



**XVI European Poultry Conference**  
VALENCIA, SPAIN 24<sup>th</sup>-28<sup>th</sup> June 2024



## Sexual dimorphism during incubation and development of ovosexing methods



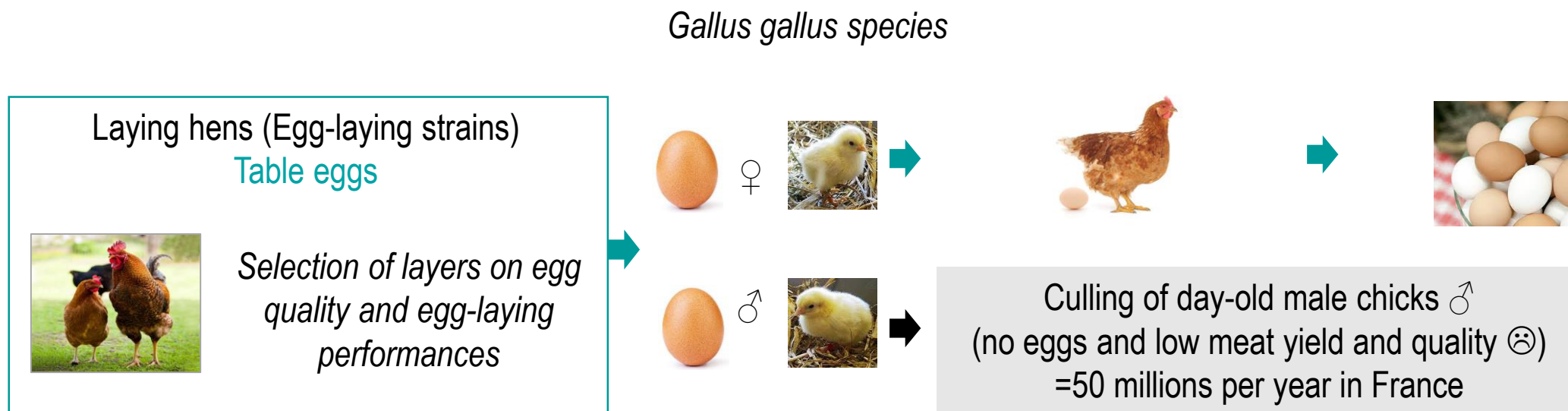
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➤ *Ethical concern related to the culling of hatched birds in poultry production (1/2)*

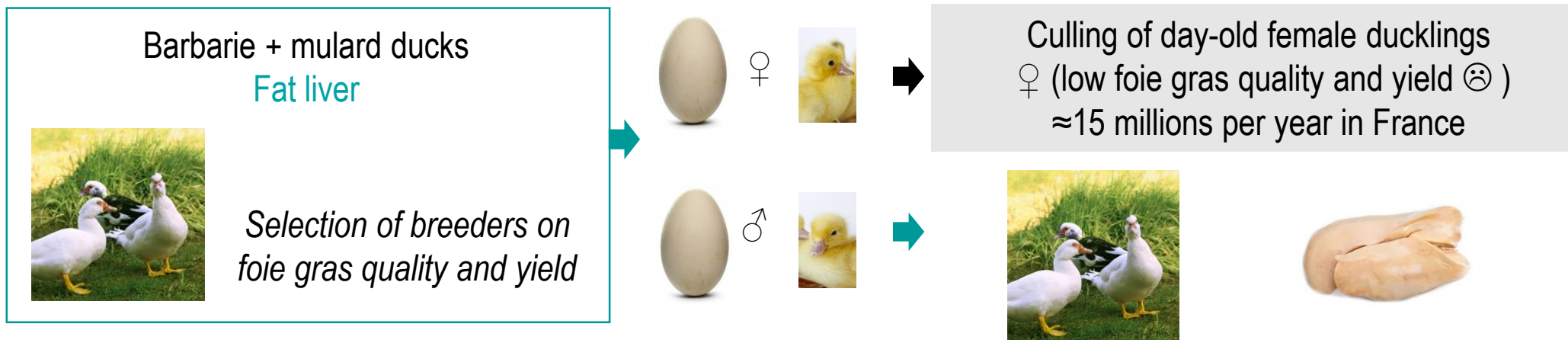
When the phenotypic and physiological characteristics of a sex do not meet the criteria for productivity and/or product quality



➤ *Ethical concern related to the culling of hatched birds in poultry production (2/2)*

When the phenotypic and physiological characteristics of a sex do not meet the criteria for productivity and/or product quality

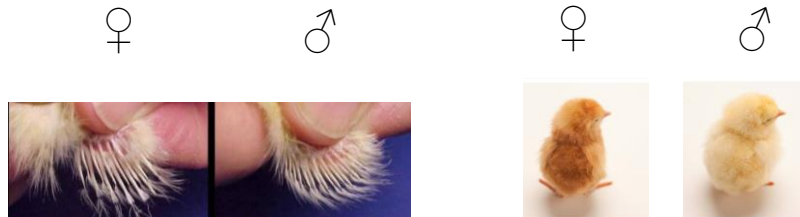
*Anas platyrhynchos* species



## ➤ Sexing day-old chicks



**Egg-laying strains, *Gallus gallus***  
-anatomical differences in the cloaca  
-size of remiges or feather colour (brown strains)



Sorting after hatching



**“Fat liver” strains, *Anas platyrhynchos***  
-autosexing strain (eye colour: red for females, black for males)



≈50% female ducklings are reared to be sold as farm cans, for roasting (export)

## ➤ *Methods used for culling chicks and management of dead birds*



Based on AVMA recommendations

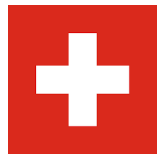
Grinding (maceration using a dedicated equipment)  
Gas inhalation (CO<sub>2</sub> at 75% for 5 minutes)

**After CO<sub>2</sub> treatment:** Wildlife parks, animal rescue associations  
**After maceration :** pet food processing plant



Ethical concerns

➤ *A highly publicized awareness, followed by ministerial announcements banning the culling of chicks in various European countries*



January. 2020



May 2021 (start in 2022)



Feb. 2022



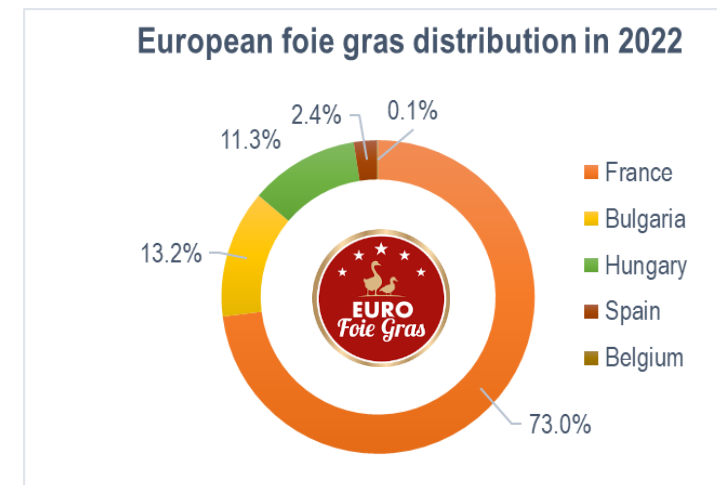
Décret n° 2022-137 du 5 février 2022 relatif à l'interdiction de mise à mort des poussins des lignées de l'espèce Gallus gallus destinées à la production d'œufs de consommation et à la protection des animaux dans le cadre de leur mise à mort en dehors des établissements d'abattage



Luxembourg, Finland, Cyprus, Ireland, Spain, Belgium, Netherlands, Greece, Slovakia, Portugal...

### What about the foie gras industry?

In ovo sexing instrumentations to be installed by July 2024



## ➤ Three alternatives to the culling of day-old male chicks

### 1 Rearing brothers of laying hens (actual layers)



Limited markets but initiatives in progress  
(reconstituted sausages/meat)

### 2 Dual-purpose breeds



Many trials assessed or being currently evaluated  
High cost, quality of by-products to be optimized  
Hardly applicable at present

### 3 *In ovo* sexing



Deployed in all five layer hatcheries in France : no major change in the organisation of the egg industry; no need to develop new markets in the broiler poultry sector; concentration of efforts at hatchery level

## ➤ Principles of ovosexing techniques

The development of ovosexing techniques is based on the detection of differences between eggs containing an ♂ embryo or a ♀ embryo  
= sexual dimorphism

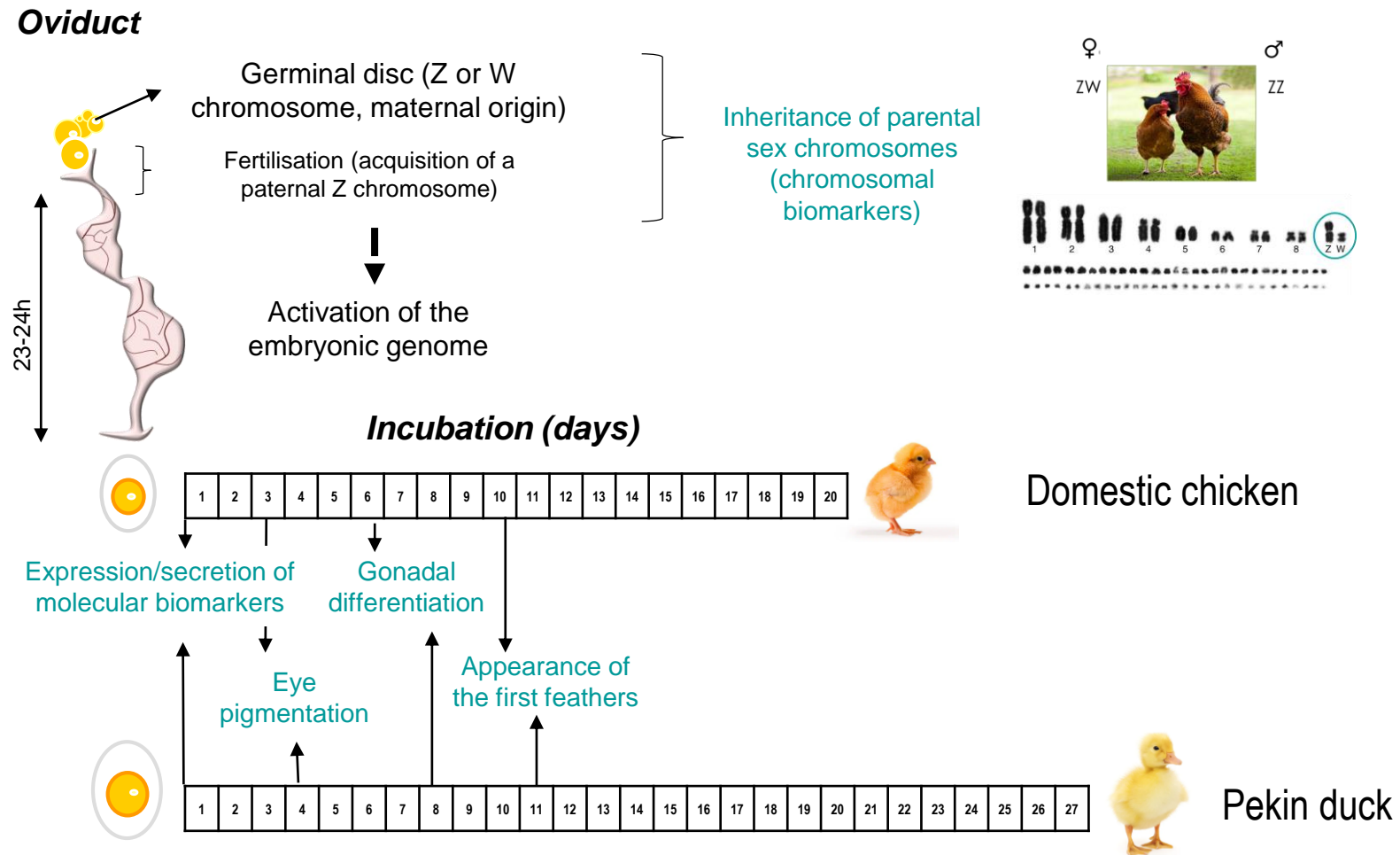
- 1 genetic biomarkers (sex chromosomes)
- 2 anatomical features (development of gonads, others ?)
- 3 phenotypic traits (feather colour and size, others ?)
- 4 molecular characteristics (lipids, proteins, genes, hormones, metabolites, volatile compounds...)

Some of these features are visible/detectable belatedly in the incubation process and result from the activation of the genome and embryonic metabolism





# ➤ Timeline for sexual dimorphism *in ovo*



For Foie Gras production: Barbarie and Mullard ducks (31 and 35 days of incubation, respectively)

## ➤ Development of *in ovo* sexing methods in practice

**Requirements** : to be able to identify the sex of the embryo at the targeted time of incubation (several techniques available depending on the developmental stage of the embryo)

### Initial procedure

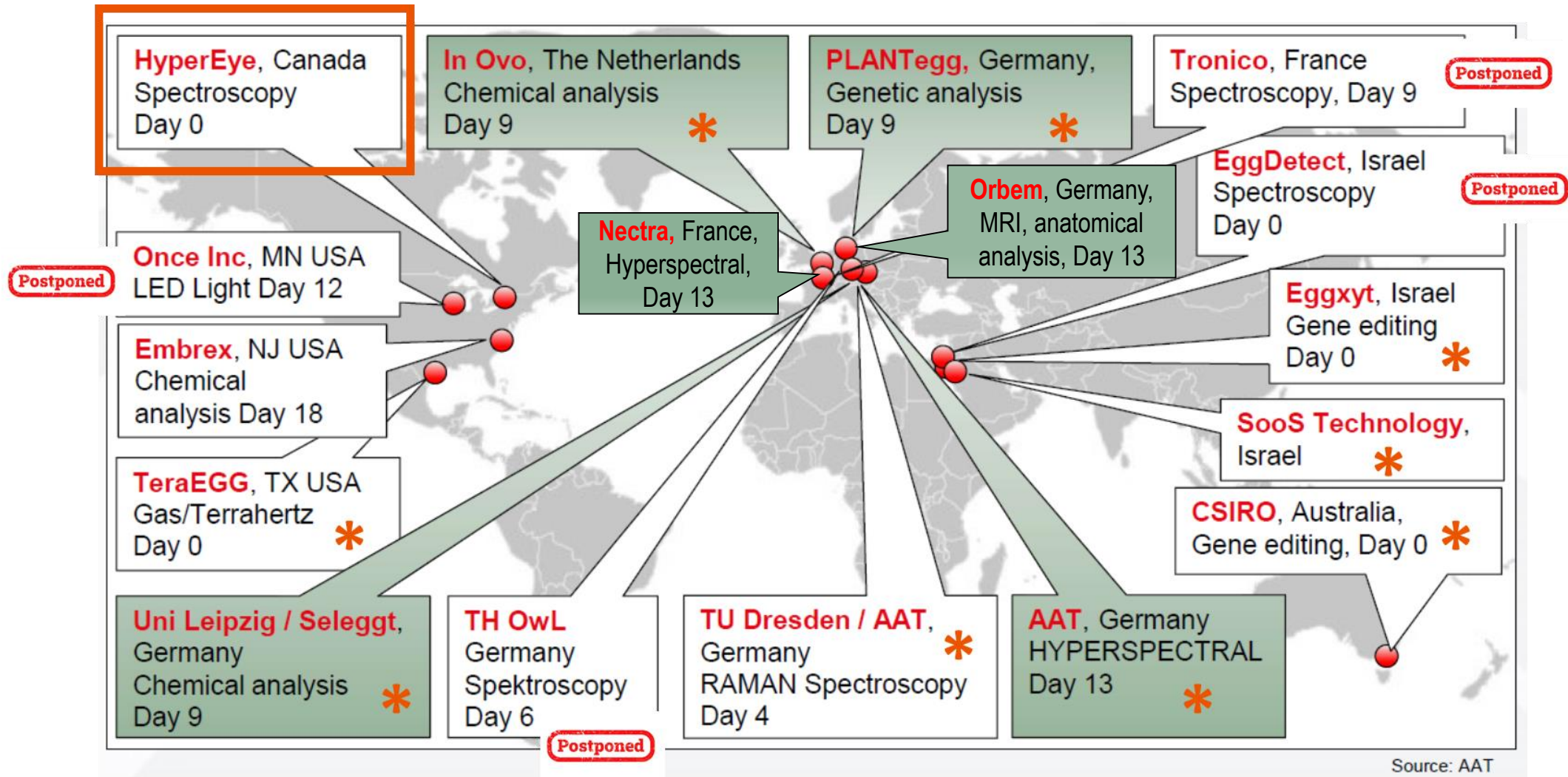
Step 1: Search for dimorphic sex indicators / markers (embryo / egg structures)

Step 2: Validate the markers on a large number of eggs, ideally from genetically different breeds and with different characteristics (weight/shape/shell colour, etc.)

### Challenges

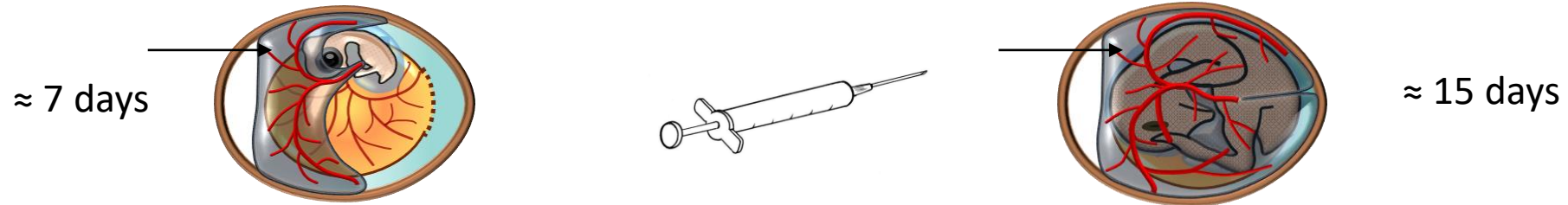
- Detection as early as possible
- Non-invasive, non-destructive (no impact on the development and viability of the embryo and animals after hatching)
- Fast, accurate, and effective
- Applicable in the field, on a variety of genetic strains
- With acceptable cost for professionals and consumers




# ➤ Overview of the methods that have been developed in the last decades



6 marketed techniques (3 semi-invasive / 3 non-invasive)

## ➤ Semi-invasive methods with *in ovo* sampling



|   | Who ?   | Country         | Method                      | Strain Specificity  | Incubation day | Precision | Speed                          |
|---|---|-----------------|-----------------------------|---------------------|----------------|-----------|--------------------------------|
| <b>Semi-invasive</b><br>(sanitary risk and chick mortality) |  PLANTegg  | Germany         | Dosage (sex-specific genes) | All chicken strains | 9              | >95%      | Low (3,000 to 6,000 eggs/hour) |
|   |  SELEGGT<br><small>responsible solutions</small> | Germany         | Dosage (hormone)            | All chicken strains | 9              | >95%      | Low (3,000 to 6,000 eggs/hour) |
|   |  INOVO   | The Netherlands | Dosage (metabolites)        | All chicken strains | 9              | >95%      | Low (3,000 to 6,000 eggs/hour) |

**Day 9 (of 21)**



# ➤ Non invasive methods based on the sampling of egg structures

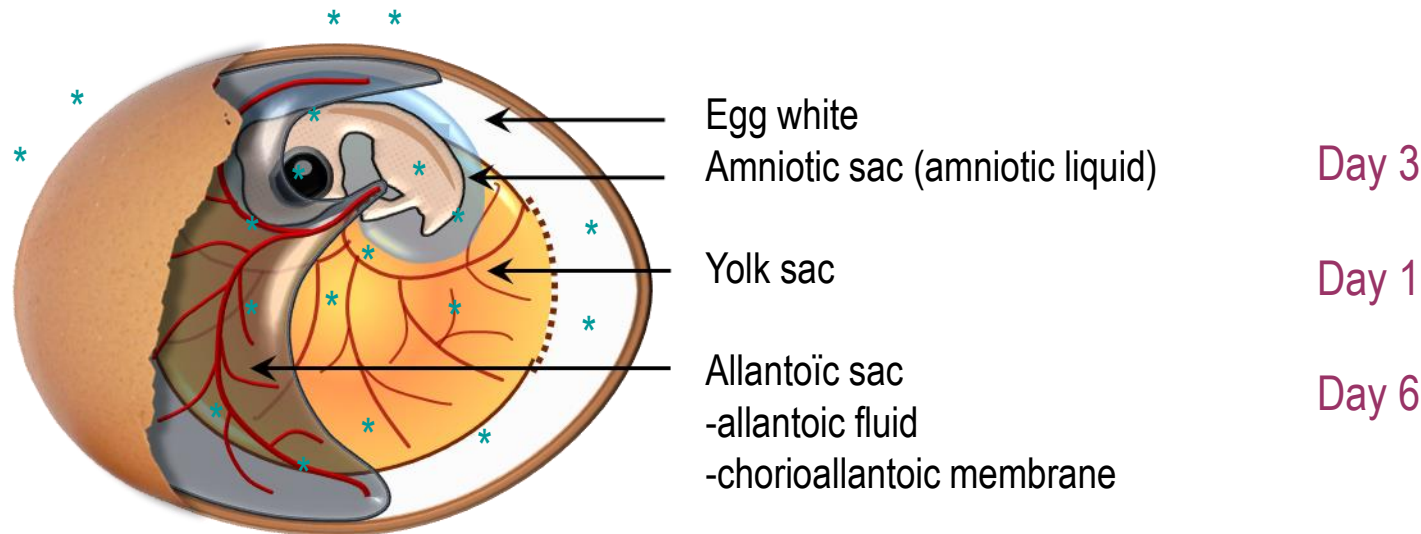
→ Difference in sex chromosomes (size, type and number of genes)



→ Difference in yolk and egg white assimilation between ♂ and ♀ (linked to different ♂ and ♀ metabolisms)

→ Excretion/secretion/diffusion of ♂ and ♀ molecules (\*) in yolk/white, through the shell, and into extra-embryonic structures

Distinct molecular profiles (hormones, metabolites, lipids, proteins, volatile compounds, etc.)



The allantoic fluid develops from the 6th day of incubation onwards

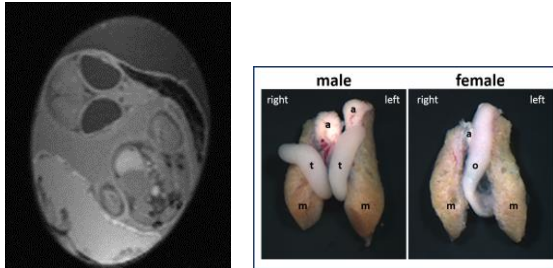
©INRAE, M. Da Silva

At very early stages, with the exception of the egg white (that can be collected through the eggshell), sampling is expected to be very complicated as it is invasive and potentially damaging to the embryo






# ➤ Non invasive methods

Magnetic resonance imaging (MRI) : gonad development



Hyperspectral imaging : chicken strains selected on the colour of feathers (« brown » strains)

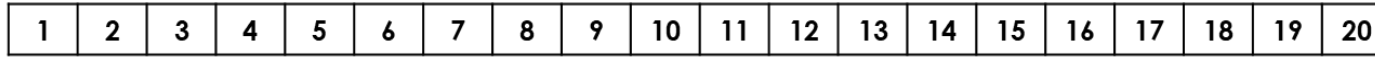


|              | Society   | Country | Method                     | Strain specificity                 | Incubation day | Precision | Speed                 |
|--------------|---|---------|----------------------------|------------------------------------|----------------|-----------|-----------------------|
| Non invasive |    | Germany | MRI                        | All chicken strains                | 12-13          | >95%      | Low (3,000 eggs/hour) |
|              |   | Germany | Hyperspectral spectroscopy | Strains with feather colour sexing | 13             | 96%       | 18-20,000 eggs/hour   |
|              |  | France  | Hyperspectral spectroscopy | Strains with feather colour sexing | 13.5           | >95%      | 20,000 eggs/hour      |

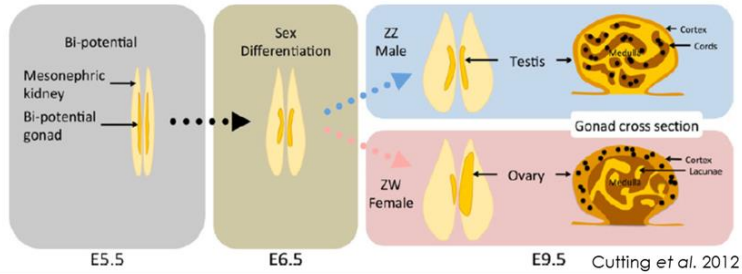
Day 13 (of 21)



# ➤ Current non-invasive methods



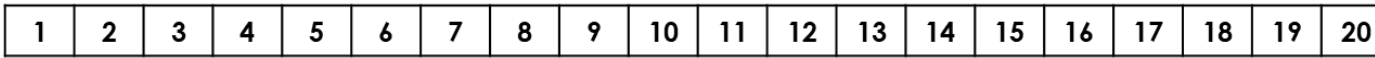
Anatomy (development of gonads)



→ Morphological difference detectable after dissection, as early as 9.5 days



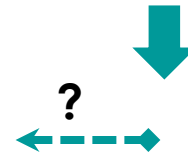
Magnetic resonance imaging (MRI)  
Characteristics: **non invasive, all breeds**



Phenotype (feather colour)

♀

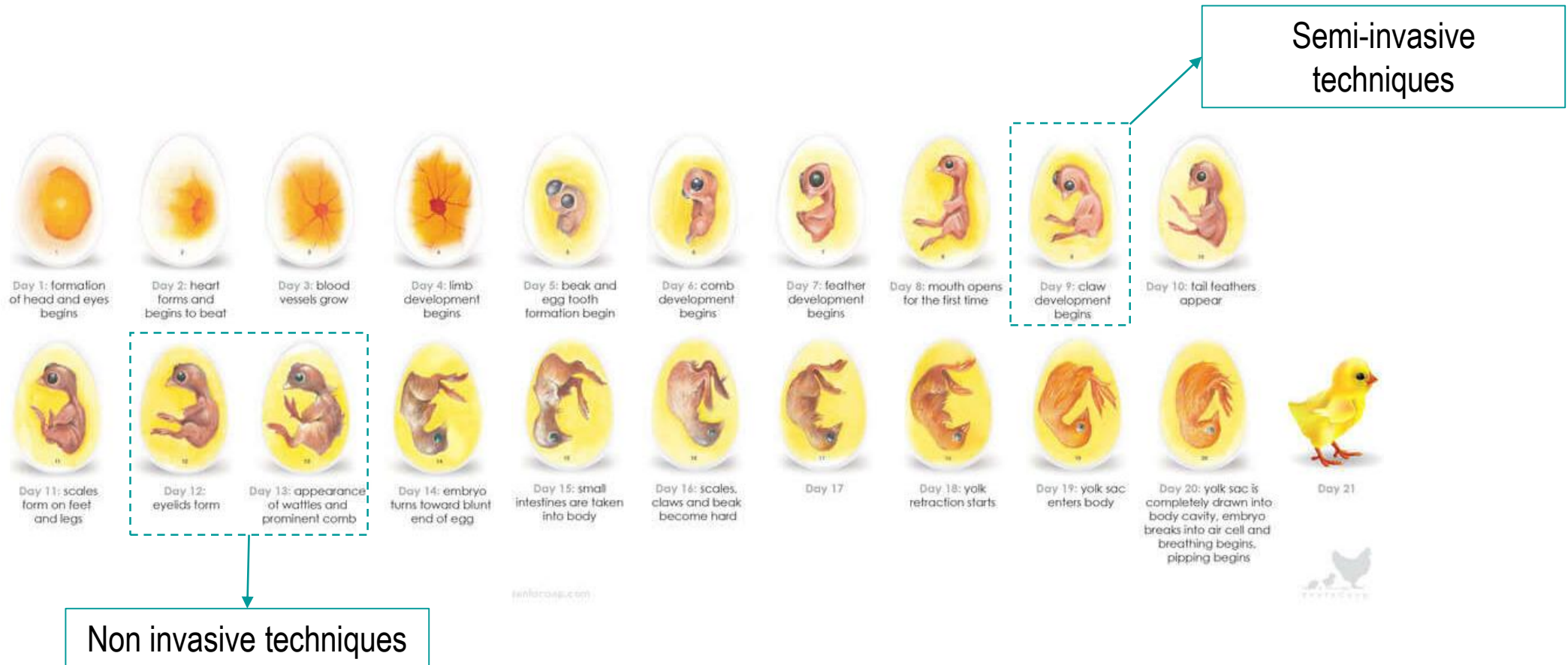
♂



Hyperspectral methods  
Characteristics: **non-invasive / « brown » breeds**

➤ Non-invasive methods enabling early detection will probably be preferred to others

The earlier, the better

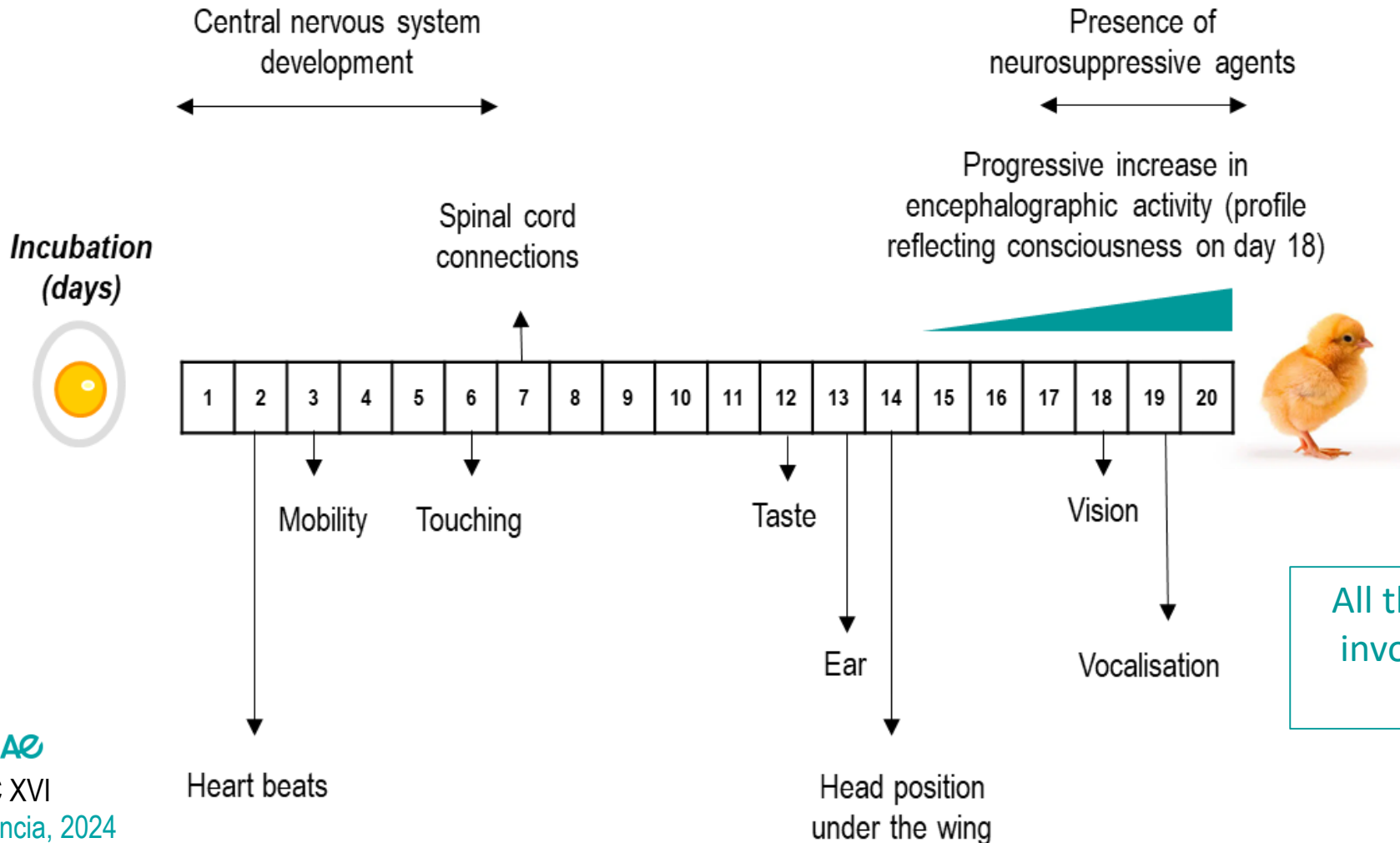


There is still room for improvement



# ➤ Onset of nociception and pain

**Controversy that depends on the parameter that is used to evaluate pain**  
(presence of consciousness, presence of stress biomarkers or neurosuppressive agents, etc.)  
*Grey zone between 8 and 13 days*



# ➤ Techniques avoiding the culling of male embryos

Sex-reversal skewed in favour of ♀ during incubation

SOOS developed an incubation system that affect the sex development process in poultry embryo and turn genetic males into functional female chicks. Our system operate an incubation protocol that control a combination of:



Temperature



Humidity



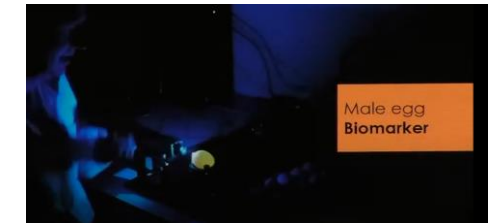
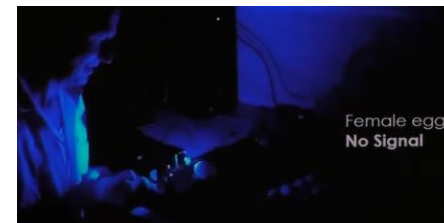
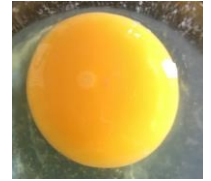
CO2 levels



Sound Vibration

« During incubation, we influence the expression of genes responsible (DMRT1) for the development of the reproductive system...(to) transform male embryos into egg laying females »

Genome editing: male eggs are detected prior to incubation



but what is the acceptability of such genetically modified animals ?  
+ dependence on "edited" strains (not applicable for traditional breeds)

Some consumers may consider the use of transgenic animals ethically preferable to the culling of the unrequired sex

## ➤ The French case : decree 2022-137



Décret n° 2022-137 du 5 février 2022 relatif à l'interdiction de mise à mort des poussins des lignées de l'espèce *Gallus gallus* destinées à la production d'œufs de consommation et à la protection des animaux dans le cadre de leur mise à mort en dehors des établissements d'abattage



French Decree 2022-137 of February 5, 2022 prohibits the killing of chicks of *Gallus gallus* breeds reared for table eggs production.

### **Out of the scope of the decree application:**

- chicks used by the pharmaceutical industry (vaccines produced against human influenza require the use of fertilized eggs) or veterinary diagnostics,
- chicks hatched for animal feed purpose
- chicks that are injured or having a disease likely to cause suffering
- hatched male chicks resulting from sexing errors
- chicks from white breeds (for which *in ovo* sexing is lacking)

Methods chosen in hatcheries need to be applicable for fertilised eggs  $\leq$  15 days of incubation

The decree stipulates that the equipment installed in hatcheries by January 2023 may not be questionable for a period of 5 years

# ➤ The French case : selection of non invasive techniques

July 2024

*G. gallus*



*A. platyrhynchos* &  
*Cairina moschata*



|                         |                  | Agri Advanced Technology (Germany)           | Orbem (Germany)  | Nectra (France)                              | Orvia/Nectra (France)                        | Grimaud frères (France)                          |
|-------------------------|------------------|--|--|--|--|--|
| <b>Day of detection</b> |                  | 13   | 12-13  | 13.5   | 8.5-9  | 9-10   |
| <b>Specificities</b>    | <b>Precision</b> | 96%  | >95%   | >95%   | 95%  | 95%  |
|                         | <b>Breeds</b>    | Brown breeds sexable on feather colour       | Any strain   | Brown breeds sexable on feather colour       | Breeds sexable on eye colour                 | Breeds sexable on eye colour                     |
|                         | <b>Speed</b>     | 18-20 000 eggs/h/machine                     | 3000 eggs/h/machine but possibility to have several ones on parallel | 20 000 eggs/h/machine                        | 20 000 eggs/h/machine                        | <i>Confidential</i>                              |
| <b>Hatchability</b>     |                  | 92.5%  | Not communicated   | No impact                                    | No impact                                    | 90-95% (to be confirmed on industrial level)     |
| <b>Objectives</b>       |                  | To improve precision, speed, early detection | To improve precision, speed, early detection                         | To improve precision, speed, early detection | To improve precision, speed, early detection | To improve speed (industrialisation in progress) |
| <b>French hatchery</b>  |                  | Lohman, Hy-line                              | Lanckriet, Hendrix Genetics  | Novoponte                                    | Orvia  | Grimaud frères sélection                         |

## ➤ Management of sorted male embryos



Effective from 01/02/21

<https://policy.brown.edu/policy/avian-embryo-use>



Embryos more than 80% developed (>16 days for chicken embryos) = live animals

### Approved euthanasia techniques

- <13 days: cool to < 4°C or -20°C for 4 hours and confirm by decapitation or crushing
- Between 13 and 17 days: cervical dislocation, decapitation, injection of barbiturates, carbon dioxide inhalation, carbon monoxide inhalation, inhalation anaesthesia and crushing using specialised equipment.
- ->18 days: carbon dioxide (20 min. 90% CO<sub>2</sub>), lethal injection of anaesthetic agents or decapitation

## ➤ Recycling hatchery by-products

CE n° 1774/2002 10/03/2002

### Hatchery by-products

Definition = stillborn chicks, chicks born and killed, eggshells from hatched chicks and unhatched incubated eggs, including eggs eliminated by in ovo sexing, clear (unfertilised) eggs; and eggs stored but not incubated.

**Category 1** = by-products that present a significant risk to public health and are therefore disposed of or can be recovered as biodiesel or fuel: **not applicable here** (ex: carcasses and all body parts of animals suspected of being infected)

**Category 2** = low-risk by-products that can be used as organic fertiliser, composting, conversion to biogas, etc.

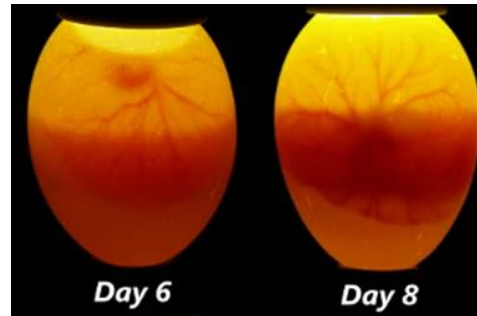
= Clear eggs, incubated eggs, certain other by-products (shells, cuticles, juice, meconium, feather).

**BUT** the use of category 2 eggs for direct use on the ground and for compost or biogas production, without prior pressure sterilisation, is strictly prohibited (possible risk of contamination of soil, crops, etc.).

**Category 3** = by-products presenting a low risk to public health that can be used in animal feed but subject to certain conditions of treatment and processing by approved facilities= **ethanised chicks (male chicks), chicks that have been slaughtered as part of the fight against zoonoses**

## ➤ Take-home messages

- Several new strategies of sexing *in ovo* are currently being developed
- It is likely that the methodology used to sex « eggs » will need to be adapted/revised according to the developmental stage of the embryo (whose position in the egg changes during incubation)



- Legislation is expected to evolve towards more « universal » methods that can be applied at early stages of development, whatever the genetic strain
- There is an urgent need to discuss more globally about the acceptability and the potential benefit/risk balance of genome editing and sex-reversal (that both avoid the culling of embryos)

Studies on the two other alternatives (rearing brothers of laying hens, development of dual-purpose breeds) are still ongoing

## ➤ Acknowledgments



[www.ppilow.eu](http://www.ppilow.eu)



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Thank you for your attention !