Evaluating the reliability of fetal electrocardiogram monitoring that acts via the abdominal wall of pregnant rabbits

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Abstract. A next generation fetal electrocardiogram (ECG) that can assess fetal well-being accurately is required in clinical settings. We developed a fetal ECG monitoring system that acts via the maternal abdominal wall and measured the ECG in our clinical studies. To assess the accuracy of the clinical fetal ECG monitoring records, it was necessary to confirm that the waveforms obtained by the indirect lead had the same accuracy as those by the direct lead. This study is translational research in which a murine fetal ECG system with the direct lead that we had already developed was applied and pregnant rabbits that had enough space to place electrodes on the maternal abdominal surface were used. In this study, fetal ECG was measured using the direct and indirect lead simultaneously using pregnant rabbits. The R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead of the same fetus was used to determine the reliability of the clinically developed fetal ECG monitoring system. The fetal ECG waveform, both with the direct and indirect lead, was obtained from three out of five pregnant rabbits. The average R-squared value was 0.99. Although one of the three pregnant rabbits presented with an atrioventricular block during the measurement, the fetal ECG waveform was successfully extracted with both the direct and indirect lead. The results of this study demonstrate that the fetal ECG monitoring system that acts via the maternal abdominal wall has the same accuracy as that of the direct lead.

Key words: fetal-electrocardiogram, reliability evaluation, direct lead, indirect lead, rabbit

Highlights

We evaluated the reliability of a fetal electrocardiogram that acts via the maternal abdominal wall of rabbits. We used three pregnant rabbits to measure and compare the direct and indirect ECG of the same fetus simultaneously. The R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead of the same fetuses in three pregnant rabbits was 0.99. The results of this study demonstrate that the fetal ECG monitoring system that acts via the maternal abdominal wall has the same accuracy as that of the direct lead.

Introduction

A cardiotocometer, a medical device that detects fetal heart rates using noninvasive ultrasound and calculates them consecutively, is widely used in clinical settings. The device observes the longitudinal fetal heart rate and estimates fetal well-being. With the device, the health of the fetal can be deduced; however, fetal malfunction cannot be detected. Consequently, unnecessary cesarean sections have occurred due to the false-positive rate. In addition, the device has not contributed to a reduction in the rate of cerebral palsy.

Electrocardiogram (ECG) waveforms were first recorded by Dutch physiologist Willem Einthoven in 1903. Since then, research has been focused on the development of devices to measure the electrical activities of fetal hearts [1]. Unfortu-
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nately, a practical fetal ECG monitoring system has not been developed as of yet. However, advances in technology have enabled fetal ECG to measure heart rate via the maternal abdominal wall. We developed a fetal ECG system that acts via the maternal abdominal wall to monitor and measure fetal ECG waveforms in our clinical studies [2].

As the system is practical in the clinical setting, the measurement data should be accurate enough for clinical demands. We must understand how the accuracy of the ECG monitoring system that acts via the maternal wall compares to that of the direct lead. A fetal ECG can be obtained by the direct lead in mice [3], but mouse maternal abdomens do not have enough space to place an adequate number of electrodes for the measurement of the fetal ECG waveforms. As a result, in mice, the accuracy of fetal ECG waveforms obtained via the maternal wall cannot be assessed by comparing those with the direct lead. Therefore, we used rabbits for this translational study from our mouse studies and to our clinical studies. The aim of this study was to assess the accuracy of the ECG waveform output of a fetus simultaneously measured using the direct and indirect lead in pregnant rabbits. The R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead of the same fetus was used to determine the reliability of the clinically developed fetal ECG monitoring system.

**Materials and Methods**

This study was approved by the Animal Research Committee of Tohoku University (approval number: 2011 idou-59).

**Animals**

Five pregnant rabbits (Kbs: JW; Kitayama Labels Co., Ltd., Ina, Japan) at 24 days were used in this study. All rabbits were exposed to a suitable environment for acclimatization (a 12-hr light/dark cycle at 24°C) before experiments.

**Anesthetic management**

Pregnant rabbits were anesthetized intramuscularly with ketamine (10 mg Ketalar/kg; Daiichi Sankyo, Tokyo, Japan) and xylazine (1.5 mg Seractar/kg; Bayer Pharmaceutical Co., Ltd., Tokyo, Japan). After intubation according to an intubation protocol by Morgan and Glowaski [4], maintenance of anesthesia by mask inhalation was achieved using 2% isoflurane (2 L Forane/min; Abbott Japan Co., Ltd., Osaka, Japan) and room air. The airway pressure was set at <15 mmHg and ventilation frequency was set to 20 breaths/min using mechanical ventilation (ARF-900; ACOMA Medical Industry Co., Ltd., Tokyo, Japan).

**Direct ECG monitoring**

After assuring that the level of anesthesia was stable, the uterus was exposed by a maternal abdominal midline incision. One fetus was chosen to measure ECG waveforms for each pregnant rabbit. A pair of needle electrodes were inserted into the uterus and fixed, with one tip located near the fetus shoulder and the other tip located at the fetus chest. The electrodes were used for the direct ECG monitoring. The signals obtained via an in vivo monitor (BSM2303; Nihon Koden, Tokyo, Japan) were recorded in the external channel of the fetal ECG monitor via maternal abdominal wall (Monitor 138: Atom medical) as Apex-Base bipolar lead (Fig. 1).

**Fig. 1.** Schematic image of fetus electrocardiogram (ECG). For the direct ECG, a pair of needle electrodes were inserted into the uterus and fixed, where one tip located near the target fetus’s abdomen (A) and the other tip located to the shoulder (B). The signals obtained via an in vivo monitor were entered into and recorded in the external channel of the monitor 138. For the indirect ECG, thirteen plate electrodes (1)–(13) were fixed on the maternal abdomen. The electrode (2) was fixed on the target fetus. The electrodes (3), (4) and (5) were fixed around the fetus. Other electrodes (6)–(12) were fixed randomly arranged. The signals were entered into and recorded in the external channel of the monitor 138 simultaneous with the direct ECG monitoring.
Indirect ECG monitoring via the maternal abdominal wall

Immediately after the fixation of the needle electrodes for direct monitoring, abdominal closure was carried out. Thirteen plate electrodes for the indirect ECG monitoring were fixed on the maternal abdomen. The signals from the indirect leads were recorded in the Monitor 138 simultaneously.

Accuracy assessment of indirect fetal ECG monitoring

Fetal cardiac electric potential obtained by the direct lead and via the maternal abdominal wall in the same fetus was obtained in the objective device at a 1,000 Hz sampling rate for 5 min. The R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead of the same fetus was used to determine the reliability of the clinically developed fetal ECG monitoring system.

Results

We successfully measured the fetal ECG waveform of pregnant rabbits both with the direct and indirect monitoring systems, as we could with pregnant mice (Table 1). The maternal abdomen of the pregnant rabbits had enough space for placing an adequate number of electrodes. Five pregnant rabbits were used in this study. In three pregnant rabbits, extracted fetal ECG waveforms obtained via the maternal abdomen wall were observed to be the same as those obtained with the direct lead (Fig. 2). In the other two pregnant rabbits, the fetal ECG waveforms could be measured by direct lead until the maternal abdomens were closed. However, the direct lead waveforms could not be extracted after the closure.

The R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead of the same fetuses in three pregnant rabbits was 0.99 (Fig. 3). Although one of the three pregnant rabbits presented with an atrioventricular block during the measurement, fetal ECG waveforms were successfully extracted from both the direct and indirect lead and showed abnormal characteristics including elongated PR-interval, ST reduction, and dropped QRS complex (Fig. 4).

Discussion

In 1980, it was attempted to calculate and monitor the fetal heart rate from a fetal ECG obtained via the maternal abdominal wall [5]. Accordingly, the history of fetal ECG is a history of the development of data processing technology because the development of fetal ECG continuously utilizes up-to-date technologies such as signal-averaging, filtering, adaptive filtering, Wiener filtering, and independent component analysis. However, bandpass filtering causes RR-interval error due to shape distortion. Adaptive filtering increases signal distortion due to convolution and noise.

### Table 1. Indication of simultaneous measurement

| Fetus ID | Direct ECG | Indirect ECG | R² value |
|----------|------------|--------------|----------|
| 1        | Simultaneous fetus waveform extraction | Fetus waveform extraction | 0.999 |
| 2        | Measurement failure due to artifacts | Fetus waveform extraction | - |
| 3        | Measurement failure due to artifacts | Fetus waveform extraction | - |
| 4        | Simultaneous fetus waveform extraction | Fetus waveform extraction | 0.997 |
| 5        | Simultaneous fetus waveform extraction | Fetus waveform extraction | 0.960 |
| Average  |            |              | 0.99     |

ECG, electrocardiogram.

Fig. 2. (a)–(c): Direct and indirect fetus electrocardiograms (ECGs). The upper ECGs are fetus waveforms obtained by the indirect lead and the lower ECGs are by the direct ECG. The range of the x-axis is changed depending on the fetus heart rates. (a) Fetus ID No. 1, (b) Fetus ID No. 2, (c) Fetus ID No. 5.
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Transient high noise levels cause accuracy deterioration of the ECG signal.

However, independent component analysis as a desirable signal extraction method under loud noise was developed by Jutten et al. in 1991 [6]. Since 2000, more research that shows the application of independent component analysis to the extraction of fetal ECG waveforms have been reported due to its applicability in a situation in which the signal-to-noise ratio is less than 1 [7]. However, some difficulties, including the stability of the fetal ECG extraction, limit the clinical application of this method. Therefore, researchers are still searching for the technical development for a novel fetal ECG. In one study, blind source separation with the reference signal (BSSR) is used to stabilize the independent component analysis [8].

In this study, 13 electrodes could be fixed on the pregnant rabbit abdomens as on the human’s in clinical setting. Moreover, we demonstrated that fetal ECG waveforms obtained with indirect leads were accurate as those with direct leads. The average R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead with BSSR of the same fetuses in pregnant rabbits was greater than 0.9, which showed a high correlation coefficient.

Electrocardiogram measurement of a human fetus requires 13 electrodes to deal with the fetal movement. In this study using pregnant rabbits, the electrocardiographic waveform of a fetus could be detected with a pair of electrodes placed near the fetus (Fig. 1). Only a pair of electrodes were needed for the measurement because there was little chance of fetal movement disturbing the measurement due to the short periods of measurement, namely 5 min, and the spatial limitation by the multiple pregnancy. Concomitantly, ECGs of multiple fetuses can be simultaneously measured if the electrodes are fixed in an appropriate manner.

Mobitz type I atrioventricular block could be diagnosed as the PR-interval gradually extended and the R wave disappeared eventually (Fig. 4). The cardiotocograph used in the clinical setting cannot classify such fetal arrhythmias because ultrasound cannot detect the electrical excitation of the myocardium. As a matter of fact, rabbits are commonly used for reproductive toxicity studies. Classification of fetal myocardium dysfunctions in rabbits will contributes to the
development of fetal medicine.

Cardiotocograph used in the clinical setting cannot detect the risk of fetal cerebral hemorrhage [9] because an average of 5 RR-intervals can be calculated from cardiotocography data. One RR-interval should be obtained to diagnose fetal malfunction. Although we successfully calculated fetal RR-intervals per every one heartbeat from indirect fetal ECG in our clinical studies using the same test device used in this study, it is unknown whether the system can detect the risk of fetal cerebral hemorrhage [8]. In a mice study, we estimated fetal autonomic nervous activities, a risk indicator of onset and severe outcome of cerebral hemorrhage, which is a cause of cerebral palsy, by subtracting fetal RR-intervals obtained from indirect ECG [3]. This study using rabbits showed that the ECG system with the indirect lead is reliable as that with the direct lead. Consequently, this translational research using rabbits shows that the clinical fetal ECG system using the indirect lead can detect the risk of fetal cerebral hemorrhage as the mouse fetal ECG system using the direct lead could.

In this study, both direct and indirect fetal ECGs successfully showed abnormal characteristics. Consequently, fetal malfunction including arrhythmia and cerebral hemorrhage could be diagnosed using an ECG that acts via the maternal abdominal wall, as in adults.

Conclusions

The average R-squared value between the RR-intervals obtained by the direct lead and those by the indirect lead with BSSR of the same fetuses in pregnant rabbits was greater than 0.9, which showed a high correlation coefficient. Our signal analysis for fetal ECG was demonstrated to be reliable in this study.

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