

Non-invasive measurement of chick embryo cardiac work

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ABSTRACT: This study used a non-invasive method of ballistocardiography to investigate cardiac work of chick embryos. In this method, an eggshell with electric charges on it is one capacitor plate, the other being a receiving antenna of the measuring equipment. Chick embryo cardiac work induces micro-movements of the whole egg, resulting in changes in the distances between the plates and thus in the difference of potentials between the shell and the receiving antenna. This is registered by the measuring equipment. The first single signals of cardiac work were registered on day 7 of incubation. Starting from day 9, the signal was recorded from all embryos. During the study, the heart rate decreased from 248 to 161 beats per minute and signal amplitude was found to steadily increase from 6.3 to 432.7 mV/m. Great disturbances in ballistocardiograms were observed on days preceding embryonic deaths.

Keywords: ballistocardiography; embryogenesis; avian embryo

Embryonic development is an important stage in the life of birds as it has a major effect on the biological value and performance of adult birds. The monitoring of embryonic development in birds is therefore highly desirable. One of the first signs of activity of an organism developing in the egg is cardiac work.

In the embryonic development of the chick, a primordial heart forms very early by the 24th hour of incubation (Bielańska-Osuchowska, 1993).

First pulsations of low frequency below 50 times per minute appear by the 29th hour of incubation (Antoni and Diliger, 1981), although blood begins to circulate at about 39th hour of hatching. This is due to the absence of links between intra-embryonic and yolk circulation (Antoni and Diliger, 1981).

Ballistocardiography is often used to study cardiac work (Lindqvist *et al.*, 1998; Benedek and Villars, 2000). This method investigates mechanical work of the heart based on graphic registration of body movements induced by the heart (Pagnacco *et al.*, 1999; Benedek and Villars, 2000).

In human ballistocardiography, Orłow (1959) found that each cycle of cardiac work induces micro-motions of the whole body (10–60 μm).

Mechanical work of the heart induces body vibrations at three levels: longitudinal, transversal and frontal. The vibrations can be registered by ballistocardiographs. A ballistocardiography technique can also be used to picture cardiac work in developing chick embryos. The mechanical impulses resulting from cardiac work make the whole embryo body vibrate, which causes micro-vibrations of the whole egg. Antoni and Diliger (1981) reported that the ejection of blood during cardiac work of an 8-day-old embryo weighing 0.2 g caused egg vibrations having an amplitude of about 8×10^{-4} mm.

Initial studies on the non-invasive method of ballistocardiography in chick embryos were carried out by Janowski and Szymański (1987, 2000) and Pawlak *et al.* (1998, 2001a,b).

These authors suggested that the antenna receiving a cardiac signal should be placed at a certain distance from the egg. In this method, the signal received from the chick embryo was very often lost in noise, which prevented interference-free registration of ballistocardiograms. Modifications to this technique by Szymański *et al.* (2002) considerably improved the sensitivity of measuring equipment, and also made the results more repeatable.

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The aim of the present study was an attempt at non-invasive registration of cardiac work of chick embryos during consecutive days of incubation.

MATERIAL AND METHODS

100 eggs from a parental flock of Rossa line (Rhode Island Red × Sussex) and 5 unfertilized table eggs were used in the study.

In the non-invasive method of chick embryo cardiac work registration, an eggshell with electric charges on it is one capacitor plate, the other being a receiving antenna of the measuring equipment. The cardiac work of chick embryo induces micro-movements of the whole egg, resulting in changes in the distances between the plates and thus in the difference of potentials between the shell and the receiving antenna. This is registered by the measuring equipment.

The measuring station consists of several blocks that fulfil different functions: Faraday cage which holds two heaters and a detector of electric field, as well as a block of amplifiers, filters and an egg polarization system, a power pack, a generator of sinusoidal signal, a vibration damper and recording equipment (analogue-digital card in a PC computer) (Szymański *et al.*, 2002; Pawlak *et al.*, 2002).

Cardiac work was measured daily at the same time, starting from egg no. 1 to egg no. 100. This was designed to retain identical time intervals between measurements. During the measurements, an egg taken out of the incubator was placed on an elastic cushion in Faraday cage.

To protect the caged embryo from a thermal stress, the cage air was heated to the incubator temperature.

The measuring antenna of the ballistocardiograph was placed 2 mm from the eggshell surface.

To minimize possible interference in ballistocardiograms due to the stress of moving an embryo into a new environment, each time measurements were made 4 minutes after the egg and embryo were placed in Faraday cage. A similar procedure was used by Cain *et al.* (1967).

After the polarization system was turned on, the signal from each egg was registered for 1.5 minutes, then the egg was put back into the incubator.

To ensure that the registered signal comes from the life function of the embryo rather than being derived from interfering signals, unfertilized eggs were subjected to measurements on each day of

the experiment. The procedure was identical to that used for eggs with live embryos.

The chick embryo cardiac signal was recorded and analysed by a computer. Digital signal processing was used to determine the number of heart contractions and the signal amplitude (Frankiewicz *et al.*, 1993).

The shape of ballistocardiograms was analysed by the methods of mathematical statistics (Statgraphics Plus, 1995) to study the relationship between the variables: amplitude (A) and heart rate (C), taking into account the weight of egg (M) and age of embryo (D). Testing was done using partial correlations. The relations between the measured values A, C, M, D were preceded by studying the normalcy of distribution according to Shapiro-Wilks test.

On day 9 of the incubation, eggs were weighed on electronic scales to the nearest 0.01 g.

Hatching was carried out in the incubator Masalles 1200-0090 TYPE 65 DIGIT. Parameters of microclimate during incubation were determined following the commonly accepted standards (Borzemska and Niedziółka, 1984).

RESULTS AND DISCUSSION

The existing methods for measuring the cardiac work of avian embryos (electrocardiography, passing a concentrated light beam through the egg with embryo, contact ballistocardiography) are invasive and subject to some error, therefore non-invasive ballistocardiography was used in the present study (Szymański *et al.*, 2002; Pawlak *et al.*, 2002).

Ballistocardiographic measurements were made from the first day of incubation. During the whole experiment, 1 814 ballistocardiographic measurements were performed. No repeatable signal that attests to cardiac work could be obtained for 6 days (Figure 1). The first cyclic signals were observed on day 7 (Figure 2) in 4 embryos. However, the signal was often lost in noise and was about the limit of equipment sensitivity. On day 8 of incubation, repeatable signals were obtained from 5 embryos. Similar problems of recording ballistocardiograms in younger embryos were faced by Suzuki *et al.* (1989). They observed that the normal and distinct signal was often subject to various interferences and sometimes even completely disappeared.

From day 9 onwards, a cardiac work signal was recorded from all fertilized eggs (Figure 3). The obtained results may attest to a considerable in-

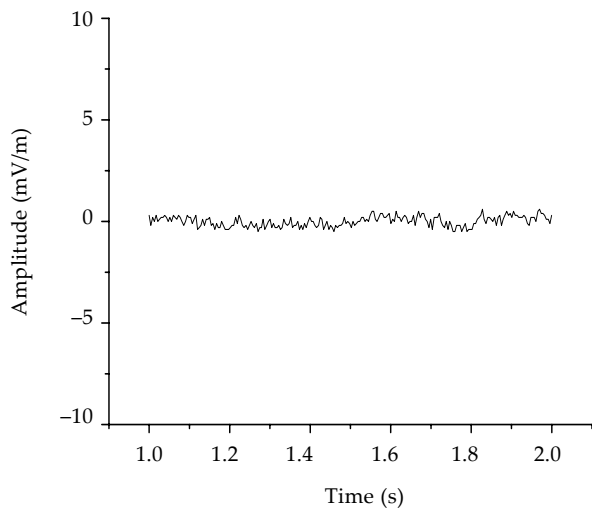


Figure 1. Signal obtained from egg with 6-day embryo

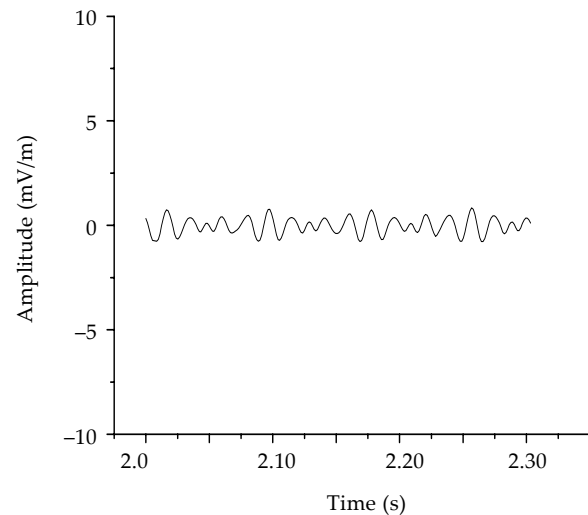


Figure 2. A sample ballistocardiogram for an embryo – day 7 of incubation

crease in the signal strength starting from day 9 of incubation. It is probably due to the fact that after eight days of incubation, the embryo produces an external circulation system in the allantoic wall that fulfils the role of the respiratory system from day nine of incubation until the adoption of lung respiration by the embryo (Borzemska and Niedziółka, 1984).

The greatest problems of measuring cardiac work were observed on the final day of incubation. Out of 52 unhatched embryos, a repeatable signal was recorded only in 39, probably due to the very high motility of embryos preparing for hatching on the

last day of incubation (Figure 4). Changes in embryo position are responsible for much greater movements of the egg than those resulting from cardiac work, which makes normal ballistocardiograms impossible (Suzuki *et al.*, 1989).

The analysis of ballistocardiograms obtained from live embryos showed a very high variation of amplitude during the measurements. The lowest amplitude was observed on day 7 of incubation. It was 1.75 mV/m and was about the limit of equipment sensitivity. Taking into account only those days in which the signal was obtained from all fertilized eggs, the lowest average amplitude was noted on

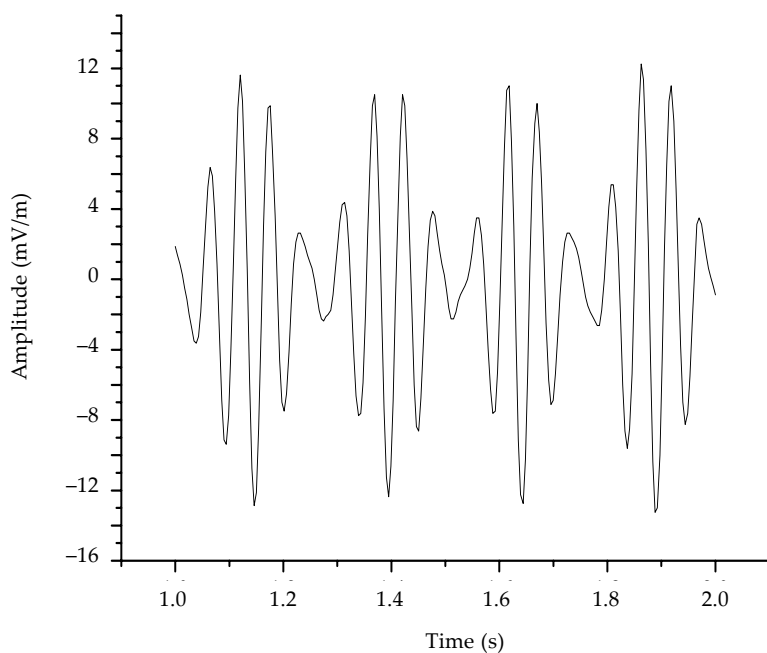


Figure 3. A sample ballistocardiogram for an embryo – day 9 of incubation

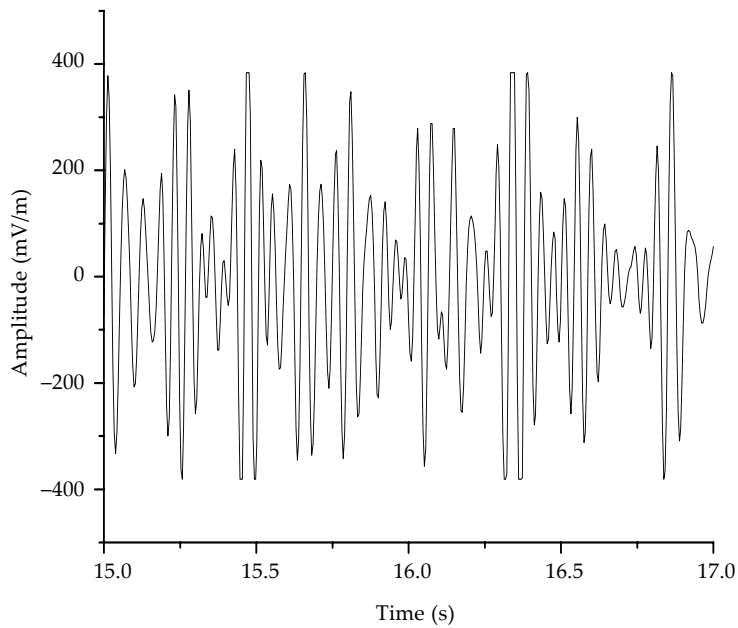


Figure 4. A sample ballistocardiogram for an embryo – day 21 of incubation

day 9 of incubation (6.3 mV/m). The analysis of amplitude size in consecutive days of embryonic development showed that it was steadily rising. The greatest leap in the signal level was found on days 20 and 21. The average value of the amplitude was 78.4 mV/m on day 19, 212.9 mV/m on day 20 and 432.7 mV/m on day 21 (Table 1).

The systematic increase in the amplitude signal was probably due to the increment in heart weight and its increased performance. As the embryo

develops, the strength of the contractile force of heart muscle and the amount of blood ejected from ventricles increase.

Based on the findings of Rahn *et al.* (1990) it is suggested that such a great leap in the amplitude value in the final days of incubation resulted from the appearance of a signal related to initiation of the embryo's lung respiration.

The number of cardiac contractions is one of the most frequent parameters used to characterize the

Table 1. Average values of the amplitude and heart rate of chick embryos according to day of incubation

Days of incubation	Amplitude		Heart rate	
	average (mV/m)	standard deviation	average (beats per min)	standard deviation
9	6.3	5.2	248	21.4
10	13.2	5.9	241	21.6
11	17.5	6.5	237	17.0
12	20.5	9.1	218	13.2
13	22.1	8.4	218	15.1
14	24.4	13.7	218	21.9
15	28.0	15.1	218	20.4
16	34.7	22.0	218	16.4
17	41.8	23.7	215	20.6
18	48.6	19.2	210	19.9
19	78.4	47.7	208	22.5
20	212.9	101.7	205	18.2
21	432.7	88.8	161	22.4

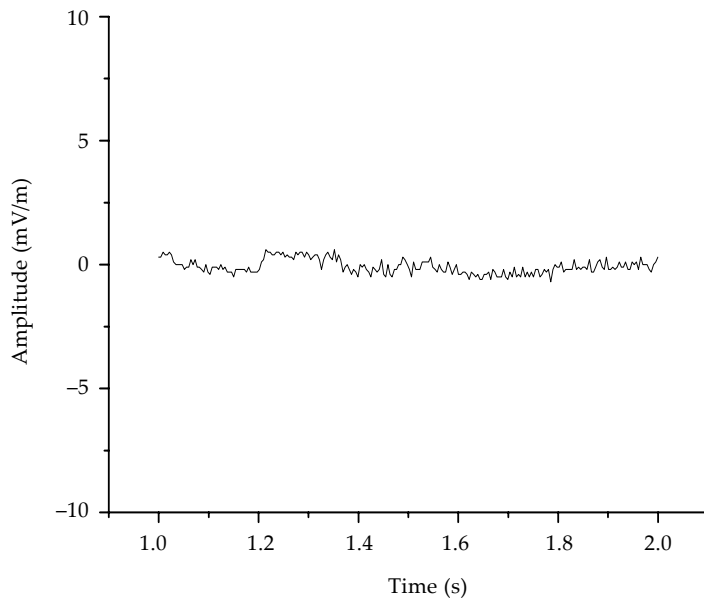


Figure 5. Signal obtained from unfertilized egg

cardiac work of an avian embryo (Suzuki *et al.*, 1989; Pearson *et al.*, 1998; Tazawa *et al.*, 2001).

In the present experiment, it was found that the highest frequency of cardiac work occurs on day 9 of incubation, averages 248 contractions per minute and is the lowest on the final day of incubation at an average of 161 contractions per minute.

When analysing the trends for average number of heart contractions on particular days of incubation, it was found that the heart rate decreased from 248 to 218 contractions per minute between 9 and 12 days (Table 1).

Measurements of cardiac work during the same period of incubation were also made by Antoni

and Diliger (1981). Using the optimal method of cardiac work measurement, they found this period of incubation to be characterized by a “flat” phase when the frequency of cardiac work is relatively stable and ranges from 264 to 277 contractions per minute.

When in the present study analysing the later period of incubation (days 12 to 16), cardiac work was found to become stable at 218 contractions per minute (Table 1). During the same incubation period Antoni and Diliger (1981) obtained similar results, while Laughlin *et al.* (1975) and Cain *et al.* (1967) recorded the number of heartbeats to decrease.

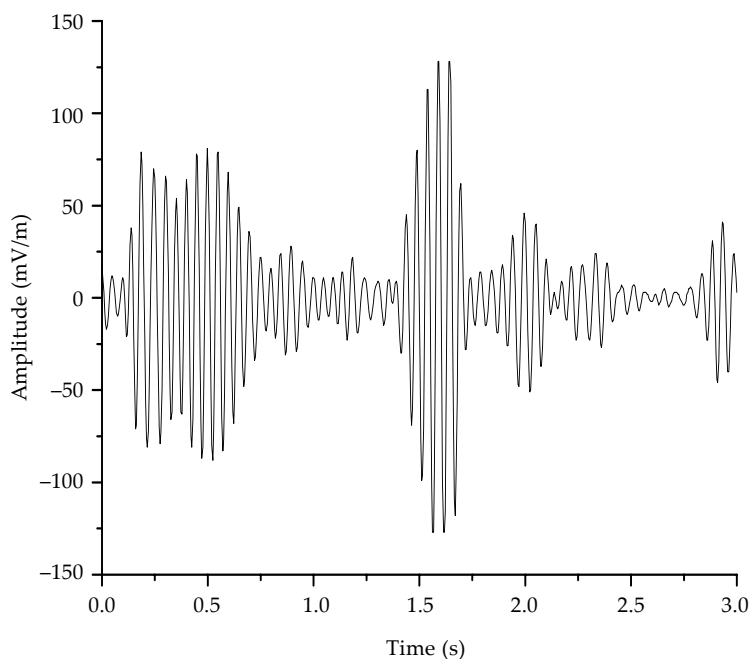


Figure 6. Ballistocardiogram of chick embryo as measured on a day preceding embryo death

As embryonic development went on (after 16 days of incubation), similarly like Laughlin *et al.* (1975) and Cain *et al.* (1967) we observed the frequency of heart work to drop from 215 to 205 contractions per minute, while the lowest values (161 per minute) were recorded on the last day of incubation when all eggs were pipped (Table 1).

A rapid decrease in the heart rate in the final period of incubation was also observed by Antoni and Diliger (1981), who reported that the frequency of cardiac work of pipped embryos may drop as low as 93 contractions per minute.

Laughlin *et al.* (1975) reported that during pipping, the heart rate increases to 270 contractions per minute.

The cardiac rhythm during the last 3 days of incubation was studied by Moriya *et al.* (2000). They made continuous measurements of cardiac work from day 18 of incubation with measuring electrodes inserted into the egg. During that period they observed the cardiac rhythm to undergo constant changes (decreases and increases), with a very high increase in the last phase of hatching.

The studies of Loffelholz and Pappano (1974) on isolated hearts of chick embryos indicated a decreased frequency of impulses from day 15 of incubation, and the lowest number of heartbeats

occurred on the last day of embryonic development.

To test the relations between the amplitude, frequency and weight of egg, coefficients of partial correlation were calculated. The calculated values evidence that in terms of linear relationship there are no significant correlations between the two variables studied, i.e. the amplitude, frequency and weight of egg, when the possible effect of other variables on the relationship is eliminated (Table 2).

To make sure that the registered signal really came from the life function of the embryo rather than being derived from some interfering signals, measurements of unfertilized eggs were made on each day of the experiment.

The signals showed considerable differences in relation to the results for live embryos. It was found that there were no repeatable signals in the ballistocardiograms recorded (Figure 5). The amplitude of the signal obtained from the measurement of unfertilized eggs was almost constant in the 2.3–3.5 mV/m range. Standard deviation for these measurements ranged from 1.9 and 4.3 (Table 3) and was always higher than 5 for eggs with live embryos (Table 1). The very low variability of signal amplitude in the case of unfertilized eggs and the high variability for live embryos indicate

Table 2. Values of partial correlation coefficients between amplitude (A), heart contraction rate (C) and egg weight (M) according to the day of embryo life

Days of incubation	A/C	A/M	C/M
9	-0.0282	0.0320	0.0548
10	0.0410	0.0659	0.0028
11	0.1954	-0.1338	-0.0437
12	0.0662	0.1533	-0.0874
13	0.1052	-0.0379	-0.0242
14	-0.0135	0.0805	0.0993
15	-0.0575	-0.0902	-0.0010
16	0.0197	0.0747	0.0217
17	-0.0219	0.0212	0.0124
18	-0.0207	-0.1430	0.0237
19	0.0512	0.0264	-0.0383
20	0.0410	-0.0659	0.0028
21	0.1569	-0.0223	0.1004

Table 3. Values depicting the amplitude of signal from unfertilized eggs

Days of incubation	Average amplitude (mV/m)	Standard deviation
9	2.5	4.3
10	2.3	3.8
11	3.4	4.2
12	2.4	3.5
13	2.8	2.0
14	3.5	3.2
15	3.0	1.9
16	2.5	4.1
17	2.9	3.9
18	2.8	2.7
19	3.1	4.1
20	3.5	3.5
21	2.9	2.8

that the variation in the amplitude level probably comes from the organism's life functions.

An embryopathological analysis of unhatched embryos was done after the end of incubation. The results of embryopathological analysis were compared with the earlier ballistocardiograms of eggs with dead embryos. This comparison showed that from the day of death determined by the embryopathological analysis there were no repeatable signals in the ballistocardiograms. It can thus be concluded that the day of embryo death determined by the analysis of ballistocardiogram matched the actual day of embryonic death.

The processing of data on ballistocardiograms of dead embryos showed that in the days preceding embryonic death there were also large disturbances in the ballistocardiograms (Figure 6). These disturbances could result from rapid movements of the embryo.

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ABSTRAKT**Neinvazivní měření srdeční činnosti kuřecího embrya**

Ke sledování srdeční činnosti kuřecích embryí byla použita neinvazivní metoda balistokardiografie. Při této metodě jednu kondenzátorovou desku představuje vaječná skořápka, na níž jsou elektrické náboje, druhou deskou je přijímací anténa měřicího zařízení. Srdeční činnost kuřecího embrya navozuje nepatrné pohyby celého vejce, čímž dochází ke změnám vzdáleností mezi deskami a vzniká rozdíl v potenciálech mezi skořápkou a přijímací anténou. Tyto změny registruje měřicí zařízení. První jednotlivé signály srdeční činnosti jsme zaznamenali 7. den inkubace. Od 9. dne byl zaznamenán signál od všech embryí. Během sledování se srdeční frekvence snížila z 248 na 161 pulsů za minutu a amplituda signálu plynule stoupala z 6,3 na 432,7 mV/m. Ve dnech, které předcházely úmrtí embryí, jsme na balistokardiogramech zjistili značné poruchy.

Klíčová slova: balistokardiografie; embryogeneze; ptačí embryo

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