

Rules for in-ovo sexing before the 13th day of incubation

Appendix 3 to the Respeggt System Manual

The 2023 study on the sensation of pain in chicken embryos conducted by the Technical University of Munich shows that embryonic brain activity can be measured from the 13th day of incubation. The calculation is represented by ED (Embryonic Development) and starts at ED0. From ED13, distinct EEG (electroencephalogram) signals were measurable for the first time, as can be seen in Figure 1.

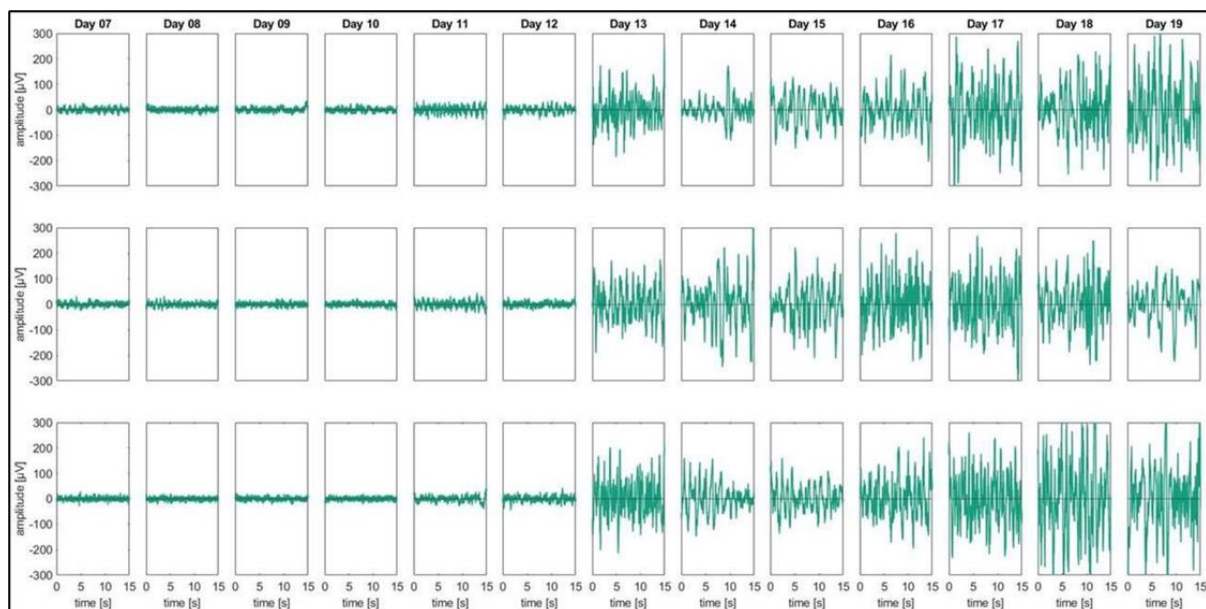


Figure 1: Raw EEG data: an overview of 15 seconds of raw EEG data from randomly selected embryo data sets in developmental stages ED07-ED19. The onset of physiological EEG signals is clearly recognisable from ED13. Source: Kollmansperger et al. (2023): *Nociception in Chicken Embryos, Part II: Embryonal Development of Electroencephalic Neuronal Activity In Ovo as a Prerequisite for Nociception*. *Animals (Basel)*. 2023 (<https://pmc.ncbi.nlm.nih.gov/articles/PMC10525651/>)

This EEG measurement illustrates that a sensation of pain can be ruled out before day 13 of incubation, as the occurrence of pronounced EEG activity can be clearly attributed to day 13 of incubation. This study is considered the basis for a ban on chick culling after day 12 of incubation in Germany. Respeggt also recognises this study as a basis for supply chain verification.

Hatching practice shows that the hatching of laying hen chicks varies greatly depending on the location and country. The duration of incubation, temperature and humidity in the incubator can vary greatly. Due to temperatural differences, the storage conditions

influence embryonic development during pre-incubation¹. Therefore, the definition of an incubation day is difficult to link to embryonic development. Respeggt has therefore developed a standard to guarantee in-ovo sexing before brain activity begins.

This offers all partners the security of being able to offer the **added value of "Free of Chick Culling" in an ethically correct** way and to establish in-ovo sexing as the best and safest solution for ending chick culling.

In order to avoid the strong fluctuation at the beginning of the incubation due to different methods of storage and preheating of hatching eggs, Respeggt calculates the limit for the application of in-ovo sexing backwards from hatching.

This assumes an incubation period of 510 hours (21.25 incubation days) with hatching or the removal of the chicks at 6 a.m. on the 21st day of incubation.

The results of the TU Munich show that 12 full days of incubation (12x24 hours) plus 23 hours and 59 minutes can be used for in-ovo sexing. Therefore, **the limit must be set at 198 hours before hatching** (see Figure 2).

Respeggt monitors this limit through digital registration and physical audits.

Incubation day	day 0	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	day 11	day 12	day 13	day 14	day 15	day 16	day 17	day 18	day 19	day 20	day 21
embryonic development (ED)*	ED 0	ED 1	ED 2	ED 3	ED 4	ED 5	ED 6	ED 7	ED 8	ED 9	ED 10	ED 11	ED 12	ED 13	ED 14	ED 15	ED 16	ED 17	ED 18	ED 19	ED 20	ED 21
total hrs of incubation	← 510 hrs →																					
brain activity*	no	no	no	no	no	no	no	no	no	no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes
brain activity hrs	← 312 →												← 198 →									
France																						
Italy (up from 01/01/2027)																						
Germany																						

* according to Study from TU Munich (2023)

Figure 2: The brain activity of a chicken embryo in hours depending on the day of incubation (according to Kollmansperger et al., 2023)

1 Sellier et al. (2006) : Comparative Staging of Embryo Development in Chicken, Turkey, Duck, Goose, Guinea Fowl, and Japanese Quail Assessed from Five Hours After Fertilization Through Seventy-Two Hours of Incubation. *Journal of Applied Poultry Research*. 2006 (<https://www.sciencedirect.com/science/article/pii/S1056617119315211>)