

Avian sexual development

References

- Activation of estrogen receptor alpha disrupts differentiation of the reproductive organs in chicken embryos, Anna Mattsson, Jan A. Olsson, Björn Brunström. *General and Comparative Endocrinology* 172 (2011) 251–259
- A suggested bisphenol A metabolite (MBP) interfered with reproductive organ development in the chicken embryo while a human-relevant mixture of phthalate monoesters had no such effects, Anna Mentor, Carl-Gustaf Bornehag, Maria Jönsson & Anna Mattsson. *J Toxicol Environ Health A* . 2020 Jan 17;83(2):66-81

Summary of the method

This test is based on fertilized White Leghorn (*Gallus domesticus*) eggs.

Early in development, the embryo has pairs of undifferentiated gonads and Mullerian ducts which subsequently differentiate in a sex-specific manner.

In this test, on incubation day 4, test substances are injected into the yolk, and embryos are dissected after 18 days of incubation.

Gross morphology of gonads and Mullerian ducts are examined under a stereo microscope. The Gonads are also histologically observed, and sex is further confirmed by genotyping. Finally, mRNA levels of two egg yolk proteins (Vitellogenin and very low-density apolipoprotein) are measured.

This test is not covered by the European Directive on the protection of animals used for scientific purposes.

Necessary equipment

- Egg incubator
 - Automatic turning of the eggs
 - Humidity: 60-70%
 - Temperature: 37± 0.5 °C
- Drill to make a hole in the egg shell (e.g. machine: https://www.amazon.se/Dremel-F0137760JA-DREMEL-Multiverktyg-7760-15/dp/B07RCHZYT6/ref=asc_df_B07RCHZYT6/?tag=shpngadsglede-21&linkCode=df0&hvadid=476515861198&hvpos=&hvnetw=g&hvrnd=6988596636271168)

[842&hvpone=&hvptwo=&hvgmt=&hvdev=c&hvdvcmdl=&hvlocint=&hvlocphy=1012595&hvtagid=pla-821982456368&pssc=1](https://www.google.com/shopping/product/10453786149403603718?hl=sv&q=tandl%C3%A44karborr&prds=epd:8605870001882006872,eto:8605870001882006872_0,prmr:1&sa=X&ved=0ahUKEwjY8MLxsZD0AhUJR_EDHUxnDwcQ9pwGCAC), drill:

https://www.google.com/shopping/product/10453786149403603718?hl=sv&q=tandl%C3%A44karborr&prds=epd:8605870001882006872,eto:8605870001882006872_0,prmr:1&sa=X&ved=0ahUKEwjY8MLxsZD0AhUJR_EDHUxnDwcQ9pwGCAC)

- Tools for injection of test solution (1-ml Hamilton syringes + dispenser or electronic pipette dosing 20 µl / injection).
- Equipment for egg candling (white light source that allows for screening if the eggs are fertilized and alive)
- Dissection tools: sharp scissors (to euthanize by decapitation and smaller for dissection), forceps
- Calipers 0.5-20 mm
- Digital camera + stand (pictures need to be analyzed by image analysis – experience in image analysis is needed) (e.g. a Canon EOS 100D (EF-S 18-55 IS STM kit) camera)
- Histology equipment (embedding, sectioning, staining, etc) alternatively, to send samples to a histology lab for sectioning?
- Real-time PCR machine
 - Genetic sex determination (PCR + gel electrophoresis can also be used)
 - Measure aromatase transcript¹⁾

1) Either histology or qPCR is feasible - if both methods are used they cannot be applied to the same individual