

Normality of the data was checked using Kolmogorov –Smirnoff test. Paired 't' test was used to compare diffrences between baseline and hypoxemia for normally distributed data. Wilcoxon signed rank test was used for the data with a non-normal distribution. SPSS v22 was used for statistical analysis. $p<0.05$ was considered as statistically significant.

Results

The mean gestational age, fetal weight and signal duration of the experimental animals are shown in Table 1. All 19 fetuses survived hypoxaemia for 30 minutes. Only 11 of the 19 animals survived hypoxaemia for 120 minutes. Seven fetuses were alive till the end of the experiment.

The results are summarised in Table 2. All the parameters except fetal serum lactate, STV, AC and DC were normally distributed. Mean (SD) Fetal pO_2 was 2.90±0.38 kPa and median (IQR) lactate were 1.70 (1.57 to 2.95) mM/L respectively at baseline. Hypoxaemia was associated with a significant reduction in pO_2 (1.62±0.37 kPa at 30 minutes and 1.51±0.16 kPa at 120 minutes, $p < 0.001$ for both). Fetal serum lactate levels were significantly higher at hypoxemia for 30 min, hypxaemia for 120 min and also at recovery ($p = 0.002$, $p = 0.008$ and $p = 0.018$ respectively, Related samples Wilcoxon signed rank test). Fetal pCO_2 did not change significantly over the study period. Fetal pH was significantly lower ($p = 0.013$) at recovery stage as compared to baseline (Table 2). Fetal heart rate was significantly lower at recovery phase as compared to baaseline ($p = 0.001$, paired sample 't' test).

At baseline, the median STV, AC and DC were 1.307 msec (IQR: 0.515 to 2.508), 1.295 (IQR: 0.990 to 2.685) BPM and 1.197 (IQR: 0.850 to 1.836) BPM respectively. Changes in STV, AC and DC with progressive hypoxaemia and recovery are seen in Table 2. Hypoxemia for 30 and 120 minutes were associated with a significant increase in the DC compared to baseline ($p = 0.014$ & 0.017 respectively). The changes in STV and AC were not statistically significant.

The effect of anaesthesia was studied in two animals before and after anaethetic was administered. The results are shown in Table 3. Reduction was seen in baseline heart rate, STV, AC and DC with anaesthesia in each of the two animals studied. No statistical tests are possible to the limited number.

Discussion

In this experiment, acute hypoxemia caused by maternal hypo-oxygenation was associated with significant an increase in fetal lactate, whereas the fetal pH remained relatively unchanged. The short-term variation as well as acceleration capacity did not change. Deceleration capacity showed a significant increase with acute hypoxaemia.

Fetal heart rate vaiability has been used for fetal monitoring in compromised pregnancies[4, 12]. Fetal heart variability is thought to be under the control of sympathetic and parasympathetic systems, but the exact mechanism of control remains unclear[13]. Chemical sympathectomy led to a reduction in fetal heart rate variability as compared to controls, but it did not alter increase in fetal heart rate variability in response to acute hypoxaemia resulting from umbilical cord occlusion[14]. Changes in sympathetic and parasympathetic influences on the heart rate are antagonistic in the fetus, but synergistic in the newborn[15].

Response to hypoxemia may vary depending on the cause and duration of hypoxemia[16]. Reduced STV has been reported in chronic hypoxemia caused by placental insufficiency[4, 12, 17]. Increased variability has been reported in hypoxemic human fetuses during intrapartum fetal monitoring[8, 18].

Murotsuki et al[1] reported on 1-hour short term variation of sheep fetuses in a group where the fetuses were exposed to hypoxia by placental embolization and compared it to fetuses with an intact placenta. The STV showed a significant increase within hours of hypoxia due to placental embolization. The mean 1-hour STV in the two groups of sheep fetuses were 6-9 msecs and 9-11 msecs in the control and embolised group of sheep fetuses respectively. In our study the STV did not change significantly with hypoxemia. The method of inducing hypoxia (maternal hypoxemia using a re-breathing circuit) in our study was different from that reported by Murotsuki et al. Moreover, the mean STV values in the current study were significantly lower as compared to that reported by Murotsuki et al (embolisation of the abdominal aorta with microparticles). The gestational age of the fetuses in Muroksuki report (104-106 days) was lower as compared to the present one (127 days). The fetal heart rate was recorded by electrodes placed on the fetal chest to obtain the ECG signal as opposed to arterial pressure waves in the current study. Animals in the Murotsuki report were not anaesthetised, whereas they were under the effect of general anaesthesia in order to perform

echocardiographic examinations in the present study. All these differences may explain why our results differ from that reported by Murotsuki at al. The number of fetuses in the present study (n = 19) was more than that of Murotsuki (n = 12). Therefore, negative results are unlikely to have resulted from the study being underpowered.

Similar findings in human fetuses have been reported by Georgieva et al[8]. They report their findings form the intrapartum cohort. PRSA DC was better than STV in the detection of fetuses with low cord pH. Intrapartum period is likely to be associated with acute hypoxemia, and their report supports the findings of current study, although they have not reported absolute PRSA DC values. Huhn et al[11], reported on STV and AC in a cohort of growth restricted fetuses and compared the values to a cohort of normally grown fetuses. They reported that both STV and AC were both significantly lower in growth restricted fetuses, although there was no significant difference in the cord arteral pH of these newborns as compared to normal fetuses. Their results are contradictory to that from the current study, but the fetuses were exposed to chronic rather than acute hypoxia. The absolute values of AC in the growth restricted and normally grown fetuses were 1.97 bpm and 2.49 bpm respectively. These values are similar to the ones in the present report, although the Huhn´s study was carried out on un-anaesthetised human fetuses. Rivolta et al[10] reported on the fetal heart rate AC and DC in response to mild, modereae and severe hypoxaemia caused by intermittent umbilical occlusions. The AC and DC were increased progressively with increasing degree of hypoxaemia. The R-R interval was calculated from the fetal ECG signal in this report. The results of the current study are in keeping with the Rivolta study.

Availability of fetal blood gas analysis, controlled level of hypoxemia and continuous access to fetal circulation are strengths of our study. The level of hypoxemia could be standardised and measured, which is not possible in human observational studies. Repeated fetal blood gas analyses and their correlation with fetal pO_2 is difficult in human setting. We utilised algorithm very similar to the one available in the commercial system (Oxford 8000 system) for consistency.

The main weakness is that the animals were under the influence of anaesthetic agents. This may have had an influence on the fetal heart rate variability. The study protocol involved examination of the fetal echocardiography with ultrasound, which is not possible in operated

pregnant ewes without anaesthesia. We studied the effect of anaesthetic on two animals by collecting the heart rate data on awake and anaethetised animals. Although the number is limited, the effect is of anaesthetic administration was a reduction in STV, AC and DC in each of the two animals. Each fetus was used as its own control, ensuring similar effects of the anaesthetic agents at baseline and with hypoxia. Had there been no difference found between normoxic and hypoxic phases, the negative finding could have been attributable to the effect of anaesthesia. However, a significantly increased DC was seen even in anaesthetised animals in this study. The results of this study are valid for these experimental conditions. It cannot be concluded that, at other degree of hypoxemia the short time variability of the fetal heart rate will not change.

Access to fetal circulation is not possible without instrumentation, which required surgery prior to the experiment. The surgical procedures may constitute a significant stress on the sheep fetuses, and it may be argued that the conditions are quite different from human fetuses exposed to hypoxaemia. Normal arterial blood gas values at the baseline phase suggest conditions close to physiologic circulatory state[19]. The study was carried out in a narrow gestational age window of 123-132 days, and may limit the validity and significance outside this time period. Extrapolation of these results to human pregnancy should be done cautiously. However, the sheep model has been extensively used for research in fetal hemodynamics.

In conclusion, acute hypoxaemia is associated with a significant increase in the deceleration capacity of the fetal heart rate in the instrumented fetal sheep model. This information will be applicable to detection of fetal compromise with acute hypoxia.

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Conflicts of Interest notification: None of the authors have any conflicts of interest to declare.

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Figure legend

Figure 1. Data capture on the polygraph. The top panel shows arterial pulses from the fetal carotid artery.

Foot note: Peak to peak pulse interval was measured in successive peaks, and averaged over 3.75 sec epochs assess fetal heart rate variability.

Table 1. Baseline characteristics of the study participants

Parameter	Mean/Median	SD/IQR
Gestational age in days	127	2.9
Fetal weight in grams	2466	301
Total duration of signal (minutes)	67.7	55.4 to 80.4
Duration of signal loss (minutes)	4.9	4.4 to 12.8

SD = Standard deviation, IQR = Interquantile range

Table 2. Fetal blood gases and Fetal heart rate parameters

Parameter	Baseline n = 19	Hypoxaemia (30 min) n = 19	Hypoxemia (120 min) n = 11	Recovery n = 7
Fetal heart rate (BPM)	174±28	171±30	163±34	141±30
Fetal pO_2 (kPa)	2.90±0.38	1.62±0.37#	1.51±0.16#	2.86±0.32
Fetal pCO_2 (kPa)	6.93±1.12	6.81±0.83	6.86±1.03	6.77±0.50
Fetal pH	7.294 ±0.060	7.295±0.044	7.236±0.250	7.193±0.060
Fetal base excess (mM/ml)	-1.33±3.34	-1.71±3.46	-10.33±6.59	-9.14±3.93
Fetal lactate (mM/ml)	1.70 (1.57 to 2.95)	3.20 (2.41 to 4.97)	9.23 (5.14 – 13.81)	9.25 (4.70 – 14.0)
STV (msec)	1.307 (0.515 to 2.508)	1.323 (0.753 to 2.744)	1.760 (0.928 – 4.656	0.962 (0.703 – 1.154
Acceleration Capacity (BPM)	1.295 (0.990 to 2.685)	1.696 (1.310 to 3.013)	3.098 (1.530 – 5.163)	1.228 (1.071 – 2.234)
Deceleration Capacity (BPM)	1.197 (0.850 to 1.836)	1.584* (1.217 to 4.132)	3.054* (1.508 – 4.522)	1.086 (0.873 – 1.568)

*Statistically significat compared to baseline (Wilcoxon Signed Ranks Test). # $p<0.001$ (paired sample 't' test).

STV: Baseline against Hypoxaemia (30 min): $p = 0.314$; Baseline against Hypoxaemia (120 min): $p = 0.285$; Baseline against Recovery: $p = 0.735$.

Acceleration capacity: Baseline against Hypoxaemia (30 min): $p = 0.335$; Baseline against Hypoxaemia (120 min): $p = 0.139$; Baseline against Recovery: $p = 0.310$

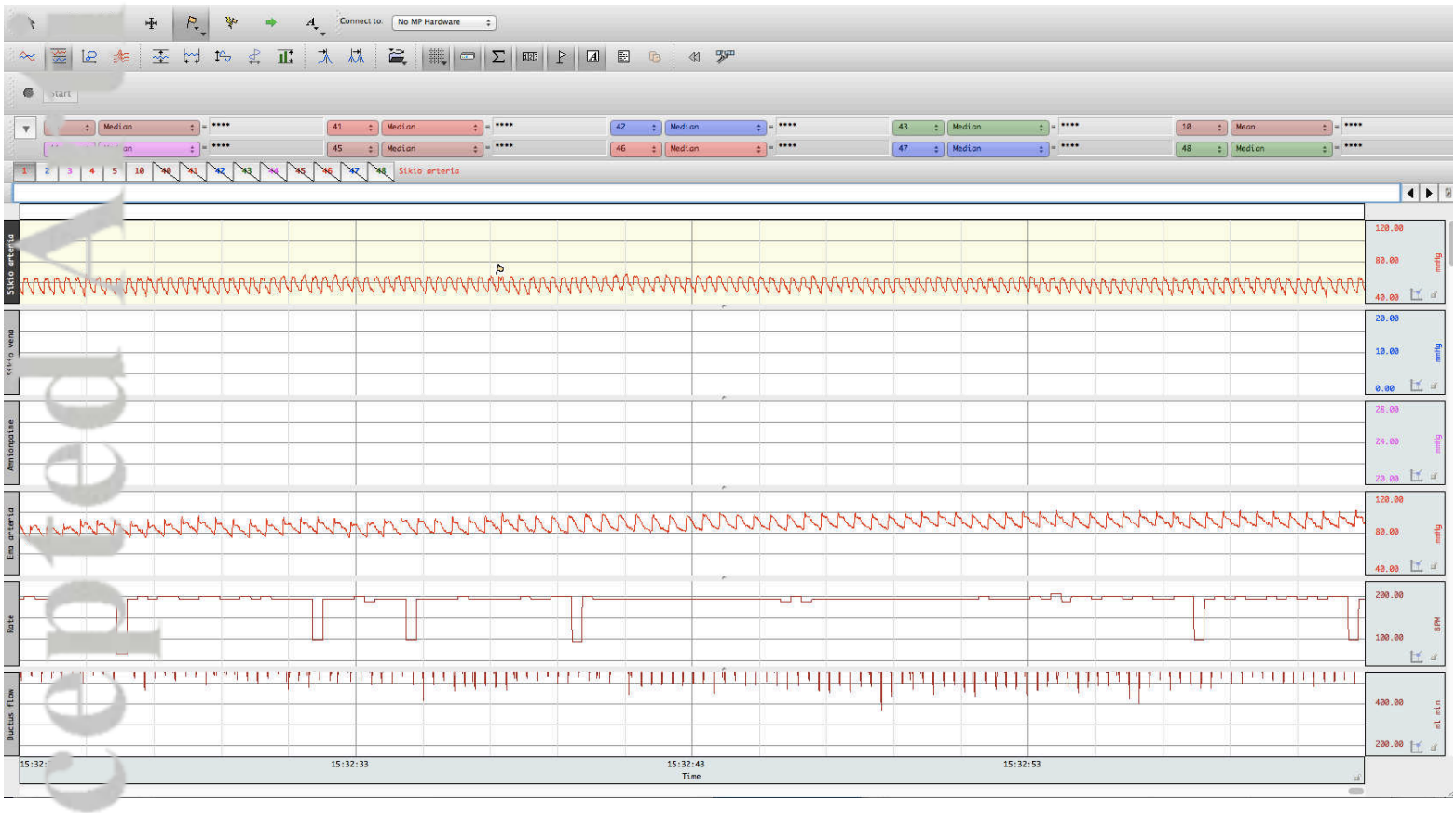
Deceleration capacity: Baseline against Hypoxaemia (30 min): **p = 0.014**; Baseline against Hypoxaemia (120 min): **p = 0.017**; Baseline against Recovery: p = 1.00

Table 3. The effect of anaesthesia on fetal heart rate and its variability assessed by computerised CTG.

Parameter	Animal no.	Awake	Anaesthetised
Fetal heart rate (BPM)	1	146	131
	2	169	119
STV (msec)	1	6.24	2.73
	2	3.63	2.87
Acceleration Capacity (BPM)	1	5.68	1.47
	2	4.87	2.41
Deceleration Capacity (BPM)	1	6.08	1.48
	2	4.01	2.43

No statistical tests have been performed due to the limited number of animals.

BPM = beats per minute, STV = Short-term variation, msec = milliseconds



UOG_20259_Fig. 1. Polygraph display.png