

# Egg Residue Analysis for *In Ovo* vaccination



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Successful application of *in ovo* vaccination requires good hatchery practices and a specific range of embryonic development parameters to provide optimum vaccine uptake by the embryo. Monitoring these is crucial for *in ovo* vaccination success.

- ▶ The *in ovo* vaccine's efficacy depends on the **embryo compartment** where it is delivered.
- ▶ The **embryo development stage** influences the frequency of which embryo compartment is injected.
- ▶ The interactions among **types of incubator, incubation time, flock age, bird type and egg storage time** impact the embryo development stage.

<sup>1</sup>Wakenell PS, Bryan T, Schaeffer J, Avakian A, Williams C, Whitfill C. Effect of *in ovo* vaccine delivery route on herpesvirus of turkeys/SB-1 efficacy and viremia. Avian Dis. 2002;46(2):274-280.

<sup>2</sup>Villalobos, T. Optimal timing for *in ovo* vaccination - understanding embryo development. International Hatchery Practice. 2014.

**Supporting the embryos journey during incubation is a requirement that starts before the eggs arrive at the hatchery, during transport to the hatchery, during egg storage, during the incubation period, up to transfer and during the hatch period.**



Proper development of the embryo to allow successful *in ovo* vaccination of the embryo during the *in ovo* vaccination window, **managing each process involved is critical to provide the embryo with optimal and uniform development to achieve each stage of their physiological development in readiness for *in ovo* vaccination and/or transfer to the Hatcher.**

All eggs are not the same, all donor flocks are not the same and all incubation systems are not the same.

**To achieve optimum disease protection from the *in ovo* application, monitoring processes and reviewing performance & hatch is needed.**

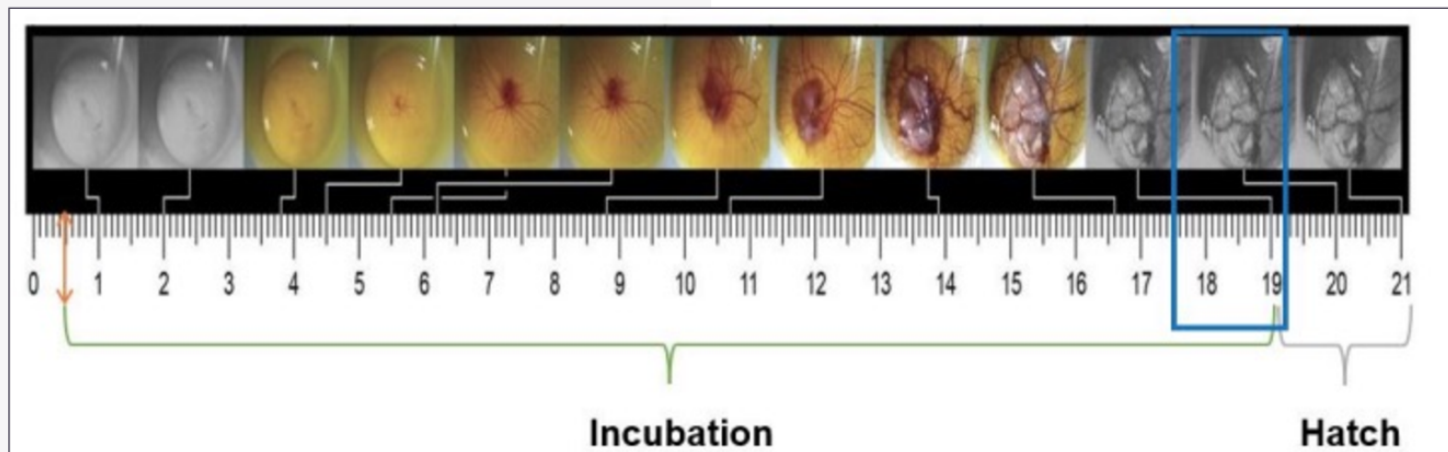
***Stage Scoring at Transfer is an accurate way to monitor embryo development stage to confirm correct time of transfer.***

Accurate Embryonic Development (ED) age at transfer assists with optimal SOI and will not adversely impact the remaining incubation/hatch period, chick quality or hatch percentages.

**Egg Residue Analysis (ERA) is used to analyse at what point the embryo ceased to develop.**

This is conducted by analysing the unhatched residue and working backwards to see at what stage the embryo stopped their development.

Egg Residue Analysis can also be used to check on improvements in incubation undertaken by the hatchery.



**ERA allows for:**

- ▶ Identification of non-hatch and classification into different segments of the incubation & transfer process.
- ▶ Review of transfer timing impact on the end hatch result.
- ▶ Incorrect transfer time impacts optimal Site of Injection (SOI) by the in ovo device which may cause:
  - ▶ Deposit of the vaccine to wrong egg compartments
  - ▶ Injury to the embryo



All eggs are not uniform in size or shell density due to different strains and breeder flock uniformity and health status.

Daily setting group numbers may not all be stored for the same amount of time prior to incubation, causing variability in reaching diapause for incubation (24-25°C). may not be uniform and incubator functionality/performance between incubators may vary.

- ▶ **All of these differences can see variance between Embryonic Development (ED) and the chronological age (incubator counter).**
- ▶ **These differences**, if not detected and addressed, **can cause non-consistent ED at transfer and hatches from day to day hatching and between incubators and hatchers where variations in performance is present.**



**Hatch residue provides us with a visual on incubation to hatch period, which includes;**

- ▶ **Too early hatch = dehydration, early losses and poor grow out**
  
- ▶ **Too late a hatch = chicks not ready, vaccine utilisation and poor start at grow out**
  
- ▶ **Transfer timing = right embryonic development for *in ovo* application**

**For this training, we will focus on our process to conduct the Egg Residue Analysis (ERA) that assists in evaluating correct timing for *In Ovo* application.**



Egg Residue Analysis (ERA) forms will vary from supplier to supplier and also vary with what information is needed from the ERA, however they all record similar stages of development that they will analyse as required.

- ▶ **Eggs in Tray** – (Non-hatch residue) – what percentage is acceptable to be above or below standard?
- ▶ **Live Chicks A** – (Average Hatch) – what percentage is acceptable to be above or below standard?
- ▶ **Cull Chicks** – (poor quality chicks) – what percentage is acceptable to be above or below standard?
- ▶ **Does excessive numbers represent a hatch timing issue?**
- ▶ **Dead in Tray** – (hatched but died) – what percentage is acceptable to be above or below standard?



Collecting the data is critical to allow dissemination of where the non-hatch issues stem from and provides data to cross reference breeder standards.

Customer:	ABC Farms		
Eggs per tray	150		
Trolley I.D./Position	S1 T10		
Flock I.D.	XYZ S2		
Eggs in Tray	18		
Live Chicks A	131		
Cull Chicks	1		
Dead in tray			
Total in basket	150	0	0

### Investigating Contaminated – Gaseous:

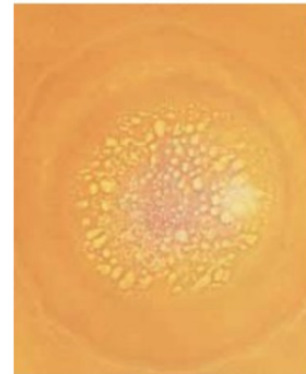
- ▶ Allows for evaluation of levels and corrective action to be taken to meet standards/requirements.
- ▶ Is it farm related? Nest boxes, cool-rooms, packaging.
- ▶ Is it transport related - temperature fluctuations or hygiene.
- ▶ Is it cool room related - Storage temperature, hygiene and humidity?
- ▶ Is it in the incubation period - Air supply, humidity, equipment or earlier contamination?
- ▶ Is it transfer related - Equipment related, process related, air or water.

- ▶ Is it in the hatcher period?
- ▶ Air, humidity, equipment or cross contamination at hatch?
- ▶ Is it in the chick process room - Air, equipment, cleaning, handling?

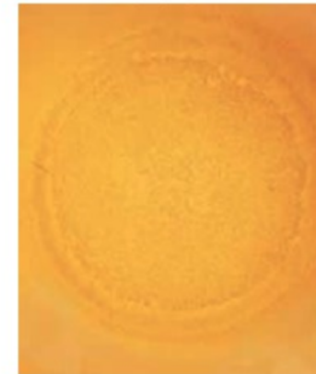
### Investigating Infertile:

#### Microscopic View

Infertile



Fertile



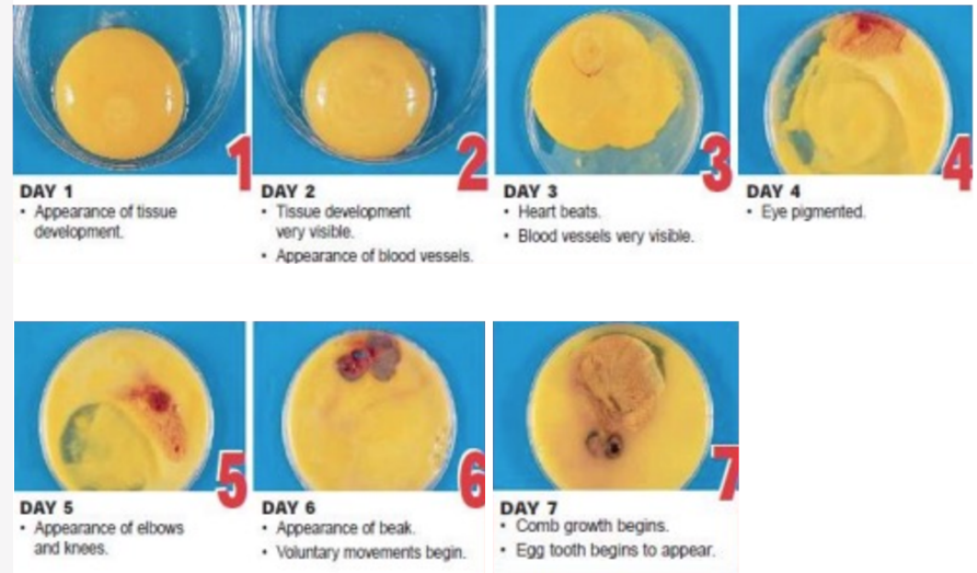
Allows for evaluation of candling accuracy if conducted or breeder fertility if no candling conducted.

- ▶ Often included with early 1-7 as identification at this incubation stage can be difficult.
- ▶ If candling accuracy at transfer is an issue, management can investigate and correct.
- ▶ If no candling is conducted, then the percentage for flock age can be checked against breeder standards and discussion on fertility undertaken.



### Investigating Early 1-7 days:

- ▶ Allows for evaluation of candling accuracy if conducted.
- ▶ Causes can vary, with some listed below;
- ▶ Breeder Farm issues (flock health, collection, storage or transport).
- ▶ Long egg storage and fluctuating egg storage temperatures.
- ▶ Early fumigation exposure.
- ▶ Slow to reach set incubation temperatures.
- ▶ Turning angle incorrect in incubator.



## Investigating Mid 8-14 days:



- ▶ Allows for evaluation of candling accuracy if conducted.
- ▶ Causes can vary, with some listed below;
- ▶ Temperature of embryo too high during early period of incubation.
- ▶ Contamination toxicity.
- ▶ Breeder flock issues.



### Investigating Late 15-18 days:

This is a critical stage of development with metabolic energy levels increasing dramatically. It also captures the pre-*in ovo* transfer period.

Causes can vary, with some listed below;

- ▶ Incubation issues (temperatures, humidity).
- ▶ Incorrect egg positioning during incubation.
- ▶ Contamination toxicity.
- ▶ Breeder flock issues.



## Investigating Post Transfer- >18 days + 12 hours:

This sector captures the Transfer period, *in ovo* vaccination, and the hatch period.

- ▶ Commonly used to set a benchmark for non-*In Ovo* & *In Ovo* comparisons.

Review of this data allows identification of:

- ▶ Correct timing of Embryonic Development at transfer.
- ▶ Vaccine aseptic and sterility of *in ovo* process.
- ▶ Transfer Cracks.
- ▶ *In Ovo* vaccination damage to the embryo.



- ▶ Late incubation issues.
- ▶ Hatcher issues.



**This stage** (from the end of incubation to the hatch) incorporates a multitude of areas throughout the embryo's journey that could have adversely affected the ability of the chick to hatch.

Int Pip live	Embryo has achieved development to the point of hatch, but not fully hatched
Int Pip dead	
Ext Pip live	
Ext Pip dead	
Set upside down	These relate to common pre incubation and incubation issues that need to be addressed if above Breeder standards/recommendations.
Head O R wing	
Head to left	
Head b'twn Legs	
Inverted in egg	
Feet too high	
No Explanation	
Moulds	
Exposed Brain	
Ectopic Viscera	
Multiple Limbs	
ANALYSIS OF NON HATCHED EMBRYOS	
Int Pip live	
Int Pip dead	
Ext Pip live	
Ext Pip dead	
Late Hatch	
Crack	
Trans Crack	
Set upside down	
Head O R wing	
Head to left	
Head b'twn Legs	
Inverted in egg	
Feet too high	
No Explanation	
Moulds	
Exposed Brain	
Ectopic Viscera	
Multiple Limbs	

- ▶ Early egg handling, breeder farm issues, early incubation issues, mid incubation issues all set the stage for correct embryonic development for successful non-*InOvo* or *InOvo* transfer and a successful hatch (to or above standard).

- ▶ **Using the Zoetis Egg Residue Analysis technique not only tells a full story from lay as stated above but assists in highlighting post incubation effect where transfer is conducted and *InOvo* application is used.**
- ▶ **Embryonic development at transfer is crucial to meet not only the changed temperature and humidity profiles from the incubator to hatcher, but the hatch window and quality of the chick.**

# Thank you!

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