When do chicken embryos start to feel pain? On the 13th day of incubation.

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From day 13 of incubation, a chick embryo responds to stimuli, which can be seen on an EEG (brain electricity). This is important to know because male chick embryos may no longer be killed in Germany after day 6 of incubation, according to the law that will come into force on 1 January 2024. But at what point can an embryo feel pain? This was the question that led to the change from day 6 to day 13.

Intro:
The news was:
Genus Focus non-invasive in-ovo (in the egg) sexing fully aligned with animal welfare standards; new German study proves pain perception does not start before day 13.

"Orbem and Vencomatic Group are excited about the fact that a recent study commissioned by the German Federal Ministry of Food and Agriculture (BMEL) confirms that the physiological neural activity of chick embryos does not start before day 13 of incubation – meaning pain cannot be processed before this time. This finding is crucial for animal welfare standards, as it provides scientific evidence to support the planned adjustment to the current German ban on chick culling after day 6 of incubation. The German federal cabinet (Bundes-kabinett) has decided to draft an aid to adapt the prohibition for ending the incubation process of chick embryos after day 12, as of January 1, 2024.

Dr. Maria Laparidou, Chief Scientific Officer (CSO) at Orbem, veterinarian, and embryo development expert comments: "The results found in this latest study of pain perception in embryos are confirming that the *Genus Focus non-invasive technology* is the way forward when it comes to in-ovo sexing in the most animal-friendly way. A perfect match with our mission to develop technology that meets high standards of animal welfare and sustainability."

EEG: Electroencephalography is a method to record an electrogram of the spontaneous electrical activity of the brain.

About a year ago the two companies announced their strategic partnership for Genus Focus: an in the egg sexing solution that can detect the sex of embryos before day 13 of incubation with ***MRI & Artificial Intelligence technology***.

The teams worked intensively to develop and install their modular trolley-to-trolley solution, combining Orbem's Genus Focus technology with a newly developed Prinzen automated egg-handling system, allowing 24,000 eggs per hour to be scanned. Given the proposed adjustment of German regulations to allow in-ovo sexing on day 12 and also after 1 January 2024, the companies expect to soon deliver their first German installation.

Now I want to know how they came to this conclusion about which day of incubation.

Basic info on the embryo and developmental interesting things you find in the book Genetics of the chicken Extremes.

PROJECT SUMMARY:
PAIN PERCEPTION IN CHICKEN EMBRYOS

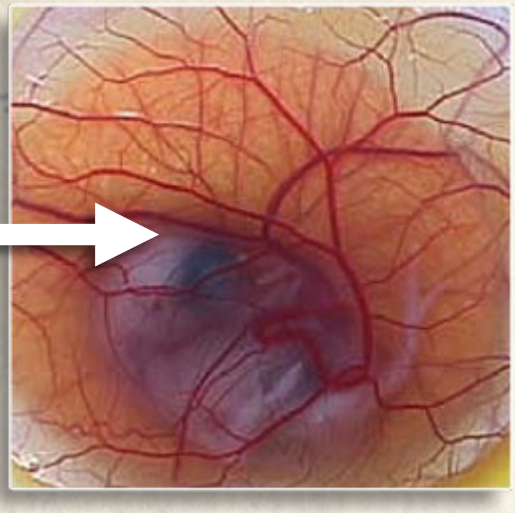
Introduction
In Germany, the ***killing*** of ***male day-old chicks*** has been ***prohibited*** by law since ***2022*** (TierSchG, 2022).
From 2024, it will also be ***prohibited*** to ***kill male chicken embryos after the 6th day*** of ***incubation*** following sex determination in the egg.
This development time was set because previous studies indicate that the beginning development of pain perception in chicken embryos from the 7th day of incubation (embryonic day, ED), and thus also the ability to perceive nociception (stimulus transmission) in the egg, cannot be ruled out (Bjørnstad et al., 2015; Krautwald-Junghanns et al., 2018). With this scientific basis

Embryos on scale 1:1 when this PDF is set ofnf 100% view

Day 11, 2 cm, here 1:1.

Day 12, 4 cm, here 1:1.

Day 13: 4,7 cm, here 1:1. Hamburger-Hamilton stage: 39. Right: yolk sack, blood vessels are app. 7 cm in cross-section.



for the law in mind, the Federal Ministry of Food and Agriculture commissioned a study with the aim of determining the period in which chicken embryos develop the ability to perceive nociception, or the ability to perceive pain from the aversive sensory experience.

Methods

In the present study, Lohman Selected Leghorn chicken embryos were used from hatching day ED7 to ED19. All measurements were performed in ovo (in the egg).
A noxious (actually or potentially tissue-damaging) stimulus and a control stimulus were applied in randomised order. A mechanical stimulus was used as the noxious stimulus for the cardiovascular parameters and the behavioural observations at the base of the beak.

The response was compared with the stimulus by touching the beak (control). As a second control group, the local anaesthetic lidocaine was applied to the base of the beak on ED18 before the mechanical stimulus.

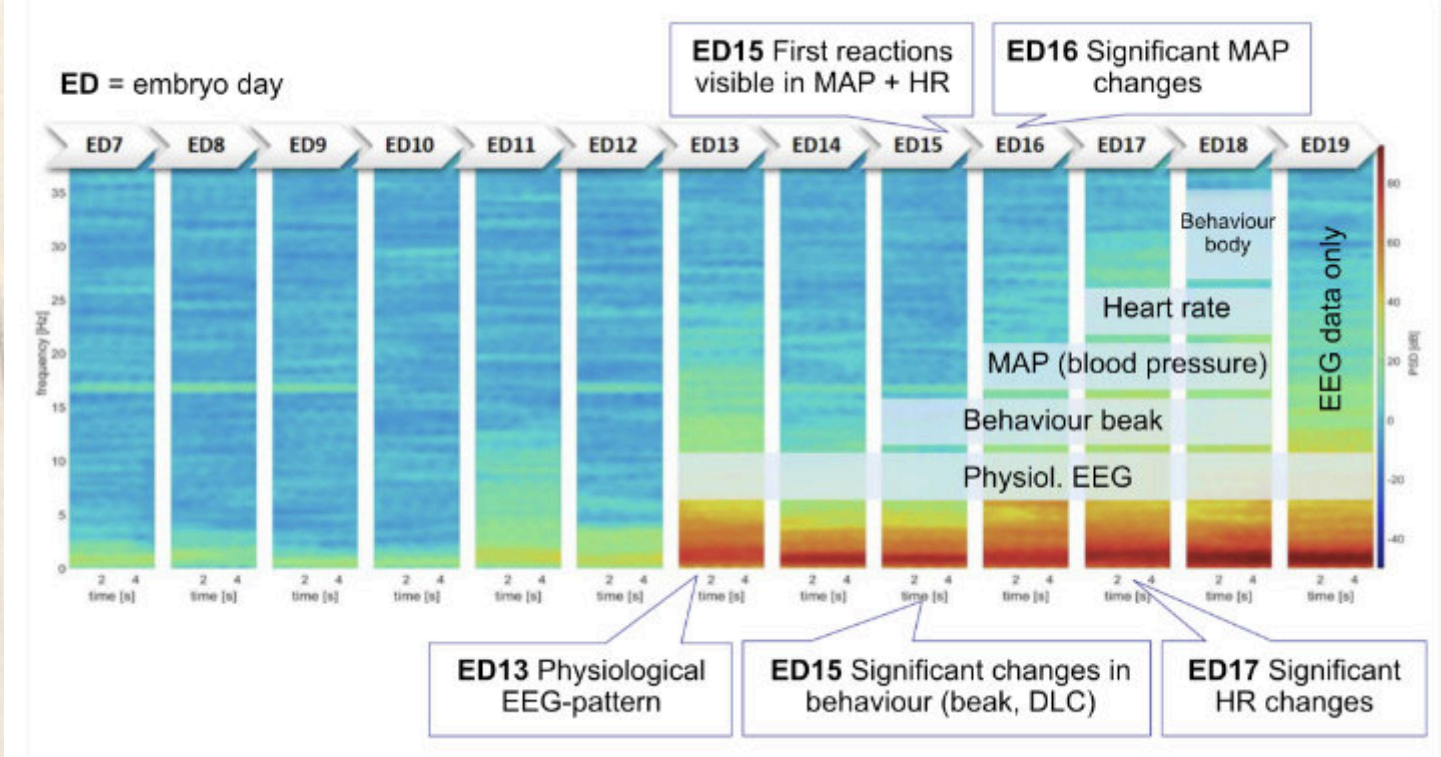
For the electrophysiological parameters, noxious stimuli (heat stimulus, electrical stimulus) were used with a Peltier element and with stimulation electrodes.

The reactions of the chicken embryos were analysed using the following parameters:
Cardiovascular parameters: Mean arterial blood pressure (MAP) and heart rate (HR) were recorded using a microcatheter inserted into an artery of the chorioallantoic membrane.

Behaviour: Movements were recorded and analysed using deep learning software (DeepLabCut, DLC) and a manual score.

Electroencephalogram (EEG): Electrical brain activity was

Overview of significant responses to mechanical stimulation in the respective parameters (MAP, HR, behaviour). Illustration based on the spectral analysis of a representative EEG of the individual breeding days, which shows the start of a physiological EEG from ED13.



measured with measuring electrodes in the hyperpallium and cerebellum.

Results

Cardiovascular parameters:
Following the mechanical stimulus, mean arterial blood pressure (MAP) of chicken embryos on embryonic day (ED) 16 to 18 increased significantly. A significant increase in heart rate (HR) was also observed after the mechanical stimulus in ED17 and ED18. Injection of the local anaesthetic lidocaine significantly reduced the response of MAP to the mechanical stimulus in ED18 embryos.
Individual embryos showed responses in blood pressure and heart rate at ED15.
However, no significant differences were observed between mechanical stimulation and touch.
No increase in blood pressure or heart rate was observed in embryos younger than ED15.

Behaviour: Beak movements increased significantly after the mechanical stimulus in ED15 to ED18 embryos compared to touch. In the first 30 seconds after the mechanical stimulus, head, elbow and metatarsal movements also increased significantly in ED18.

EEG: From ED7 to ED12, no physiological EEG of the chicken embryos in the egg could be detected. From ED13 onwards, physiological brain activity could be reliably recorded. An adequate EEG response to the thermal or electrical stimulus could not be recorded from ED13 onwards.

Summary
In summary, cardiovascular responses to a mechanical stimulus occurred significantly from ED16, and in individuals from ED15. A significant behavioural response to a mechanical stimulus was also observed in ED15 to ED18 embryos. The ability to transmit stimuli (nociception) cannot be ruled out on the basis of the results at ED15 and can be assumed from ED16 onwards.
With regard to the EEG analysis, physiological neuronal activity of the brain (EEG) was measurable from ED13 onwards. On the one hand, this allows the conclusion that the ability to transmit stimuli (nociception), or the ability to perceive aversive sensory experiences as pain, is potentially present from this point onwards. On the other hand, it shows that the processing of a noxious stimulus in the brain does not appear to be possible up to and including ED12.

Links:
Link to the original German pain-paper: https://www.bmel.de/SharedDocs/Downloads/DE/_Tiere/Tierschutz/bericht-21-6a-tierschg-anlage.html

Link to the manufacturer of the in-ovo sexing machine: https://www.vencomaticgroup.com/genusfocus-inovosexing