Original Research *Highlight article*

In-ovo imaging using ostrich eggs: Biomagnetism for detection of cardiac signals and embryonal motion

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Impact Statement

The role of biomagnetism has been described for neurological and cardiac diseases in humans. The current work applies biomagnetism to ostrich eggs and investigates the influence of isoflurane anesthesia with regard to embryonal motion and cardiac signals. This information is needed for planning and execution of in-ovo imaging using ostrich eggs, a recently advancing method to avoid classical animal testing using rodents. The current work shows that application of isoflurane anesthesia is effective and safe.

Abstract

In-ovo imaging using ostrich eggs has been described as a potential alternative to common animal testing. The main advantage is its independence from small animal imaging devices as ostrich eggs provide good image quality on regular CT, MRI, or PET used in examinations of humans. However, embryonal motion during dynamic imaging studies produce artifacts. The aims of this study were (1) to explore the feasibility of biomagnetism to detect cardiac signals and embryonal motion and to use these findings (2) to investigate the effect of isoflurane anesthesia on ostrich embryos. A standard magnetoencephalography developed for brain studies was used to detect embryonal signals of ostrich eggs on developmental day 34. Signals were instantly shown on a screen and data were also postprocessed. For assessing the effects of anesthesia, nine ostrich eggs were investigated using isoflurane 6% for 90 min. Biomagnetic signals were recorded simultaneously. A control group consisting of eight different ostrich eggs was also investigated. Cardiac signals similar to electrocardiography were observed in all eggs. Postprocessing revealed frequent motion of embryos without anesthesia. The exposure to isoflurane led to a significant decrease in motion signals in 9/9 ostrich embryos after 8 min. Motion was significantly reduced in the isoflurane group versus control group. There were no isoflurane-related deaths. This study shows that biomagnetism is feasible to detect cardiac signals and motion of ostrich embryos in-ovo. Application of isoflurane is safe and leads to a rapid decrease in embryonal motion, which is an important prerequisite for the implementation of in-ovo imaging using ostrich eggs.

Keywords: In-ovo imaging, ostrich eggs, biomagnetism, alternative animal testing, isoflurane anesthesia

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Introduction

In-ovo imaging using ostrich eggs has been described as a potential alternative concept to common animal testing using rats or mice.[1] Preliminary studies report on the feasibility and technical success of positron emission tomography/computed tomography (PET/CT) and computed tomography (CT) examinations; however, embryonal motion was identified as a major limitation regarding imaging.[1,2]

The advantages of using ostrich eggs for preclinical imaging instead of common animal testing consist of the following: (1) Regular scanners commonly used in clinical routine in humans are sufficient to provide good image quality. Due to the large size of approximately 20 × 15 cm, there is no need for dedicated small animal imaging devices, thus bypassing costly investments.[1] (2) According to national and international legislation, research using eggs does not qualify as animal testing as long as all experiments are carried out before hatching.[3–5] (3) Incubation and storage of ostrich eggs requires less space and care compared to common animal testing.[1] Summarizing the use of ostrich eggs complies with basic principles of animal research: replacement, reduction, and refinement.[6]

Biomagnetism describes the properties of magnetic fields that surround living viable cells or tissues that are electrically charged. Axons of neural cells, muscle fibers, and an entire muscle represent compartments which produce magnetic fields.[7–9] Magnetoencephalography (MEG) was designed to assess magnetic fields within the brain in neurology studies, comprising epilepsy, cognition, and

post-septic encephalopathy.[8,10,11] Also, heart actions originating from cardiac innervation and myocardial contraction and infarction have been investigated using biomagnetism.[12,13] Immobilization regarding in-ovo imaging has been successfully achieved using isoflurane anesthesia, however, using chicken eggs and small animal PET/CT.[14] Considering the similar dimensions of an ostrich egg and the human head, we hypothesized that an MEG system is suitable to detect ostrich embryonal motion and cardiac signals. The detection of biomagnetic signals from eggs is hereafter referred to as magnet-ovography (MOG).

This study aims at (1) exploring the feasibility of biomagnetism to detect embryonal motion and cardiac signals and using these findings (2) to investigate the effect of isoflurane anesthesia on ostrich embryos. The conclusions drawn from this study are needed to plan and execute further experiments regarding in-ovo imaging using ostrich eggs, that is, in terms of establishing a protocol for successful immobilization.

Materials and methods

Ethics approval and consent to participate

This embryo study did not qualify as an animal research study according to the Federal German Animal Protection Act. Registration took place with the Office for Consumer Protection of the Thuringia State of Germany, Registration No. 22-2684-04-02-114/16.

Ostrich eggs

Experiments were carried out using ostrich eggs which were obtained from a local ostrich farm 15 km from the research facility between April and September. Artificial incubation was carried out using a multistage egg incubator (Sofie 3, Hemel, Verl, Germany) with a capacity of 80 ostrich eggs. Incubation properties were kept constant at 36.5°C and 25% air humidity, and eggs were automatically turned every 2 h to prevent sticking of embryonal structures to the egg membrane and thus simulating natural breeding conditions. If artificially incubated, ostrich eggs usually hatch after 42 days. For pilot experiments, eggs of different development days (DD) were used. As it was a requirement to end all experiments before hatching, isoflurane studies were performed on DD 34. This time point represents a compromise between complete embryonal development (as expected on DD 42) and guaranteed prevention of hatching. A viable fertilized egg with regular embryonal development was defined as an egg producing carbon dioxide on DD 15 and 34, and showing bone structures on a CT scan on DD 34.

Magnet-ovography

MEG methodology and system setup The MEG system consists of up to 300 magnetometers, that is, superconducting quantum interference devices (SQUID) arranged in a semi-concentric pattern around an object. Each SQUID consists of a wound coaxial coil measuring magnetic flux and a second wound coaxial coil for background noise correction.[15] To detect the very small magnetic fields (5×10^{-14} T) produced by neuronal activity, adequate shielding of external magnetic fields (e.g. earth geomagnetic field: 5×10^{-5} T; cars/electronic devices: 1×10^{-6}–1×10^{-9} T) is utmost important.[15,16] Each magnetometer produces a graph (channel) showing the magnetic field change over time (Figure 1).

The system setup is shown in Figure 2. The MEG system is located in a shielded room used for brain studies reducing external magnetic fields to a minimum. As ferromagnetic objects result in magnetic fields, it is a necessity to design all kinds of material metal-free. Thus, a remote control of isoflurane anesthesia and gas monitoring was designed to be located outside of the shielded room, enabling continuous investigation. The gas-tight system holding the ostrich egg was printed with a 3D-printer (Ultimaker 2+, Ultimaker, Geldermalsen, Netherlands) and equipped with a sealed polymethylmethacrylate lid secured with plastic screws.

Pilot experiments Ostrich eggs of different DD were investigated with the MEG system to visualize cardiac signals and motion. These experiments were surprisingly immediately successful as MEG signals instantly shown on a computer screen were identified as ECG-like patterns and non-periodically occurring strong artifact-like signals representing embryonal motion (Figure 3). The visual identification of ECG-like signals and motion signals did not require data processing.

Effect of isoflurane anesthesia

Time schedule for isoflurane experiments is shown in Figure 4. Two groups were investigated comprising Group A (isoflurane anesthesia, nine eggs) and Group B (control group without isoflurane anesthesia, eight eggs). Eggs were carefully placed in the MEG system and data acquisition was started. To exclude embryonal arousal by transport, the eggs were allowed to rest for 15 min (resting phase) before starting isoflurane anesthesia (Group A) (Figure 5). Subsequently, isoflurane (Piramal Healthcare, Mumbai, India) was applied by remote control using a proprietary vaporizer with a maximum concentration of 6% according to the manufacturer's instructions. Continuous monitoring of isoflurane concentration within the gas-tight system holding the egg was achieved using a standard gas measurement module for anesthetic care (Scio Four Oxi, Dräger, Lübeck, Germany). After 90 min of isoflurane exposition (interventional phase), gas inflow was stopped.

Eggs of Group B underwent 105 min of data acquisition without intervention (time intervals were chosen identical to Group A: resting phase 0–15 min, interventional phase 15–105 min).

Data acquisition and postprocessing

Chronological steps of data acquisition and data postprocessing are shown in Tables 1 and 2, respectively.

Toxicity

Toxic effect of isoflurane was assessed by continuous recording of MOG signals during isoflurane exposure. Furthermore, biomagnetic signals were checked on DD 35

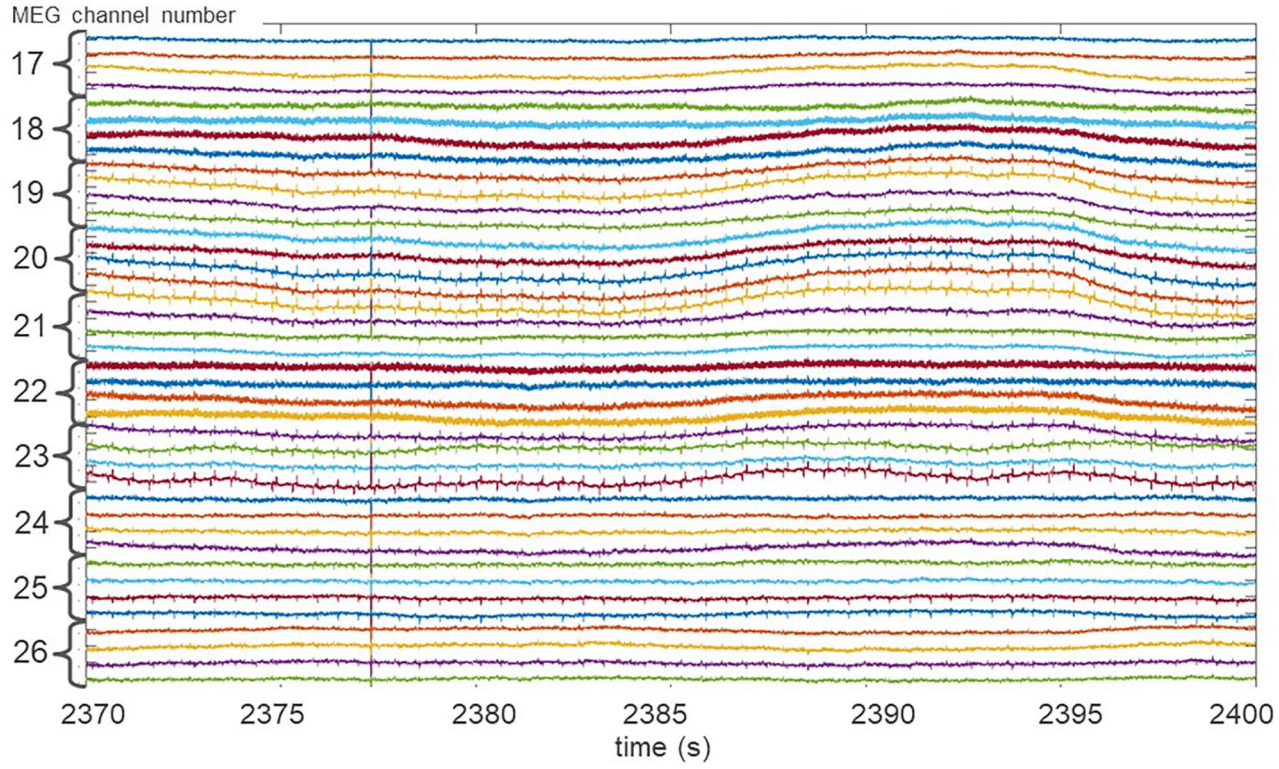

Figure 1. A total of 36 channels (nine detectors with four channels each) of biomagnetic signals of an ostrich egg on DD 31 over 30 s (x-axis). Examples of periodically occurring spikes are depicted in Detectors 20 and 23. Detectors 18 and 22 show noisy signals without the detection of spikes. At 2377 s, an artifact produced a high amplitude signal in all channels. (A color version of this figure is available in the online journal.)

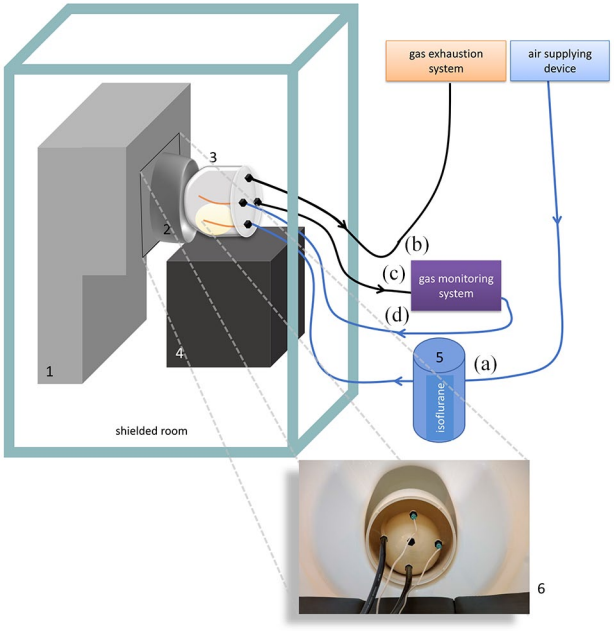

Figure 2. MEG-system setup. (1) The helium-cooled MEG system detects magnetic field changes by multiple SQUID, arranged in a semi-concentric pattern similar to a helmet, so-called dewar (2). The gas-tight system (3) holding the ostrich egg is set on a stretcher (4) and is connected to four gas tubes: (a) an air supply, optionally equipped with a vaporizer (5) for narcotic gases (e.g. isoflurane). (b) Outflow and exhaustion of air and – if applied – narcotic gases is realized by a wall-mounted gas exhaustion system for general anesthesia. (c) Part of the gas in the gas-tight system holding the ostrich egg is analyzed by a gas monitoring system and subsequently re-transferred into the gas-tight system (d). Photography of the air-tight system holding the ostrich egg is seen in (6). (A color version of this figure is available in the online journal.)

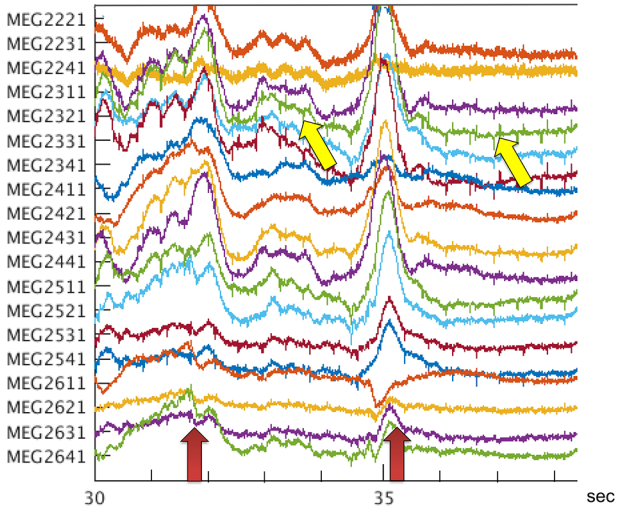

Figure 3. A total of 19 MOG channels over time of an ostrich egg on DD 31 identifying periodically occurring low-amplitude spikes (yellow arrows), representing cardiac signals. In addition, signals with larger amplitude and longer duration of signals are depicted (red arrows) representing embryonal motion.

to verify survival until the day after isoflurane exposure. After toxicity evaluation, ostrich embryos were sacrificed by cooling or underwent experimental PET/CT studies.

Statistics

Heart rate was evaluated in 10-min intervals comparing initial heart rate (0–15 min) and intervals of 40–50, 50–60, 60–70,

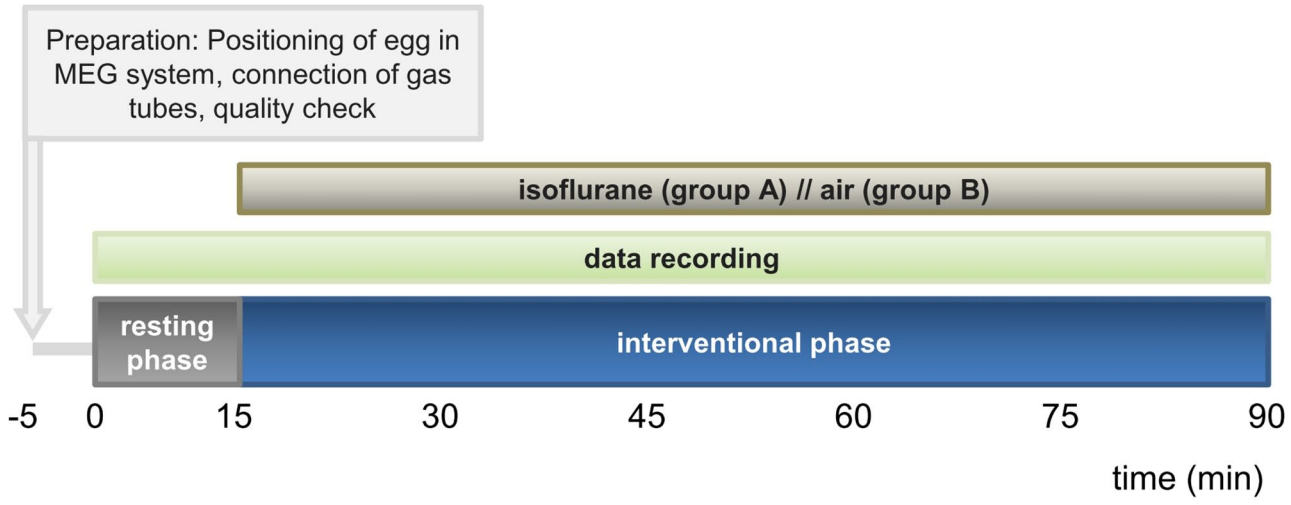

Figure 4. Time schedule for isoflurane anesthesia (Group A) and control group (Group B). (A color version of this figure is available in the online journal.)

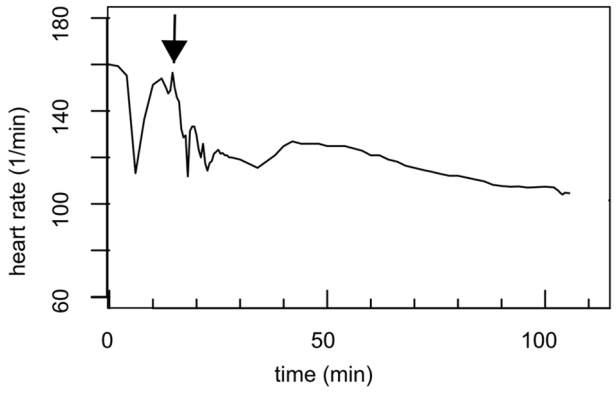

Figure 5. Heart rate over time of an ostrich egg on DD 34. The black arrow marks the beginning of isoflurane anesthesia at 15 min.

70–80, and 80–90 min (delta representing heart rate change). Welch's test was applied to compare Groups A and B.

Motion analysis was carried out using the level of activity, Figure 6(c) and (f), during resting phase and interventional phase (delta representing motion rate change). After logarithmic calculus, Student's *t*-test was used to describe the differences between resting phase and interventional phase, and Groups A and B. The level of significance was defined at $p < 0.05$.

Results

A total of 22 fertile eggs were investigated. Five eggs were used for pilot experiments, nine eggs were investigated in Group A (isoflurane), and eight eggs were observed in Group B (control).

Pilot experiments

Starting on DD 31, periodically occurring ECG-like signals were detectable immediately without post-processing. On later DD (32–37), cardiac signals showed higher amplitudes over time (data not shown). Motion was detectable by non-periodically, occasionally occurring signals at frequencies of 0.1–3 Hz.

Heart rate

During isoflurane exposure, heart rate was significantly lower in Group A as compared to Group B without isoflurane exposure (Figure 7).

Motion analysis

During resting phase, level of activity in Groups A and B did not differ significantly. After starting exposure to isoflurane, Group A showed a significant lower level of activity (Figure 8).

The level of biomagnetic signal intensity differed between subjects. Therefore, data underwent normalization according to resting phase activity, comparing the level of activity at an arbitrary time point and the average level of activity during resting phase. These values were used for further analysis. Eggs of Group A did not show significant motion anymore after 7.7 ± 3.1 min (range 3.0–11.8 min).

Toxicity

After experiments on DD 34, 9/9 (100%) embryos of Group A and 8/8 (100%) embryos of Group B produced clear cardiac signals on DD 35, indicating short-term survival after exposure to isoflurane.

Discussion

Biomagnetism prove feasible to detect embryonal cardiac signals and motion in ostriches. Furthermore, it was shown that isoflurane anesthesia leads to a significant reduction of embryonal motion without lethal side effects. This is an important step in planning and conduction of further experiments with ostrich embryos, enabling motion-artifact-free imaging, for example, for testing of radiopharmaceuticals and their biodistribution using PET/CT or single-photon emission computed tomography (SPECT/CT).

MOG

Surprisingly, MOG signals revealed clear cardiac signals even without post-processing as shown in Figures 1 and 3.

Table 1. Chronological steps of MOG data acquisition.

Action	Specific name	Purpose	Duration (min)	Remark
Start acquisition software	DANA 3 data acquisition software, Elekta/Neuromag, Elekta Instrument AB, Stockholm, Sweden	Proprietary software to detect signals	2	-
Check settings according to manufacturer's specifications	Sensors current status of proper operation Reset of sensors, if necessary	Provide optimal measuring conditions	5	No egg within shielded room
Insert egg in gas-tight system, seal with screws and place in MEG system in shielded room		Insert egg	2	-
Connect isoflurane and air tubes to the gas-tight system holding the egg		Enable remote anesthesia	5	After connecting and egg positioning, persons leave shielded room
Check sensors' live signals	DANA 3 data acquisition software	Ensure correct positioning of egg	1	If no adequate signal is detected, turn egg by 90°–180° to ensure close positioning of embryo to the sensors and repeat checking sensors' live signals
Start data acquisition according to Figure 4				

MEG: magnetoencephalography.

Table 2. Chronological steps of data post-processing.

	Action	Specific name	Purpose	Duration (min)	Remark
General	Elimination of artifacts and signals outside the shielded room	Neuromag maxfilter Rev. 2.2.10	Proprietary software to eliminate artifacts	10	-
General	Data transformation into MATLAB (The Mathworks Inc., Natick, MA, USA) compatible format	*Ad hoc* developed script		25	-
Heart rate analysis	Identification of suitable channels	*Ad hoc* developed script	Graphical presentation of different channels' signals	1	-
Heart rate analysis	Automated calculation of heart rate/time in one suitable channel	*Ad hoc* developed script	Graphical presentation of heart rate over time	16	If signal is smooth without motion artifacts. Result: heart rate every 120 s
Heart rate analysis	Manual analysis of heart rate/time in one suitable channel	*Ad hoc* developed script	Graphical presentation of heart actions over time, zoom for individual counting of 30 R-R peaks	35	If automated heart rate analysis is hampered by embryonal motion, manual analysis is necessary. Result: heart rate every 120 s
Heart rate analysis	Graph of heart rate over time	-	-	1	Figure 5
Motion analysis	Frequency analysis	*Ad hoc* developed script	Graphical presentation of magnetic field changes in different frequencies over time (Figure 6(a) and (d))	30	
Motion analysis	Translation of frequency analysis to motion signal	*Ad hoc* developed script	Table and graphical presentation of either positive (motion) or negative (no motion) values for each frequency band separately over time, Figure 6(b) and (e)	2	
Motion analysis	Elimination of artifacts	-	Manual analysis of graphical presentation (frequency analysis) over time to identify SQUID artifacts	15	
Motion analysis	Determination of successful immobilization	-	Level of activity: comparison of resting phase and interventional phase		The time point of successful immobilization was determined by inflection point of a sigmoidal curve fitting, Figure 6(c) and (f)

Previous studies on magneto-cardiography (MCG) showed its potential in detection of myocardial injury in adult humans[12] and human fetuses;[17,18] however, these objects produce much stronger signals as compared to the magnetic field changes obtained from ostrich embryo cardiac signals. Given the comparable dimensions of the human head and an ostrich egg, a similar setup to MEG was deemed appropriate for the detection of ostrich embryo cardiac signals and this approach was shown to successfully producing electrocardiogram (ECG)-like signals, enabling the determination of

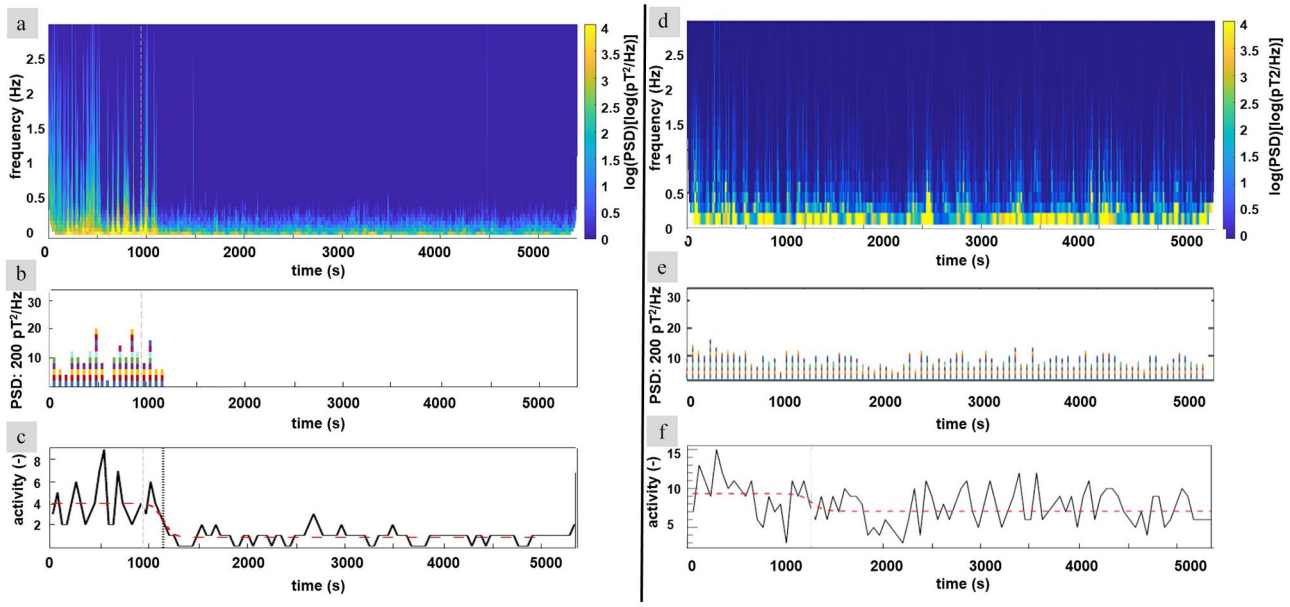

Figure 6. Motion analysis of two ostrich eggs of Group A (intervention group) with isoflurane (a–c) and of Group B (control group) without exposure to isoflurane (d–f) on DD 34. (a) Time-Frequency-Spectrogram, representing power (color scale) of signals at different frequencies (y-axis) over time (x-axis). After 15 min, isoflurane 6% was started (gray dashed line) and after 90 s, signal intensity decreased and did not increase during constant isoflurane exposure. (b) Graph representing dichotom scale classification of either positive (colored box) or negative (no box/white) of different frequencies bands at 0.05 Hz intervals (range 0–3 Hz) over time. Each frequency band is represented by a different color. The threshold for differentiating positive from negative signals was defined at a power spectral density (PSD) of 200 pT2/Hz. Data were analyzed with a time resolution of 1 min. (c) Based on the sum of positive frequency bands, see (b), mean embryonal activity was calculated during resting phase (0–15 min) and interventional phase (15–90 min). Both values were graphically shown and connected using a sigmoidal curve fitting (red graph). The inflection point of this sigmoid curve function was determined as the time point of successful immobilization (black dashed line). (d) Analogous to (a): Ostrich egg (Group B; control group) without isoflurane exposure. Note the missing decline of signal intensity after 15 min. (e) Analogous to (b): Note the constant positive signals above 90 min without distinct discrimination of resting phase and interventional phase. Periodical phases of higher (around 250 s) and lower (1800–2100 s) activity. (f) Analogous to (c): Note the higher embryonal activity above 90 min with only slight decrease after 1300 s, attributable to continuous cooling. Comparing (c) and (f), the graph in (f) has much higher values on y-axis.

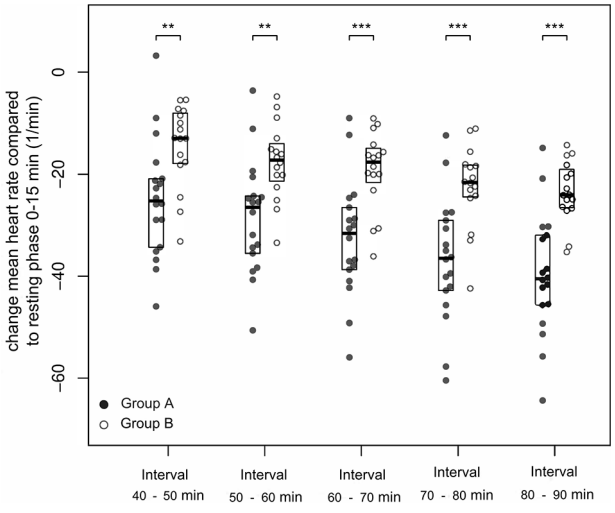

Figure 7. Mean heart rate change of different time intervals during interventional phase of Group A (isoflurane exposure, black dots) and Group B (control group, white dots). Mean heart rate during time intervals of 40–50, 50–60, 60–70, 70–80, and 80–90 min is compared to the mean heart rate during resting phase of 0–15 min. $^{**}p < 0.01$, $^{***}p < 0.0001$.

heart rate and its change over time. Although the setting is rather elaborate considering room shielding, MOG is a tool to non-invasively evaluate cardiac signals in ostrich eggs. This has been described with only one alternative method, that is, acoustocardiography.[19,20] Automated heart rate analysis was achieved by an *ad hoc* developed script detecting R-R-peak distance and required about 17 min for a dataset of 90 min. However, if motion artifacts hampered automated analysis, visual evaluation and manual counting were necessary to determine the heart rate. This procedure was more time-consuming and required 35 min for a dataset of 90 min.

Motion, that is, contraction of muscle fibers, their neuronal activation, and the movement of a body part are usually considered an artifact in imaging, electrocardiography, and other methods to detect biosignals. One goal of this study was (1) to quantify this "artifact" to (2) measure the effect of isoflurane. Quantification was achieved by investigating different frequency bands and categorizing into "positive" or "negative," based on an empirically derived threshold (Table 2, Figure 6(b) and (f)). This categorization was repeated at 30-s intervals; thus, quantification over time was realized. Motion analysis with biomagnetism has been described for human fetuses;[17] however, analyzing the magnetic heart axis and its relative position change over time which represents a more direct approach that the one described in the current setup with ostrich embryos. Motion analysis was more elaborate than heart rate evaluation, requiring about 45 min post-processing. Embryonal motion in chicken embryos occurs as early as DD 10, showing repetitive leg flexion and extension.[21–23] As the embryonal development of ostriches is about twice as long as the one of chicken, it is conceivable that ostrich embryonal motion is detectable on DD 20 and later. The experiments in this study were conducted on DD 34, thus it was assumed that embryonal motion occurred frequently. MOG revealed irregularly occurring signals at

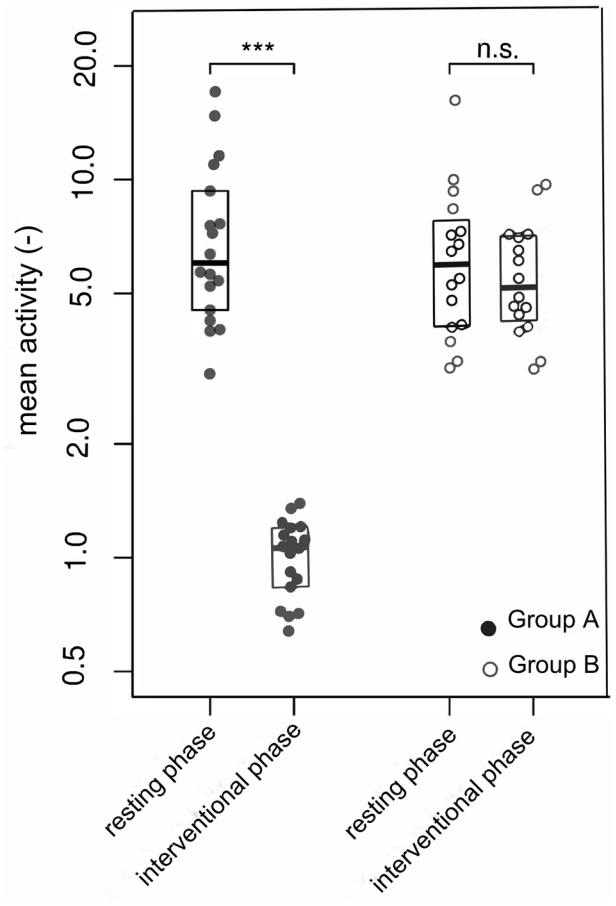

Figure 8. Level of activity during resting phase and interventional phase of Group A (isoflurane exposure, black dots) and Group B (control group, white dots). $^{**}p < 0.01$; n.s. not significant.

0.1–3 Hz which is clearly below signals resulting from muscle contraction at 20–40 Hz. Slow movement of legs in chicken embryos (before and after hatching) has been described to occur at 1–4 Hz.[21,24,25] Even slower signals of < 1 Hz are attributable to slow mass movements, possibly caused by liquid motion within the albumen or yolk.

Isoflurane anesthesia

Heart rate was lower in ostrich embryos which have been exposed to isoflurane (Figure 7). The use of isoflurane in ostrich embryos has not been described before. In adult and juvenile ostriches, isoflurane anesthesia is known to increase the heart rate due to vasodilation and subsequent decrease in blood pressure.[26,27] Heart rate of chicken embryos is not significantly affected by isoflurane as reported by Wojtczak in 2000.[28] We assume that decreasing heart rate observed in this study is attributable to two major factors. First, reduction of embryonal motion and arousal by anesthesia leads to lower need for oxygen and thus, reduction of heart rate represents a physiological reaction. Second, given the temperature difference of the ostrich egg (36.5°C) and the shielded room (25°C), continuous cooling of the eggs is apparent (data not shown). However, this aspect is present in the control group B also and was observed in a significantly less pronounced fashion (Figure 7); therefore, cooling cannot be the reason for the difference between Group A and Group B.

Embryonal motion was significantly reduced by isoflurane exposure as compared to intraindividual level of activity before starting isoflurane (resting phase) and as compared to control group B (Figure 8), indicating effective immobilization by isoflurane. Mean 8 min of isoflurane exposure was necessary to significantly reduce level of activity. This is a highly relevant finding regarding the primary goal of this study: Establishing a protocol for non-invasive effective immobilization of ostrich embryos to enable motion-artifact-free imaging. Similar approaches have been reported for chicken embryos successfully applying isoflurane.[14,29] Different studies investigated the use of cooling,[30] muscle relaxing agents[31–34] and ketamine;[32] however, these concepts usually requiring invasive preparation, that is, partially removing the egg shell to get access to the chorion-allanois-membrane and/or vitelline vessels for intravasal agent application. Also, the aforementioned studies consider chicken embryos. The presented data are the first to investigate immobilization in ostrich eggs.

Isoflurane anesthesia of ostrich eggs is safe and non-toxic as no experiment-related deaths were detected on next-day follow-up.

Limitations

This study aimed at investigating the general ability of biomagnetism to depict cardiac signals and embryonal motion in ostrich eggs. Defining criteria for motion signals was empirically derived as no comparable studies exist and therefore, these criteria need verification, that is, direct comparison of imaging with and without isoflurane anesthesia which is planned in future studies. The rather low sample size hampered further analysis regarding interindividual differences, that is, different level of level of activity during the resting phase. In addition, the detection of biomagnetism requires cost-intensive premises, rooms, and trained personnel. Alternative approaches comprise the analysis of capacitive signals, which is independent from external shielding[35–37] and optically pumped magnetometers.[38]

Future studies should aim at the investigation of different concentrations of isoflurane and the performance of other narcotic gases as desflurane and sevoflurane.

Conclusions

Biomagnetism in ostrich eggs (MOG) using standard MEG is feasible to detect embryonal cardiac signals and embryonal motion. Effective embryonal immobilization was reached within 8 min after isoflurane exposure. The use of isoflurane is safe and does not lead to experiment-related deaths of ostrich embryos.

AUTHORS' CONTRIBUTIONS

MF and TW contributed in conceptualization; JG, CK, and TW participated in methodology; HH and OP performed the formal analysis and investigation; MF and OWW contributed in resources and supervision; TW contributed in writing the original draft preparation; MF and TW contributed in project administration and funding acquisition. All authors contributed in writing – review and editing.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES

1. Freesmeyer M, Kuehnel C, Opfermann T, Niksch T, Wiegand S, Stolz R, Huonker R, Witte OW, Winkens T. The use of ostrich eggs for in ovo research: making preclinical imaging research affordable and available. *J Nucl Med* 2018;**59**:1901–6
2. Winkens T, Christl A, Kuehnel C, Ndum F, Freesmeyer M. In-ovo imaging using ostrich eggs—evaluation of physiological embryonal development on computed tomography. *Acta Zool* 2021;**0**:1–11
3. United States Department of Agriculture. Animal welfare act and animal welfare regulations, 2013, https://www.aphis.usda.gov/animal_welfare/downloads/bluebook-ac-awa.pdf
4. Europäisches Parlament und Rat. Richtlinie 2010/63/EU zum Schutz der für wissenschaftliche Zwecke verwendeten Tiere, 2010, https://eur-lex.europa.eu/legal-content/DE/TXT/HTML/?uri=LEGISSUM:sa0027
5. Bundesministerium der Justiz und für Verbraucherschutz. Tierschutzgesetz, 2016, https://www.gesetze-im-internet.de/tierschg/BJNR012770972.html
6. Russell W, Burch R. Principles of humane experimental-technique. *Roy Soc Health J* 1959;**79**:700
7. Gross J. Magnetoencephalography in cognitive neuroscience: a primer. *Neuron* 2019;**104**:189–204
8. Park CK, Hwang SJ, Jung NY, Chang WS, Jung HH, Chang JW. Magnetoencephalography as a prognostic tool in patients with medically intractable temporal lobe epilepsy. *World Neurosurg* 2019;**123**:e753–9
9. Yin C, Zhang X, Xiang J, Chen Z, Li X, Wu S, Lv P, Wang Y. Altered effective connectivity network in patients with insular epilepsy: a high-frequency oscillations magnetoencephalography study. *Clin Neurophysiol* 2019;**131**:377–84
10. Gotz T, Baumbach P, Huonker R, Kranczioch C, Witte OW, Debener S, Klingner C, Brunkhorst FM, Günther A. Slowed peak resting frequency and MEG overactivation in survivors of severe sepsis and septic shock. *Clin Neurophysiol* 2016;**127**:1247–53
11. Zhou YJ, Perez-Bellido A, Haegens S, de Lange FP. Perceptual expectations modulate low-frequency activity: a statistical learning magnetoencephalography study. *J Cogn Neurosci* 2019;**32**:691–702
12. Goernig M, Liehr M, Tute C, Schlosser M, Haueisen J, Figulla HR, Leder U. Magnetocardiography based spatiotemporal correlation analysis is superior to conventional ECG analysis for identifying myocardial injury. *Ann Biomed Eng* 2009;**37**:107–11
13. Nowak H, Leder U, Pohl P, Brauer H, Tenner U, Haueisen J. Diagnosis of myocardial viability based on magnetocardiographic recordings 5 Biomagnetic Centre, University of Jena, Jena, Germany. *Biomedical Engineering/Biomedizinische Technik* 1999;**44**:174
14. Heidrich A, Würbach L, Opfermann T, Saluz HP. Motion-artifact-free in vivo imaging utilizing narcotized avian embryos in ovo. *Mol Imaging Biol* 2011;**13**:208–14
15. Hämäläinen M, Hari R, Ilmoniemi RJ, Knuutila J, Lounasmaa OV. Magnetoencephalography theory, instrumentation, and applications to noninvasive studies of the working human brain. *Rev Mod Phys* 1993;**65**:413–97
16. Proudfoot M, Woolrich MW, Nobre AC, Turner MR. Magnetoencephalography. *Pract Neurol* 2014;**14**:336–43
17. Schmidt A, Schneider U, Witte OW, Schleußner E, Hoyer D. Developing fetal motor-cardiovascular coordination analyzed from multichannel magnetocardiography. *Physiol Meas* 2014;**35**:1943–59
18. Kiefer-Schmidt I, Lim M, Wacker-Gussmann A, Ortiz E, Abele H, Kagan KO, Kaulitz R, Wallwiener D, Preissl H. Fetal magnetocardiography (fMCG): moving forward in the establishment of clinical reference data by advanced biomagnetic instrumentation and analysis. *J Perinat Med* 2012;**40**:277–86
19. Tazawa H, Nakagawa S. Response of egg temperature, heart rate and blood pressure in the chick embryo to hypothermal stress. *Journal of Comparative Physiology B* 1985;**155**:195–200
20. Tazawa H, Ar A, Pearson JT, Moriya K, Gefen E. Heart rate in developing ostrich embryos. *Br Poult Sci* 1998;**39**:161–6
21. Bradley NS, Ryu YU, Lin J. Fast locomotor burst generation in late stage embryonic motility. *J Neurophysiol* 2008;**99**:1733–42
22. Bradley NS, Bekoff A. Development of coordinated movement in chicks: I. Temporal analysis of hindlimb muscle synergies at embryonic days 9 and 10. *Dev Psychobiol* 1990;**23**:763–82
23. Chambers SH, Bradley NS, Orosz MD. Kinematic analysis of wing and leg movements for type I motility in E9 chick embryos. *Exp Brain Res* 1995;**103**:218–26
24. Bradley NS, Solanki D, Zhao D. Limb movements during embryonic development in the chick: evidence for a continuum in limb motor control antecedent to locomotion. *J Neurophysiol* 2005;**94**:4401–11
25. Johnston RM, Bekoff A. Patterns of muscle activity during different behaviors in chicks: implications for neural control. *J Comp Physiol A* 1996;**179**:169–84
26. Al-Sobayil FA, Ahmed AF, Al-Wabel NA, Al-Thonayian AA, Al-Rogibah FA, Al-Fuaim AH, Al-Obaid AO, Al-Muzaini AM. The use of xylazine, ketamine, and isoflurane for induction and maintenance of anesthesia in ostriches (*Struthio camelus*). *J Avian Med Surg* 2009;**23**:101–7
27. Cornick-Seahorn J. Ratite management and surgery. In: Tully TN, Shane SM (eds) Ratite management and surgery. Malabar, FL: Krieger Publishing; 1996, p.88
28. Wojtczak JA. The hemodynamic effects of halothane and isoflurane in chick embryo. *Anesth Analg* 2000;**90**:1331–5
29. Gebhardt P, Würbach L, Heidrich A, Heinrich L, Walther M, Opfermann T, Sørensen B, Saluz HP. Dynamic behaviour of selected PET tracers in embryonated chicken eggs. *Rev Esp Med Nucl Imagen Mol* 2013;**32**:371–7
30. Bain MM, Fagan AJ, Mullin JM, McNaught I, McLean J, Condon B. Noninvasive monitoring of chick development in ovo using a 7T MRI system from day 12 of incubation through to hatching. *J Magn Reson Imaging* 2007;**26**:198–201
31. Boss A, Oppitz M, Wehrl HF, Rossi C, Feuerstein M, Claussen CD, Drews U, Pichler BJ, Schick F. Measurement of T1, T2, and magnetization transfer properties during embryonic development at 7 Tesla using the chicken model. *J Magn Reson Imaging* 2008;**28**:1510–4
32. Waschkies C, Nicholls F, Buschmann J. Comparison of medetomidine, thiopental and ketamine/midazolam anesthesia in chick embryos for in ovo Magnetic Resonance Imaging free of motion artifacts. *Scientific Reports* 2015;**5**:15536

33. Levillain A, Rolfe RA, Huang Y, Iatridis JC, Nowlan NC. Short-term foetal immobility temporally and progressively affects chick spinal curvature and anatomy and rib development. *Eur Cell Mater* 2019;**37**:23–41
34. Hammond CL, Simbi BH, Stickland NC. In ovo temperature manipulation influences embryonic motility and growth of limb tissues in the chick (*Gallus gallus*). *J Exp Biol* 2007;**210**:2667–75
35. Asl SN, Oehler M, Schilling M. Noise model of capacitive and textile capacitive noncontact electrodes for bioelectric applications. *IEEE Trans Biomed Circuits Syst* 2018;**12**:851–9
36. Oehler M. *Kapazitive Elektroden zur Messung bioelektrischer Signale*. Braunschweig, Germany: Technische Universität Carolo-Wilhelmina zu Braunschweig; 2009
37. Oehler M, Ling V, Melhorn K, Schilling M. A multichannel portable ECG system with capacitive sensors. *Physiol Meas* 2008;**29**:783–93
38. Oelsner G, Schultze V, Ijsselsteijn R, Stolz R. Performance analysis of an optically pumped magnetometer in Earth's magnetic field. *EPJ Quant Tech* 2019;**6**:6

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