Europ.Poult.Sci., **84.** 2020, ISSN 1612-9199, © Verlag Eugen Ulmer, Stuttgart. DOI: 10.1399/eps.2020.315

Electrical anaesthesia of male chicken embryos in the second third of the incubation period in compliance with animal welfare

Tierschutzkonforme elektrische Betäubung männlicher Hühnerembryonen im zweiten Drittel der Brut

L. Zumbrink[1], **B. Brenig**[1], **A. Foerster**[2], **J. Hurlin**[3]* and **M. von Wenzlawowicz**[4]

[1] University of Goettingen, Institute of Veterinary Medicine, 37073 Goettingen, Germany

[2] Lohmann Breeders GmbH, 27472 Cuxhaven, Germany

[3] Agri Advanced Technologies GmbH, 49429 Visbek, Germany

[4] bsi Schwarzenbek, Training and consultancy institute for animal welfare at transport and slaughter, 21493 Schwarzenbek, Germany

*Correspondence: joerg.hurlin@agri-at.com

Manuscript received 7 August 2020, accepted 21 September 2020

Abstract

The aim of *in ovo* sex determination in chicken hatching eggs is to detect male embryos and terminate their development at an early stage of incubation to avoid killing day-old-chicks after hatch. Since there is no secure scientific knowledge on presence or absence of pain perception between day 7 and 15 of incubation, it is necessary to develop animal welfare friendly and consumer acceptable procedures to end the incubation process. In this study, electrical current flow was investigated as a method for anaesthesia of chicken embryos. In two test series a current flow at 110 V AC was applied for 2 seconds to 304 and 56 embryonated eggs, respectively. Under these experimental conditions embryonic reactions associated with possible awareness of pain were absent in 99.3% of the examined embryos after exposure (results of trial series 1). The results suggest that application of an electric current is a practical and animal welfare-compliant procedure for chicken embryos in the second third of the incubation period.

Key words

in ovo sex determination; stunning; male chicken embryo; animal welfare; hyperspectral imaging; laying hen

Zusammenfassung

Die *in ovo* Geschlechtsbestimmung dient zur frühen Erkennung männlicher Hühnerembryonen, sodass eine Bebrütung rechtzeitig unterbrochen werden kann. Da über das Schmerzempfinden von Hühnerembryonen zwischen dem 7. und 15. Bebrütungstag jedoch keine gesicherten Daten vorliegen, gilt es ein Verfahren zu entwickeln, welches eine tierschutzkonforme Betäubung und anschließende Beendigung des Brutvorgangs erlaubt. Dies kann außerdem zu einer besseren gesellschaftlichen Akzeptanz des Verfahrens beitragen. In der vorliegenden Studie wurde die elektrische Durchströmung als Betäubungsverfahren untersucht. Dabei wurden in zwei Versuchsreihen 304 bzw. 56 bebrütete Eier jeweils 2 Sekunden mit 110 V durchströmt. Unter diesen Versuchsbedingungen bewirkte die Durchströmung, dass Reaktionen, die mit einer möglichen Empfindungsfähigkeit vereinbar sind, bei 99,3% der untersuchten Embryonen nicht mehr auftraten (Ergebnis Versuchsreihe 1). Die Ergebnisse zeigen, dass die elektrische Betäubung bei Hühnerembryonen im zweiten Drittel der Brut ein praktikables und tierschutzkonformes Verfahren darstellt.

Stichworte

in ovo Geschlechtsbestimmung; Betäubung; männliche Hühnerembryonen; Tierschutz; hyperspektrale Messtechnik; Legehenne

Introduction

Intensive research on various methods for *in ovo* sex determination has been undertaken during the past years. Given that killing of day-old male chicks should be ended as soon as possible, practical methods are being sought which can reliably and quickly identify the sex of the embryo before the embryo develops pain sensitivity. Some of the methods already applied (e.g. rearing of males, hyperspectral imaging, endocrinological methods, genetic diagnostic procedures) are currently quantitatively not sufficient to end killing of male chicks completely. However, none of the currently used market-ready methods of *in ovo* sex determination is applicable before day 8 of incubation (BOURIN et al., 2020; RICHARD, 2020).

In the discussion about the onset of pain perception, the question arises as how to handle the rejected male embryos in methods that are used from day 8 of incubation onwards. Although numerous studies point to a definite absence of pain perception of the chicken embryo in the first trimester of incubation, there are no reliable findings on pain perception for the period between day 8 and 15 of incubation (CHUMAK, 1961; MELLOR and DIESCH, 2007; BJØRNSTAD et al., 2015; DEUTSCHER BUNDESTAG, 2017). For methods of sex determination applied after day 7 of incubation, a solution is sought to interrupt the development of the male embryos in an animal welfare friendly and consumer acceptable manner (ALEKSANDROWICZ and HERR, 2015). The EC Regulation No. 1099/2009 generally allows simultaneous stunning and killing by mechanical disruption, which however has met little societal acceptance (HIRT, 2004; LEENSTRA et al., 2011). According to its general definition in EC Regulation No 1099/2009, the term stunning means "[…] any intentionally induced process which causes loss of consciousness and sensibility without pain […]". A practical method for embryo stunning in accordance with animal welfare requirements thus represents a further relevant step towards better animal welfare. The novel method of electrical anaesthesia of male embryos in the egg presented in this study is intended to close a gap precisely at this point. The aim of this study was to investigate the application of an electric current in order to achieve unconsciousness in chicken embryos until the assured termination of development by mechanical means occurs.

Methods

Preliminary trials for experimental set-up design

In ovo sex determination of chicken embryos is possible on incubation day 13 by hyperspectral imaging in layer chickens from genotypes with gender-specific down feather colour, as described by GÖHLER et al. (2017). In preliminary trials, different approaches were tested to find an applicable method for stunning of embryos at this incubation stage. Analogous to PRINZ et al. (2012), REIMERS (2008) and RETTER (2014) with broiler chickens, rainbow trouts or carps, a water bath stunning system was tested (Fig. 1), whereas a second approach tested a perfusion with electrodes applied to the outside of the eggshell (Fig. 2). Already after a small number of eggs these methods turned out to be not applicable, because the calcareous shell isolates the egg interior effectively.

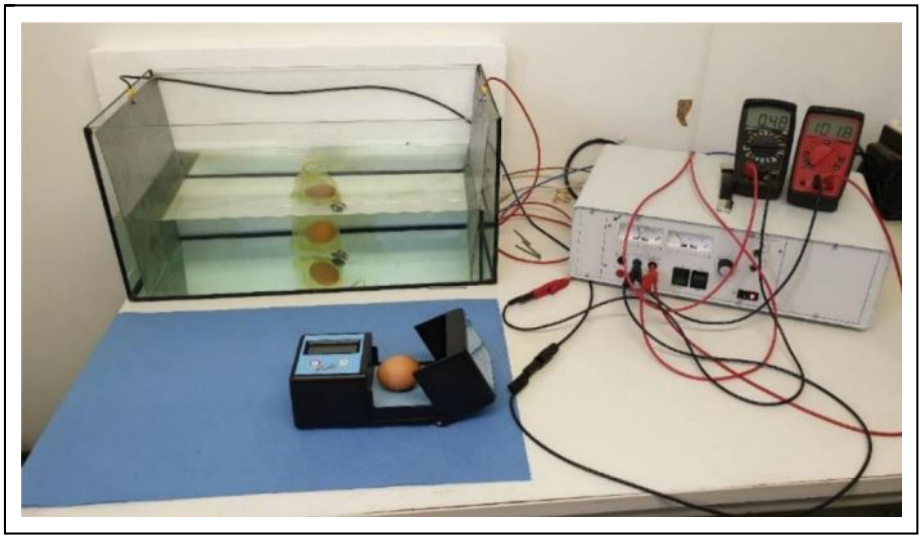

Figure 1. Initial experiments on waterbath stunning

Initialversuche mit Wasserbadbetäubung

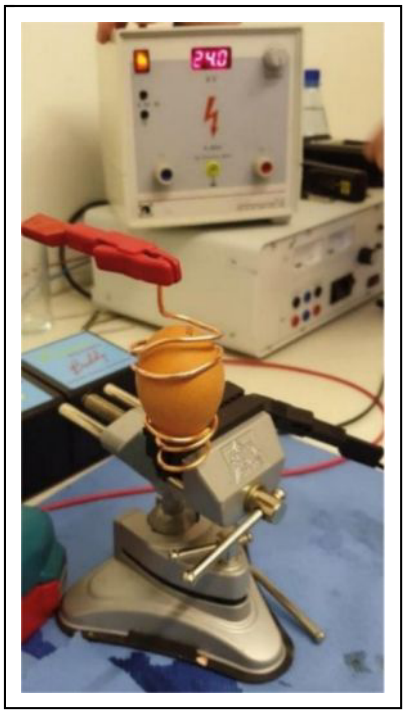

Figure 2. Initial experiments with externally applied electrodes

Initialversuche mit außen angelegten Elektroden

A reliable current flow could be achieved with electrodes inserted through the eggshell on the pole sides. The penetration depths were set to 22 mm (top) and 12 mm (bottom) to pierce the air cell, but not to come too close together to avoid electric short circuit. The initial experiments for the selection of parameters showed the most promising results with a 2-second current flow at 110 V and 50 Hz. For application of an electric current a manually operated test stand with a special fixing system for a definite penetration depth of the electrodes at various egg sizes was used (Agri Advanced Technologies GmbH) (Fig. 3).

Figure 3. Test stand by Agri Advanced Technologies GmbH with a special fixing system

Versuchsstand der Firma Agri Advanced Technologies mit Arretierungssystem zur einheitlichen Durchströmung

The visual observation of treated and control embryos in these preliminary trials were used to establish the final criteria of monitoring the reactions in accordance with literature.

Trial series 1

350 hatching eggs (Lohmann Brown, incubation day 13) were incubated under practical conditions at 37.8°C in mobile incubators (incubato, RCom Maru). The hatching eggs were removed from the incubator in small batches to ensure the same temperature conditions during testing. Each egg was given a consecutive number by a sticker. The heartbeat of each embryo in the closed egg was measured for 43 seconds (avg.) using a digital egg monitor "Buddy" (Avitronics, Cornwall, UK). Hatching eggs without detectable heartbeat were rejected. Eggshell temperature was measured with an infrared thermometer (Testo 830-T1). Each hatching egg was placed into the test stand and the electrodes were inserted through the eggshell at both poles with a standardised depth of 22 mm at the blunt end with the air cell and 12 mm at the sharp end. Maximum current intensity during the 2-second application was read off and documented. The eggshells were opened and the embryo, including egg liquid and yolk, was placed in a petri dish and positioned under a video camera within 20 seconds (avg.). Embryos were visually assessed for at least 60 seconds based on five criteria (beak opening, movement of head, wings, toes/legs and reaction on mechanical irritation with forceps) and the time interval between the end of the current flow and reactions of the embryo was recorded.

An embryo was considered successfully anesthetised if it did not show any spontaneous movements and reactions. The visual observation was recorded on video in addition to the tabular data recording (Fig. 4, experimental set-up).

Figure 4. Experimental set-up of trial series 1

Versuchsaufbau Versuchsreihe 1

As controls 13 randomly chosen vital embryos (4.35%) without current flow were observed accordingly. A total of 304 embryos were examined. Five records were eliminated because they did not meet the standardised experimental conditions (death during early stage of incubation, double current application, no treatment performed, no current flow, current intensity not documented).

Trial series 2

A second series of experiments was conducted to investigate the following questions:

1. What kind of reactions occurs in the embryos during the electrical flow and in the time window of the first 15 seconds thereafter?
2. Can the embryos be hit and injured by the electrodes?

For this purpose, the electrical current flow was carried out on windowed eggs in the horizontally oriented test stand (Fig. 5). The procedure remained largely similar to that of the first test series: hatching eggs were taken from the incubator in small batches, the eggs were labelled, and heartbeat and temperature were measured. Each egg was manually placed into the test stand with a penetration depth of the electrodes of 22 mm (top) and 12 mm (bottom). Before the onset of current flow, parts of the eggshell were removed using forceps to create a large hole in the egg for observation of the embryo. If necessary, the embryo was rotated slightly within the egg by using forceps so that it was clearly visible. For each egg, it was recorded whether the electrodes touched the embryo or not. The maximum

current was recorded during the current application. The time interval between the end of current flow and observation of reactions was measured with a stopwatch.

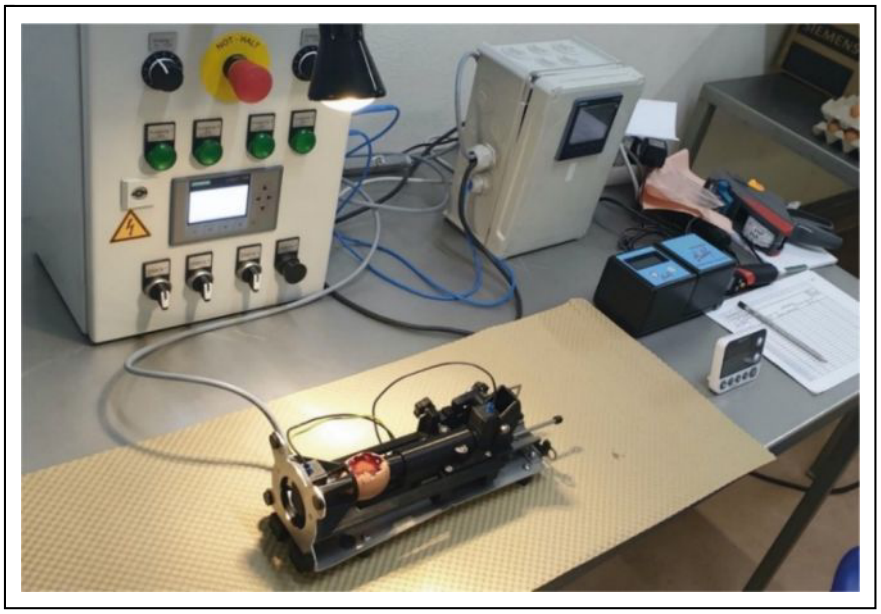

Figure 5. Experimental set-up trial series 2

Versuchsaufbau Versuchsreihe 2

The five above mentioned criteria were monitored on the embryo within the egg during the first 15 seconds after treatment. This corresponded approximately to the time span that occurs in practical implementation until the embryos are transferred to mechanical comminution after anaesthesia. After the 15-second interval from the end of the current flow, the embryos were removed from the egg and further observed. Three embryos without electrical treatment were examined as controls (5.77%). A total of 56 records were collected, of which four were discarded because they did not meet the standardised experimental conditions (incorrect electrode setting at the beginning, current intensity not documented). Additionally, video records were taken to retrace the visual assessment in retrospective.

Statistical Analysis

IBM SPSS Statistics 26 was used for the statistical analysis, and descriptive studies were used to determine the location parameters and measures of variation of the variables.

By applying contingency table, chi-square test and measures of association phi and Cramer's V, it was determined whether the independent variables “heartbeat”, “maximum current” and “egg temperature” each had a significant impact on the dependent variable, the anaesthesia result. Only the groups “motionless/anesthetised” and “motions/anesthetised” were considered, because the group “motions/non-anesthetised” had too few accounts for a statistical analysis (n = 2). The binary logistic regression examined the association of the three independent variables (heartbeat, current, temperature) with the binary coded dependent variable (anaesthesia result: 0 = movement/anesthetised, 1 = movement/non-anesthetised).

Furthermore, the five criteria were evaluated and compared with each other using the measures of association phi and Cramer's V. Level of significance was set at $p < 0.05$.

Results

In the first series of experiments average heartbeat of the embryos was 205 beats per minute (BPM) before treatment and mean eggshell temperature was 32.2°C. The average of maximum current measured during electrical flow was 3.82 A (Table 1). 255 embryos of 286 treated eggs were classified as successfully anesthetised due to the absence of any movement or reaction. Although in 29 embryos (10.1%) movement was observed, these embryos were also

considered anesthetised because the movements differed significantly from those of an untreated embryo in the control group. The movements were jerky and lasted only a few seconds (avg. 3.5 seconds) after the embryos were retrieved from the egg and were therefore considered to be a reflex and not voluntary or conscious movements. In total 284 embryos (99.3%) were successfully anesthetised (Table 2, Fig. 6). Table 3 shows the occurred movements in the different groups. Even after prolonged observation up to three minutes no recovery was determined.

Table 1. Location parameters and measures of variation of heartbeat, eggshell temperature and current in trial series 1

Deskriptive Statistik mit Lage- und Streuungsparametern der Variablen Herzschlag, Eitemperatur und Stromstärke in Versuchsreihe 1

		Heartbeat BPM	Egg temperature °C	Current A
N		299	299	286
Average		205	32.2	3.82
Median		204	32.5	3.83
Standard deviation		44	1.70	0.716
Variance		1926	2.88	0.512
Minimum		74	25.0	0.65
Maximum		297	35.5	5.65
Percentiles	25	176	31.0	3.45
	50	204	32.5	3.83
	75	242	33.5	4.29

Table 2. Absolute and relative frequency distribution of anaesthesia results in trial series 1

Absolute und relative Häufigkeitsverteilung der Betäubungsresultate in Versuchsreihe 1

	Absolute	Relative (%)	Cumulative (%)
Motionless/anesthetised	255	89.2	89.2
Motion/anesthetised	29	10.1	99.3
Motion/non-anesthetised	2	0.7	100.0

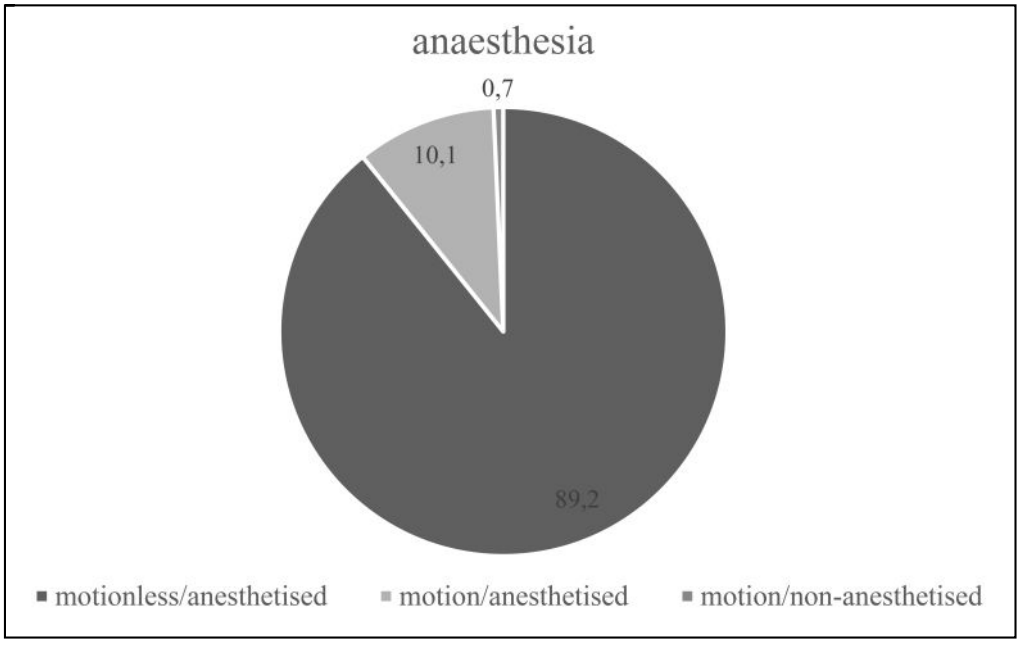

Figure 6. Frequency distribution of anaesthesia results in percent

Häufigkeitsverteilung der Betäubungsergebnis-Gruppen der Versuchsreihe 1 in Prozent

Table 3. Frequencies of the occurred movements in the experimental groups

Häufigkeiten der aufgetretenen Bewegungen in den Versuchsgruppen

	n	Beak opening	Head movement	Movement of wings	Movement of toes/legs	Reaction on mechanical irritation
Motionless/anesthetised	254	0 0%	0 0%	0 0%	0 0%	0 0%
Motion/anesthetised	29	4 13.8%	22 75.9%	6 20.7%	7 24.1%	5 17.2%
Motion/non-anesthetised	2	1 50%	1 50%	2 100%	2 100%	1 50%
Control	13	10 76.9%	12 92.3%	12 92.3%	12 92.3%	12 92.3%

For the variable “heartbeat” no significant association with the anaesthesia result was found by contingency table, chi-square test and correlation measures phi and Cramer's V ($p = 0.593$). For the variables “maximum current” ($p = 0.263$) and “egg temperature” ($p = 0.138$) no significant effects could be found in the same tests either. The binary logistic regression resulted in a significant model with all three variables ($p = 0.001$). However, none of the variables achieved a significant coefficient (heartbeat $p = 0.713$; egg temperature $p = 0.354$; current $p = 0.179$). In general, at higher temperatures and currents, there is a higher chance that the embryo is motionless. For the heartbeat, lower values tended to lead to a higher chance of achieving motionlessness. However, these tendencies were not significant.

Treatment and control groups differed significantly ($p = 0.001$) in all five evaluation criteria. Absence of head movements, wing movements, leg and toe movements after electrical flow as well as reaction to tactile stimulations were highly associated to the treatment with association values phi and Cramers V of 0.959 each. The association value for beak opening was slightly weaker at 0.872 still corresponding to a strong association according to COHEN (1988).

In the second series of tests, the embryos' heartbeat before treatment averaged 267 BPM; the mean egg temperature was 34.2°C. An average maximum current intensity of 4.03 A was achieved during the electrical current flow (Table 4). In none of the observed embryos injuries caused by the electrodes were detected. In 49 treatments, 48 embryos were classified as successfully anesthetised due to the absence of movements (Table 5). Through the window in the eggshell it was observed that the embryos showed a short cramping during the current flow, after which they were motionless. One embryo was classified as not anesthetised due to a low current flow of 0.4 A resulting from incorrect penetration depth of the electrodes. On the other hand, another embryo, which was also treated with a low current flow (0.7 A) showed the known jerky movement pattern and was therefore classified as anesthetised.

Table 4. Location parameters and measures of variation of heartbeat, eggshell temperature and current in trial series 2

Deskriptive Statistik mit Lage- und Streuungsparametern des variablen Herzschlags, der Eitemperatur und Stromstärke in Versuchsreihe 2

		Heartbeat BPM	Egg temperature °C	Current A
N		52	52	49
Average		267	34.2	4.03
Median		254	34.3	4.2
Standard deviation		63.2	0.787	1.16
Variance		3992	0.620	1.36
Minimum		117	32.7	0.4
Maximum		375	36.1	5.78
Percentiles	25	236	33.7	3.87
	50	254	34.25	4.2
	75	325	34.7	4.62

Table 5. Absolute and relative frequency distribution of anaesthesia results in trial series 2

Absolute und relative Häufigkeitsverteilung der Betäubungsresultate in Versuchsreihe 2

	Absolute	Relative (%)	Cumulative (%)
Motionless/anesthetised	47	95.9	95.9
Motion/anesthetised	1	2.0	97.9
Motion/non-anesthetised	1	2.0	100.0

Discussion

In EC Regulation 1099/2009 Annex I, Chapter I, Table 2, point 2, electrical stunning is characterised by head-to-body electrical flow with an epileptic form on the EEG and the fibrillation or stopping of the heartbeat. Since EEG activity is not detectable in the embryo before day 15 of incubation (MELLOR and DIESCH, 2007) and methods of electrical anaesthesia of embryos have not yet been scientifically investigated, ventricular fibrillation/cardiac arrest was chosen as preferred criteria for evaluating the effects of electrical flow. Ventricular fibrillation is dependent on various factors, which include the path of current flow through the body, the region of the heart where the current arrives, the phase of the heart rhythm, and the duration, frequency and form of the current (GREGORY et al., 1991). GREGORY (1998) described that it is difficult to induce ventricular fibrillation with electric current in animals with high heart rates. EFSA (2004) also describes that in small animals (piglets, young lambs, rabbits), it may be difficult to induce cardiac ventricular fibrillation due to the small size of the heart resulting in the current to pass through tissues surrounding the heart rather than through the heart directly. The electrical resistance of various other tissues in the pathway may also a play role. The embryos are still rather small and surrounded by relatively large amounts of egg fluid and yolk on day 13 of incubation and hence it is unclear, which path the current takes in the egg or embryo.

The parameters voltage or current intensity and the duration of current flow were varied in initial experiments, while for technical reasons the frequency could not be changed. In none of the investigated variants ventricular fibrillation or cardiac arrest was observed. Preliminary tests with very high voltage ranges (400 V, 1000 V) could not trigger ventricular fibrillation or cardiac arrest. In the 1000 V variant, the body of the embryo was even structurally dissolved and the eggshell destroyed, while the heart continued to beat. Therefore, the heartbeat cannot be used as a criterion to prove a possible anaesthetic effect of the electrical current flow.

Reflexes such as the corneal reflex, which can be used in birds to evaluate the narcotic level (HEATLEY, 2008 according to LIERZ and KORBEL, 2012), are not yet developed in embryos on day 13 of incubation. In contrast to this PRINZ et al. (2012) came to the conclusion that reflexes cannot be used to assess the state of anaesthesia in adult chickens. However, this trial deals with embryos and an EEG cannot be measured at this age (MELLOR and DIESCH, 2007). According to CHUMAK (1961) and ALEKSANDROWICZ and HERR (2015), five criteria can be used as a basis for evaluating successful anaesthesia: Absence of beak opening, movement of head, wings, toes or feet and reaction to

tactile stimulation. The five selected criteria proved to be very suitable for the assessment and differences between controls and treated embryos. The statistical evaluation with phi and Cramers V showed a significant, strong association between treatment and all five criteria.

According to ALEKSANDROWICZ and HERR (2015) embryos are considered to be anesthetised if no spontaneous and coordinated movements can be detected. Muscle jerks or reflexes can occur, for example, during a clonic phase triggered by electrical current and therefore do not indicate consciousness (JENNINGS, 2004 according to VON HOLLEBEN et al., 2010; GRILLNER et al., 2008). Other than in mammals, grand-mal epilepsy is usually not observed in poultry after electrical stunning (GREGORY and WOTTON, 1987). SCHUTT-ABRAHAM et al. (1983, according to EFSA, 2004) state that a short period of epileptiform activity immediately followed by a profoundly suppressed or quiescent EEG is typical. This is in line with our observations of brief muscle spasms during electrical flow followed by motionlessness. Hence, motionlessness can be considered as an indicator for effective anaesthesia and elimination of pain perception. Presumably the current flow in the embryos leads to a severe functional disorder of muscles and nerves, which could be further investigated in a histological or neurological study.

The analyses showed a reliable and repeatable effect of electrical anaesthesia. 99.3% of the embryos were successfully anesthetised. Heartbeat, current intensity or temperature does not affect the anaesthetic result significantly. The average maximum current intensity was relatively high compared to the minimum current intensities specified in EC Regulation 1099/2009. In the EC Regulation 1099/2009 Annex I Chapter II Table 2, minimum currents for waterbath stunning in various animals are mentioned, for instance 45 mA for small poultry such as quails. It can be assumed that not all of the current affects the embryo, as it is surrounded by a high volume of egg mass in the second third of the incubation period. Probably a certain threshold value, which was not examined in detail here, was exceeded in our study, which might have led to the observed motionlessness. Possibly the measurement of the root mean square would have been more reliable (as e.g. applied by PRINZ et al., 2012) but for technical reasons only the maximum value could be measured with the given experimental set-up. The eggshell temperatures were slightly lower than normal incubation temperature and so could be a reason for reduced movements. However, since the statistical analysis found no significant impact of the temperature on the anaesthesia result, it is unlikely that the motionlessness was caused by reduced vitality due to lower eggshell temperature.

For a targeted practical application, it is important to find the reasons for incorrect current flow and insufficient treatment results. However, a full reliability of 100% for a novel method is not easy to achieve in biological systems like embryos.

The group of 29 embryos in the first trial, where movements or reactions were observed after treatment, were monitored more precisely in retrospect. The movements occurred immediately after egg retrieval, lasted only a few seconds until they ended abruptly and were uncoordinated and jerky. The control group in contrast showed more coordinated movements which lasted longer. CHUMAK (1961) described that tactile, algetic, cold and warmth receptors of the skin can cause motor reactions already from incubation day 7 on. Therefore, various factors may be involved as triggers for the jerk-like movements, such as the removal of the embryo from the egg and positioning on a petri dish, damage of the embryonic vascular structures, contact with the forceps or even the temperature difference between egg inside and ambient temperature.

In this study a sample of 300 similar hatching eggs was examined. For further testing and development of a practicable method, a greater variation of experimental samples should be investigated. The eggshells may differ in terms of breaking strength, shell thickness and shell density. These characteristics are influenced by genotype, production system, bird age, feeding, water quality, stress and diseases of the laying hens (LEYENDECKER et al., 2002; ROBERTS, 2004). For example, the breaking strength of eggs decreases over the laying period. As shell stability and bone stability are negatively correlated, differences between genetics may be present (LEYENDECKER et al., 2002). Technical adjustments might be necessary, as thin eggshells could break more easily, e.g. when the electrodes penetrate.

Conclusion

In summary, a very promising "proof of concept" has been developed to use application of an electrical current for anaesthesia of chicken embryos in the second third of incubation. The results of this study should be verified to a greater extent in routine use under practical conditions and can be used as a basis for the development of a practice-ready high-throughput system in hatcheries.

Acknowledgements

The authors are grateful to bsi Schwarzenbek (Training and Consultancy Institute for animal welfare at transport and slaughter) for support.

Authors´Contribution

All authors provided critical feedback and helped shape the research, analysis and manuscript. J. Hurlin, L. Zumbrink and A. Förster planned the study with contribution of M. v. Wenzlawowicz and carried out the experiments. L. Zumbrink analysed data and wrote the manuscript with A. Förster in consultation with J. Hurlin, B. Brenig and M. v. Wenzlawowicz.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

ALEKSANDROWICZ, E., I. HERR, 2015: Ethical euthanasia and short-term anesthesia of the chick embryo. ALTEX **32**(2), 143-147. DOI: 10.14573/altex.1410031.

BJØRNSTAD, S., L.P.E. AUSTDAL, B. ROALD, J.C. GLOVER, R.E. PAULSEN, 2015: Cracking the egg: potential of the developing chicken as a model system for nonclinical safety studies of pharmaceuticals. J. Pharmacol. Exp. Ther. **355**, 386-396.

BOURIN, M., J. PUTERFLAM, I. BOUVAREL, 2020: Avancées concernant les méthodes d'ovosexage des poussins. Tema **54**, 1-8.

CHUMAK, V.I., 1961: Dinamika reflektomykh reaktsii i vklyuchenie retseptornykh apparatov u embriona kuritsy (Dynamics of reflex reactions and initiation of receptor systems in the chick embryo). In: SBORNIK: Voprosy fiziologii i patologii tsentral'noi nervnoi sistemy cheloveka i zhivotnykli v ontogeneze, Moskva, 63-68.

COHEN, J., 1988: Statistical power analysis for the behavioral sciences (2nd ed.). New Jersey, Lawrence Erlbaum Associates, ISBN: 9780805802832.

DEUTSCHER BUNDESTAG, 2017: Zum Schmerzempfinden von Hühnerembryonen. Wissenschaftliche Dienste, Sachstand WD 8 - 3000 - 030/17.

European Food Safety Authority (EFSA), 2004: Scientific Report of the Scientific Panel for Animal Health and Welfare on a request from the Commission related to welfare aspects of animal stunning and killing methods.

GÖHLER, D., B. FISCHER, S. MEISSNER, 2017: *In-ovo* sexing of 14-day-old chicken embryos by pattern analysis in hyperspectral images (VIS/NIR spectra): A non-destructive method for layer lines with gender-specific down feather color. Poult. Sci. **96**, 1-4. DOI: 10.3382/ps/pew282.

GREGORY, N.G., 1998: Physiology of Stress, Distress, Stunning and Slaughter. In: GREGORY, N.G.: Animal welfare and meat science (2nd ed.). Wallingford, Oxon, UK, ISBN: 9781845932152, 64-92.

GREGORY, N.G., L.J. WILKINS, S.B. WOTTON, 1991: Effect of electrical stunning frequency on ventricular fibrillation, downgrading and broken bones in broiler, hens and quails. Brit. Vet. J. **147**, 71-77. DOI: 10.1016/0007-1935(91)90069-y.

GREGORY, N.G., S.B. WOTTON, 1987: Effect of electrical stunning on the electroencephalogram in chickens. Brit. Vet. J. **143**, 175-183. DOI: 10.1016/0007-1935(87)90009-1.

GRILLNER, S., P. WALLEN, K. SAITOH, A. KOZLOV, B. ROBERTSON, 2008: Neural bases of goal-directed locomotion in vertebrates - An overview. Brain Res. Rev. **57**, 2-12. DOI: 10.1016/j.brainresrev.2007.06.027.

HEATLEY, J.J., 2008: Anesthesia and analgesia. In: CHITTY, J., M. LIERZ: BSAVA Manual of Raptors, Pigeons and Passerine Birds. Gloucester, UK, BSAVA Publishing, ISBN: 9781905319046, 97-113.

HIRT, H., 2004: Töten männlicher Legeküken - Situationsanalyse Schweiz 2004. Forschungsinstitut für biologischen Landbau (FiBL). Archived at http://orgprints.org/14779/.

JENNINGS, D.P., 2004: Supraspinal control of posture and movement. In: REECE, W.O.: Dukes' Physiology of Domestic Animals, 12th edition. Cornell University Press, Ithaca, ISBN: 9780801442384, 820-842.

LEENSTRA, F., G. MUNNICHS, V. BEEKMAN, E. VAN DEN HEUVEL-VROMANS, L. ARAMYAN, H. WOELDERS, 2011: Killing day-old chicks? Public opinion regarding potential alternatives. Animal Welfare **20**(1), 37-45. ISSN: 0962-7286.

LEYENDECKER, M., H. HAMANN, J. HARTUNG, G. GLÜNDER, M. NOGOSSEK, U. NEUMANN, C. SÜRIE, J. KAMPHUES, O. DISTL, 2002: Untersuchungen zur Schalenfestigkeit und Knochenstabilität von Legehennen in drei verschiedenen Haltungssystemen. Lohmann Information **2**/2002, 1-6.

LIERZ, M., R. KORBEL, 2012: Anesthesia and Analgesia in Birds. J. Exotic Pet Med. **21**, 44–58. DOI: 10.1053/j.jepm.2011.11.008.

MELLOR, D.J., T.J. DIESCH, 2007: Birth and hatching: Key events in the onset of awareness in the lamb and chick. New Zealand Vet. J. **55**(2), 51-60. DOI: 10.1080/00480169.2007.36742.

PRINZ, S., A. COENEN, F. EHINGER, G. VAN OIJEN, W. BESSEI, 2012: Stunning effectiveness of broiler chickens using a two-phase stunner: pulsed direct current followed by sine wave alternating current. Arch. Geflügelk. **76**(1), 63-71. ISSN 0003-9098.

REIMERS, U., 2008: Untersuchung zur Elektrobetäubung von Regenbogenforellen (*Oncorrhynchus mykiss*). Tierärztliche Hochschule, Hannover. Retrieved from https://elib.tiho-hannover.de/receive/etd_mods_00001652.

RETTER, K., 2014: Untersuchung zur Elektrobetäubung von Karpfen (*Cyprinus carpio L.*). Tierärztliche Hochschule, Hannover. Retrieved from https://elib.tiho-hannover.de/receive/etd_mods_00000604.

RICHARD, L., 2020: Vorfahrt für gesexte Legehennen, DGS-Magazin **31**/2020, 14-19.

ROBERTS, J.R., 2004: Review: Factors affecting egg internal quality and egg shell quality in laying hens. J. Poult. Sci. **41**(3), 161-177. DOI: 10.2141/jpsa.41.161.

SCHUTT-ABRAHAM, I., H.J. WORMUTH, J. FESSEL, J. KNAPP, 1983: In: G. EIKELENBOOM: Stunning of Animals for Slaughter. The Hague: Martinus Nijhoff, 154.

VON HOLLEBEN, K., M. VON WENZLAWOWICZ, N. GREGORY, H. ANIL, A. VELARDE, P. RODRIGUEZ, B. CENCI GOGA, B. CATANESE, B. LAMBOOIJ, 2010: Report on good and adverse practices - Animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary sciences. Schwarzenbek, bsi Schwarzenbek.