In ovo sex determination

H&N Distributor Conference

11th May 2022

Jörg Hurlin, Laura Zumbrink

KEY FACTS EW GROUP

- Founded in 1928
- 100% family-owned
- > 180 subsidiaries in > 45 countries
- Core business:
  - Animal Genetics (Poultry & Fish)
  - Animal Health (Vaccines & SPF / Clean Eggs)
  - Animal Nutrition (Feed additives)
- Employees: > 17,000 (2020/21)

AAT GmbH
EW GROUP COMPANY

BUSINESS ACTIVITIES – EW GROUP

Segment	Subsidiaries	Market position
Broiler breeding	Aviagen	Global leading
Turkey breeding	Aviagen Turkeys	Global leading
Layer breeding	Lohmann Tierzucht, Hy-Line, H&N International, Novogen	Global leading
Fish breeding	AquaGen, GenoMar, Aquabel	Global leading
SPF & Clean eggs	VALO BioMedia	Global leading
Animal Vaccines	vaxxinova, IZO	Regional leading
Feed additives	ew | nutrition	Regional leading
Technical Solutions	AAT GmbH EW Group Company, Innovatec	Regional leading
Food / Grain storage	Pilzland aus gutem Grund, EIPRO, MGG	National leading

ABOUT AAT

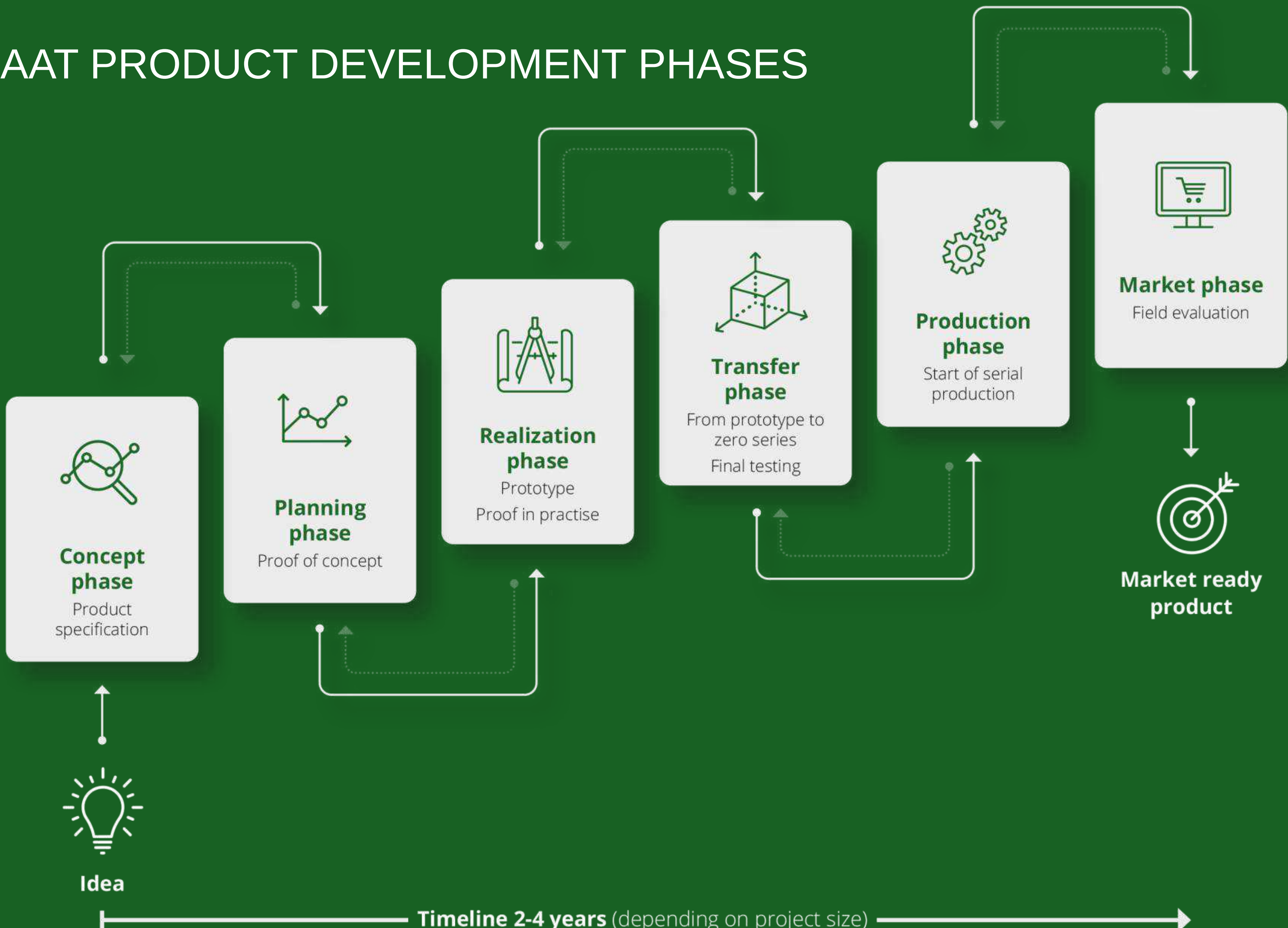
Hatchery Solutions

Farm Equipment

Feed Disinfection

Founded in 2015 | Family owned | Based in Germany

AAT PRODUCT DEVELOPMENT PHASES



AGENDA

01 Introduction & overview of the situation in Europe

02 In ovo sex determination research projects

03 The Cheggy and Stunny process

04 Market developments in Europe

THE PROBLEM

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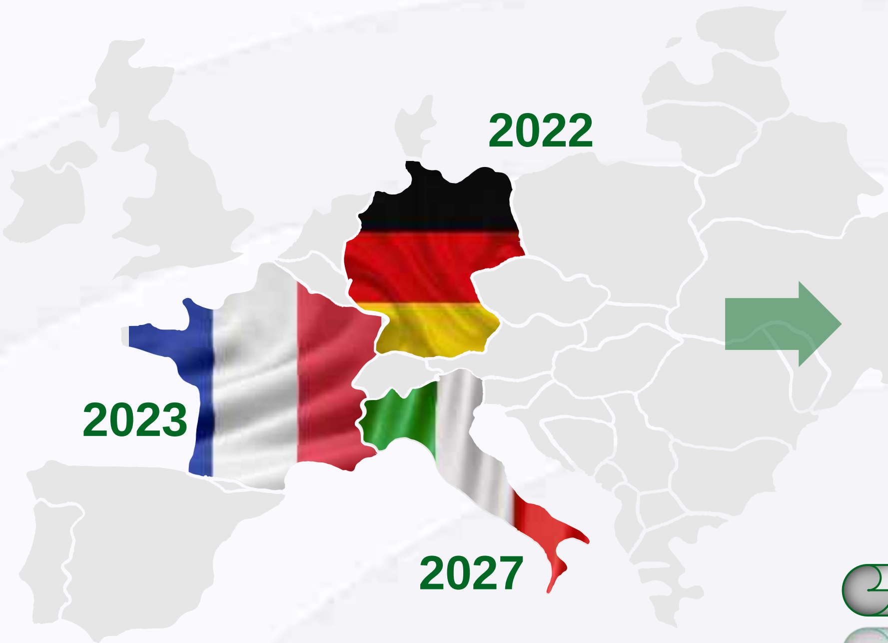
SITUATION IN EUROPE



§

no more culling of day old chicks

?

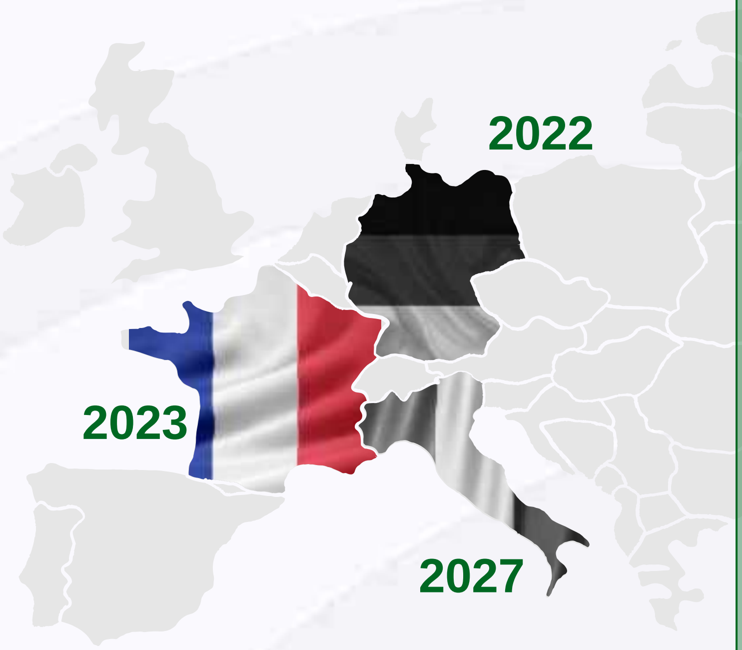
SITUATION IN EUROPE

Germany:

- Ban of chick culling since 1.1.2022
  - AAT as only technology provider for in ovo sexing in a German hatchery
- Adaptation of the law 2024: in ovo sex determination only before day 7 of incubation
  - Probably there will be no in ovo sexing technology available!

(Tierschutzgesetz)

SITUATION IN EUROPE

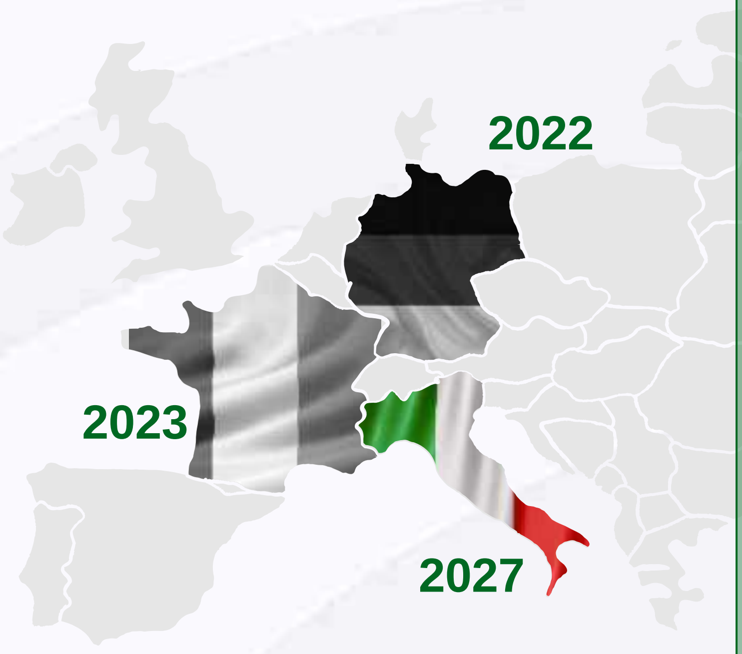


France:

- Ban of chick culling from 1.1.2023 onwards
  - Transition phase in 2022
  - All technologies until day 15 of incubation allowed
  - Five years security for the currently available technologies
  - State subidies for the hatchery investments

(Decret n° 2022-137)

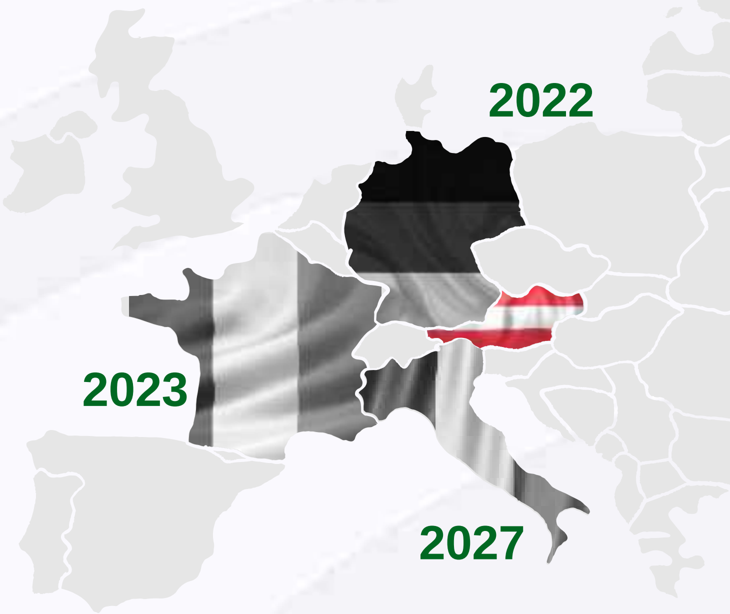
SITUATION IN EUROPE



Italy:

- Amendment to end culling of chicks in the layer industry by the end of 2026 passed the Italian parliament end of 2021
- The Senate, as the second chamber, still has to vote

SITUATION IN EUROPE



Austria:

- Industry Agreement to end unnecessary chick culling, law in progress
- Production of frozen DOC for feeding for zoos etc. will still be allowed
- In ovo sex determination in the second third of incubation has to be done in combination with a welfare-friendly stunning method

(Austrian Industry Agreement)

OPTIONS TO STOP CULLING DAY-OLD

Carcasses of different origin (70 days of age)

Slow growing broiler
~ 2,8 kg carcass weight

Male layer hybrid
~ 1 kg carcass weight

Male dual purpose breed
~ 2 kg carcass weight

SEX DETERMINATION: FUNCTIONAL REQUIREMENTS

- Early
- Accurate
- Fast
- No negative impacts
  - Embryo
  - Layer performance
- Use of male embryos/eggs
- Inexpensive
- Sustainable
- **Consumer acceptance: not too late/best before incubation**

chicken embryo day 3
Photo: AAT

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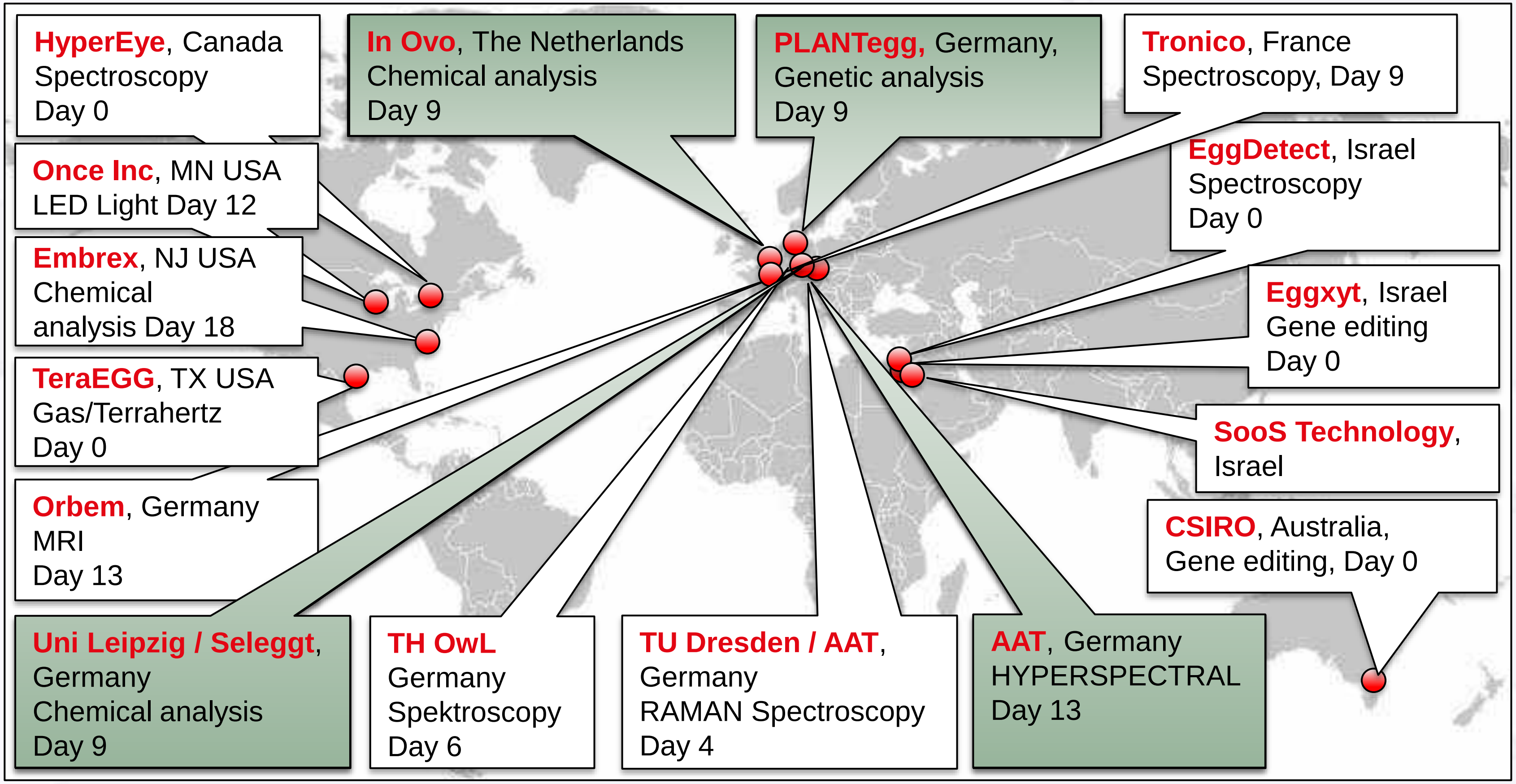
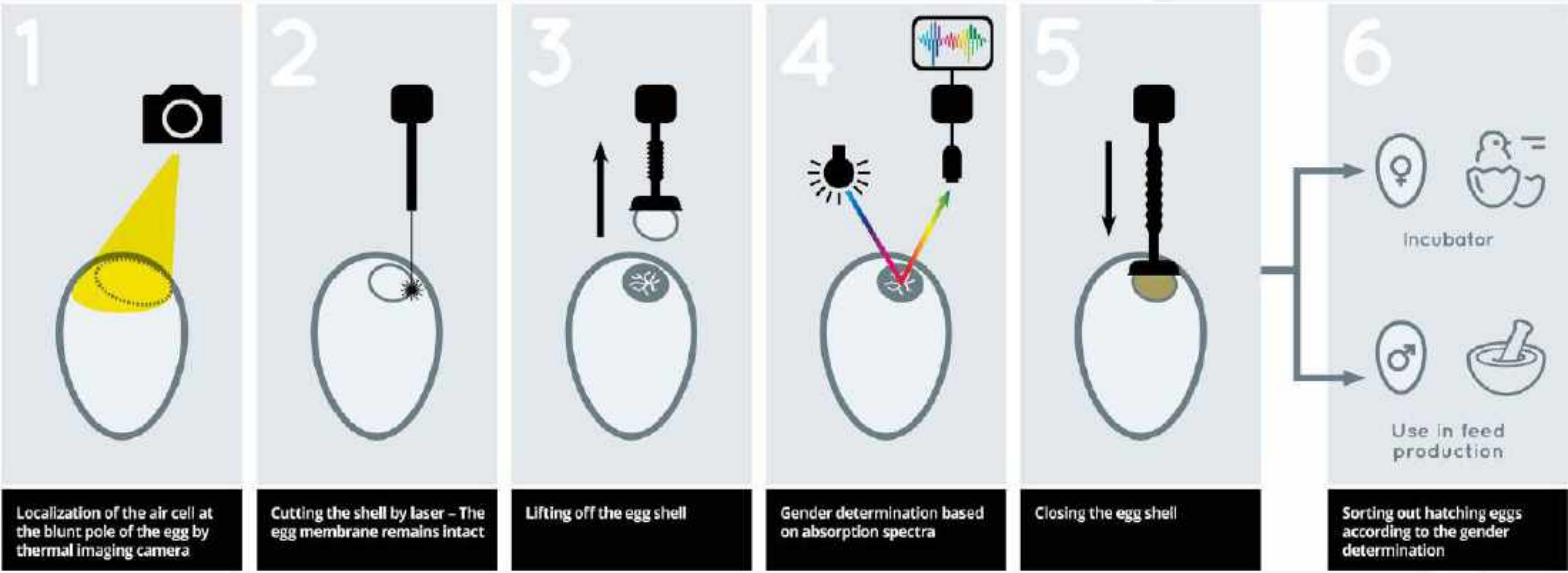
LOCATIONS OF SEX DETERMINATION TRIALS



Source: AAT

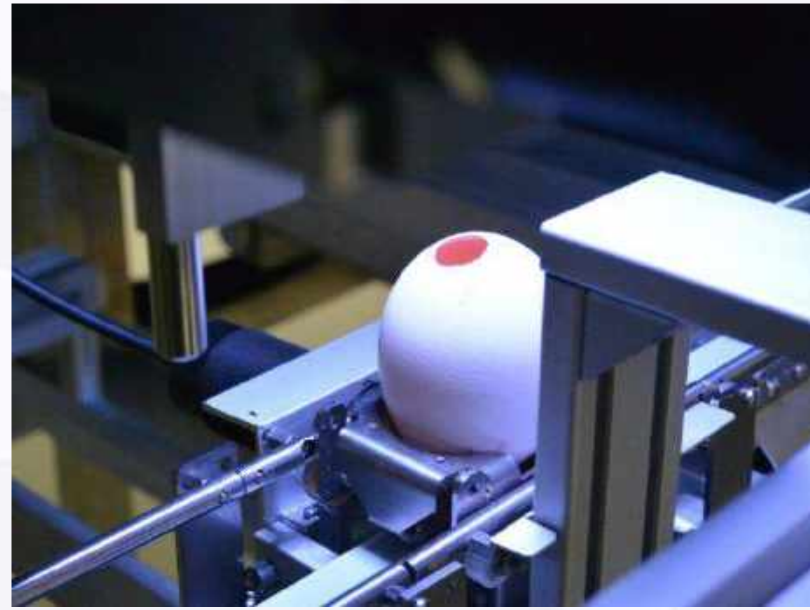
RAMAN-SPECTROSCOPY AUTOMATION



Source: AAT

based on [WO 2017/017277 PCT](WO 2017/017277 PCT)patent publication „METHOD AND DEVICE FOR INTRODUCING AN OPENING INTO THE CALCAREOUS SHELL IN THE REGION OF THE BLUNT END OF INCUBATED BIRD EGGS WITH AN EMBYRO "

RAMAN SPECTROSCOPY



- For further information visit www.agri-at.com/en/products/
- Collaboration with University of Dresden and Ministry of Lower Saxony
- Proof in practice studies in the next 12 months

MARKET-READY ALTERNATIVES

no pain perception | pain perception not proven | definite pain perception

1 2 3 4 5 6 7 | 8 9 10 11 12 13 14 | 15 16 17 18 19 20

Day 21

Invasive (sampling allantoic fluid)
Measurement out of the egg

SELEGGT | IN OVO | PLANTegg

started 06/2018 | 11/2020 | 11/2020

3.3 – 3.5 €/DOC

Non-invasive (optical analysis)
Measurement inside the egg

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12/2019

1.2 €/DOC

Growing of the males

~3.5 – 3.8 €/DOC

AAT GmbH EW GROUP COMPANY

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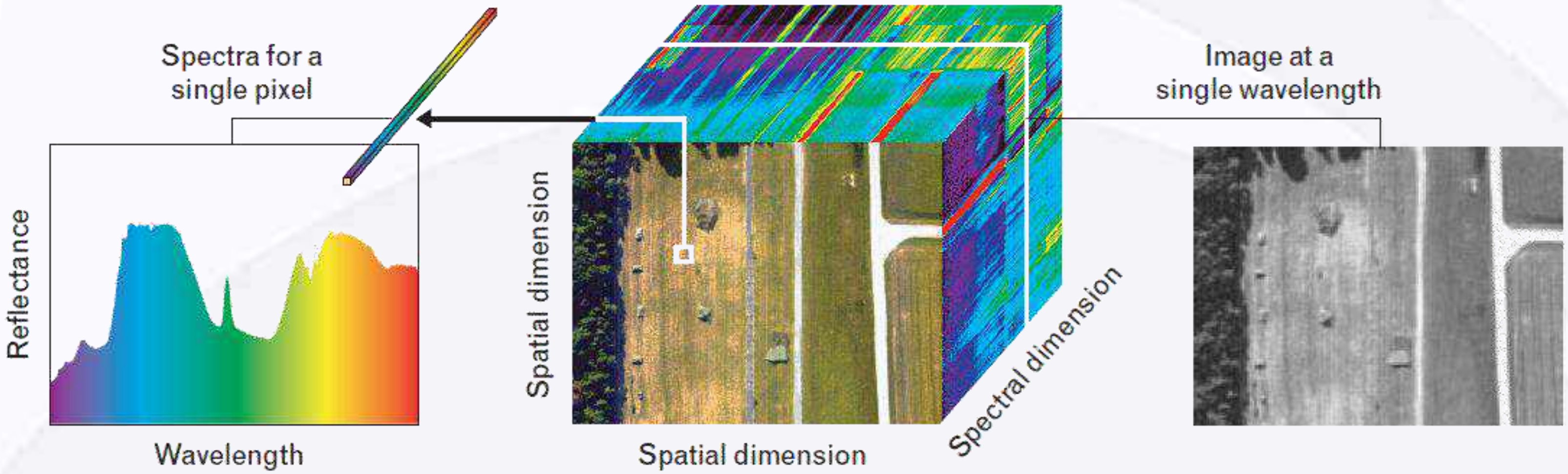
DEFINITION AND APPLICATION OF HYPERSPECTRAL IMAGING

- Three dimensions: two spatial, one spectral
- hyperspectral = imaging of the whole spectrum

(BELLON-MAUREL und GORETTA, 2014; MANOLAKIS et al., 2013)



MANOLAKIS et al. (2013)

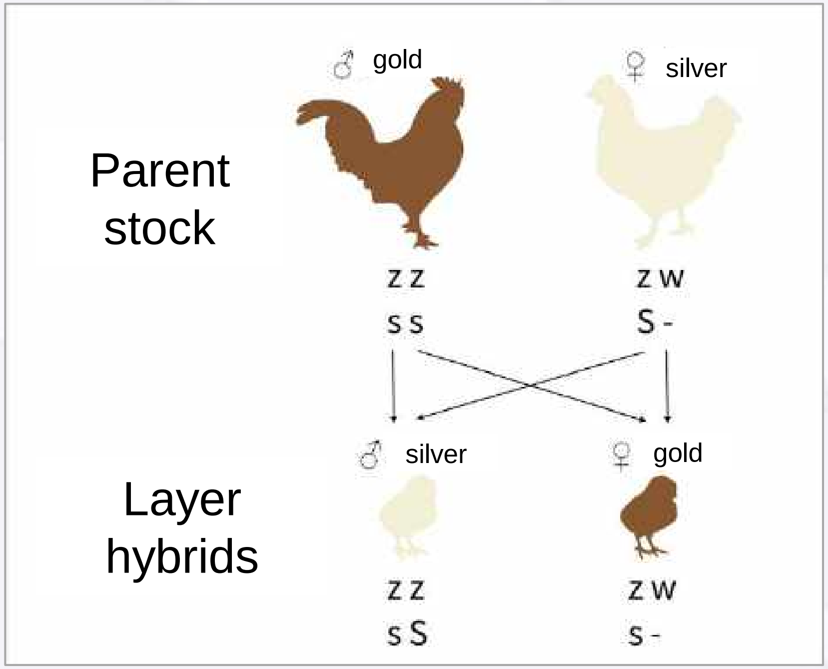
EXCURSUS: REGULAR COLOR SEXING

(hatching day)

FEATHER COLOR SEXING

thanks to systematic crossbreeding, brown layers are color-sexable

(TAYLOR, 1949; SMYTH, 1990)



FEATHER COLOR SEXING

Brown chicken can be sexed as day old chicks by feather colour

Males are white

Females are brown

FEATHER COLOR SEXING

There are some variations:

Males with one dark stripe

Females with one light stripe

FEATHER COLOR SEXING

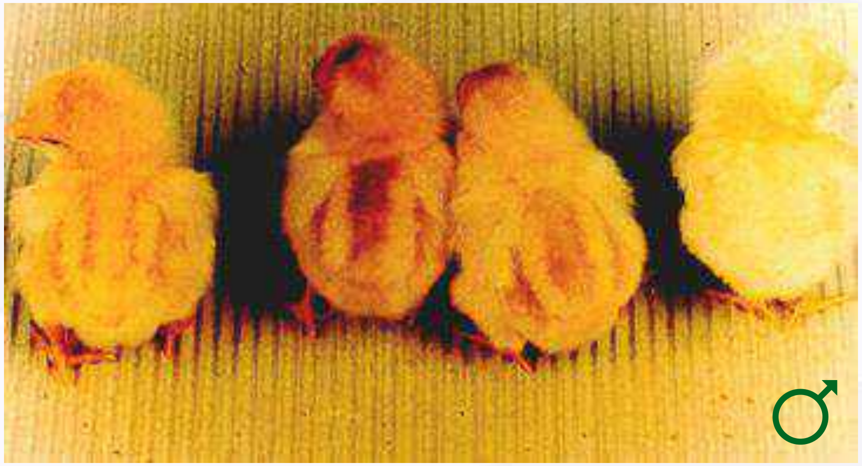
There are some variations:

Males with distinct light stripes

Light females with one light stripe with brown edging

FEATHER COLOR SEXING

There are doubtful chicks:



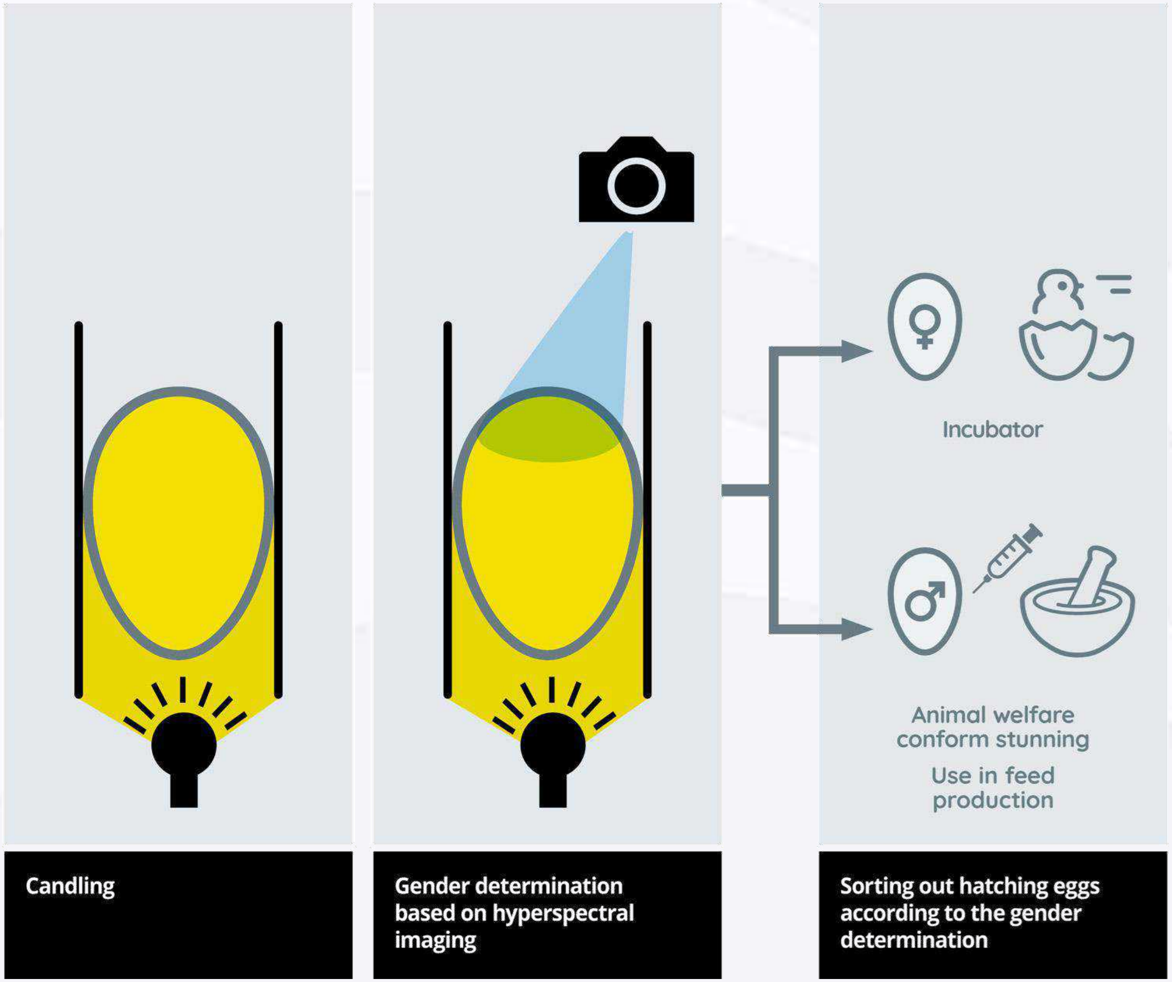
Males

Females

in the colour sexing of chicks by hatchery specialists, an accuracy of 99.5 % is achieved

- variation between the breeds possible
- doubtful chicks are sorted out as males to avoid sexing errors in the female flock

SEX DETERMINATION – HYPERSPECTRAL



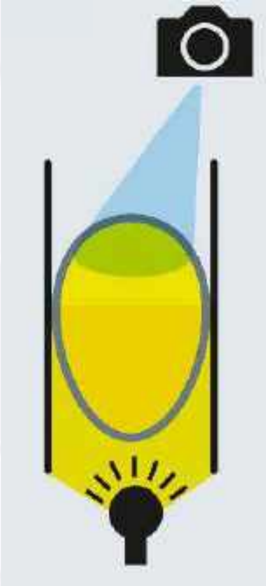
Source: AAT

based on WO 2014/033544 A9 PCT patent publication „Spectrophotometric analysis of embryonic chick feather color“

HYPERSPECTRAL MEASUREMENT

Optical Measurement on incubation day 13

- Non-invasive process
  - no risk of contamination
  - no risk of injury to the embryo
  - no influence of the measurement on the embryonic development
- Hatching eggs robust in handling on day 13
  - no system-related hatch reduction
- No expensive consumables (chemicals)
  - environmentally friendly
- Specific for brown layers



FULLY AUTOMATED CHEGGY MACHINE

- In ovo sex determination with high accuracies (> 96%)
- Fully automatic high-speed measurement (20,000 eggs per hour)
- Cost-efficient: only approx. 1/3 compared to the cost of other alternatives
- Easy to operate
- Online documentation of the results

VIDEO

www.Cheggy.de

FIELDSTUDY IN FRANCE

ANESTHESIE ELECTRIQUE D'EMBRYONS DE POUSSINS MALES AU DEUXIEME TIERS DE L'INCUBATION DANS LE RESPECT DU BIEN-ETRE ANIMAL

-

DE LA RECHERCHE FONDAMENTALE A LA PRATIQUE A HAUT RENDEMENT

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RÉSUMÉ

ABSTRACT

Electrical anaesthesia of male chicken embryos in the second third of the incubation in compliance with animal welfare – from basic research to high-throughput operation

- AAT is the **only technology provider** that publishes detailed practical results
- Long-term study in France over 6 months
- Results are published at Itavi Poultry Research days in Tours, FR

FIELDSTUDY IN FRANCE

Key figures to evaluate in ovo sexing technologies

% sexing error	number of hatched males / all chicks hatched, as a measure of the accuracy of the technique
% total hatch	number of all chicks hatched / number of eggs set, as a measure for hatchability
% hatch of transferred eggs	number of chicks hatched / number of eggs further incubated after sexing, as a measure for hatchability after sexing
% female hatch	number of female chicks hatched / number of eggs set, as impotant parameter of animal welfare, sustainability and production planning
hatching eggs (HE)/fem	1 / % female hatch, as impotant parameter of animal welfare, sustainability and production planning

FIELDSTUDY RESULTS

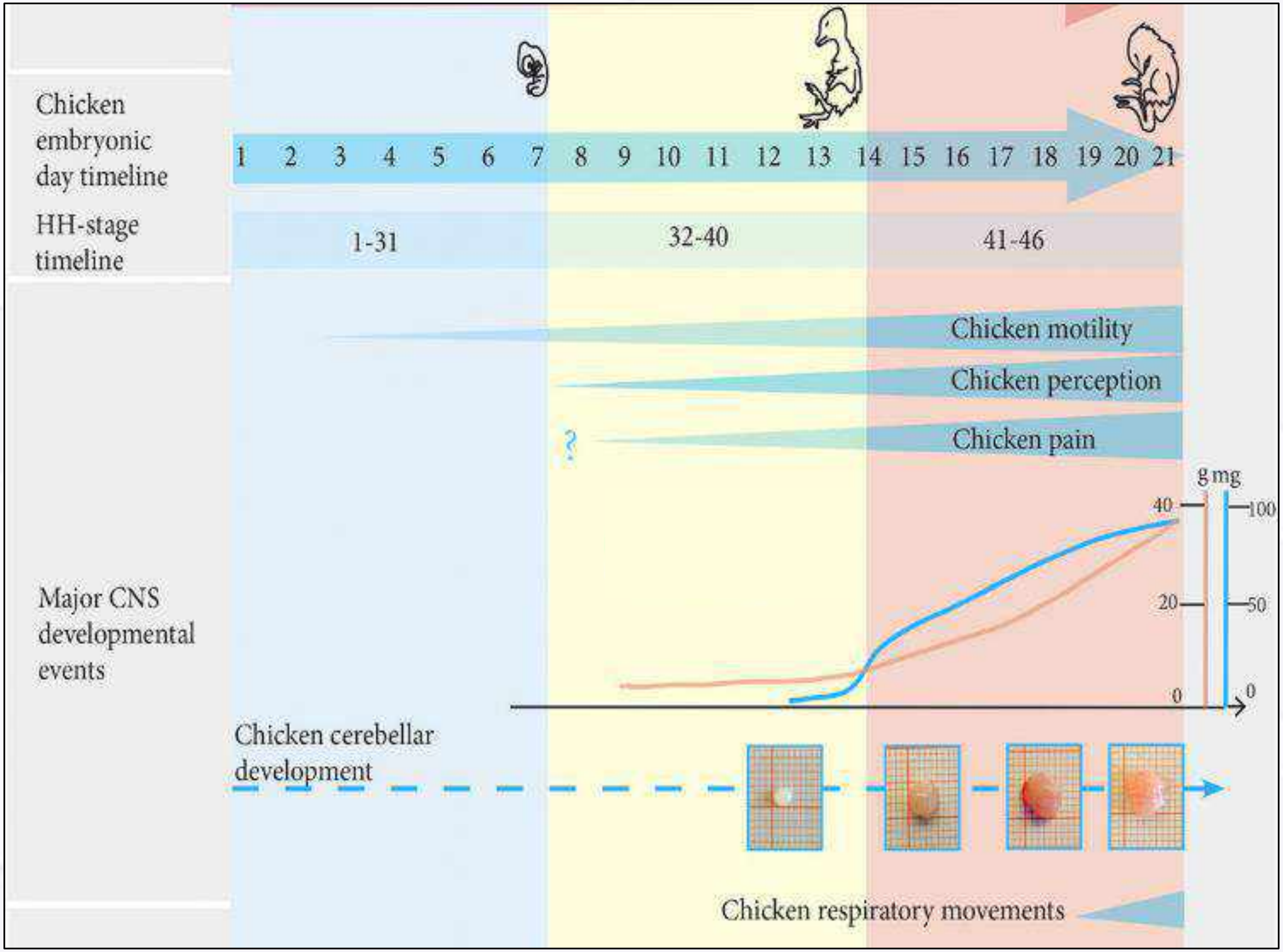
AVERAGE*		
	hatched female chicks	38 %
	sexing error	4.1 %
	hatching eggs per female chick	2.6
	hatching eggs per female chick (standard)	2.4
	extra demand of hatching eggs	8 %
	hatch after CHEGGY sexing	92.5 %

Average of 50 commercial flocks (six strains: HyLine Brown, Lohmann Brown, H&N Brown Nick, ISA Brown, Bovans Brown, Novogen Brown) in France between January and July 2021

RESPECTFUL HANDLING

OF MALE EMBRYOS

BJØRNSTAD ET AL. (2015), MODIFIED:



The Journal Of Pharmacology And Experimental Therapeutics

In order to have an extra guarantee that the embryos do not feel pain an approved stunning method is essential.

Concept of an animal-welfare approved method for stunning chicken embryos

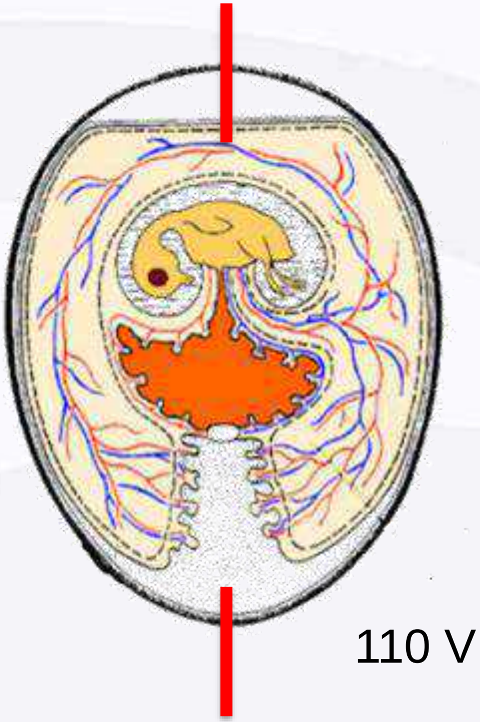
STUNNING METHODS FOR EMBRYOS
– TECHNICAL FEASIBILITY

Method	Problem or Conflict
Injection of approved narcotic-agents	Approved to use only by veterinarians, needed volumes, waste disposal, cost
Carbon dioxide (> 80%)*	Investigate duration needed, no automation equipment available, space requirement, labour intensive (manual sorting)
Maceration*	Often considered unaesthetic
Cooling (>4h at 4°C) / Freezing*	No defined exposure for stunning, Space requirement, energy cost
Heating (>45°C)	No defined exposure for stunning
LAPS (Low Atmospheric Pressure Stunning)	Space requirement, no automation equipment available
Electricity	Ensure electricity flow

* Recommended for embryos after AVMA Guidelines for the Euthanasia of Animals: 2013, page 63

AAT CONCEPT FOR EMBRYO STUNNING

Stunning



Source: modified after Kalweit et. Burmeister 1995

RESEARCH EMBRYO STUNNING

Joined research project with University of Goettingen (Institute of Veterinary Medicine) and bsi Schwarzenbek (Consultancy Institute for animal welfare)

Europ.Poult.Sci., 84. 2020, ISSN [illegible], © Verlag Eugen Ulmer, Stuttgart. DOI: [illegible]

Electrical anaesthesia of male chicken embryos in the second third of the incubation period in compliance with animal welfare

Tierschutzkonforme elektrische Betäubung männlicher Hühnerembryonen im zweiten Drittel der Brut

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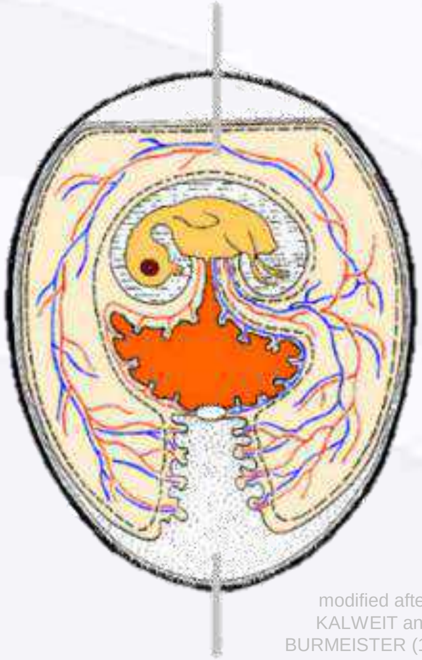
Manuscript received 7 August 2020, accepted 21 September 2020

Abstract

The aim of in ovo sex determination in chicken hatching eggs is to detect male embryos and terminate their development at an early stage of incubation to avoid killing day-old-chicks after hatch. Since there is no secure scientific knowledge on presence or absence of pain perception between day 7 and 15 of incubation, it is necessary to develop animal welfare friendly and consumer acceptable procedures to end the incubation process. In this study, electrical current flow was investigated as a method for anaesthesia of chicken embryos. In two test series a current flow at 110 V AC was applied for 2 seconds to 304 and 56 embryonated eggs, respectively. Under these experimental conditions embryonic reactions associated with possible awareness of pain were absent in 99.3% of the examined embryos after exposure (results of trial series 1). The results suggest that application of an electric current is a practical and animal welfare-compliant procedure for chicken embryos in the second third of the incubation period.



modified after KALWEIT and BURMEISTER (1995)

Photo: AAT

99.3% successfully anesthetized

https://www.european-poultry-science.com/Electrical-anaesthesia-of-male-chicken-embryos-in-the-second-third-of-the-incubation-period-in-compliance-with-animal-welfare

HANDLING OF MALE EMBRYOS

Fully automated electrical anaesthesia

- Camera-based position detection of hatching eggs
- Special egg fixation system for equal penetration depths with different egg sizes
- Measurement and documentation of the achieved current flow **per individual egg**
- Throughput of 10.000 eggs per hour
- Surveyed by independent veterinary institute regarding animal welfare aspects

HIGH-QUALITY PROTEIN SOURCE

- Male embryos sorted out are classified in **category 3** for further use in accordance with EC Regulation 1069/2009
- Further processing into dried egg powder, which can be used as feed for **pets and livestock** or for applications in the cosmetics sector
- The sensible usage of the sorted hatching eggs as a high-quality protein source makes the in ovo sexing a **resource-saving and thus sustainable process**

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OVERVIEW OF Cheggys

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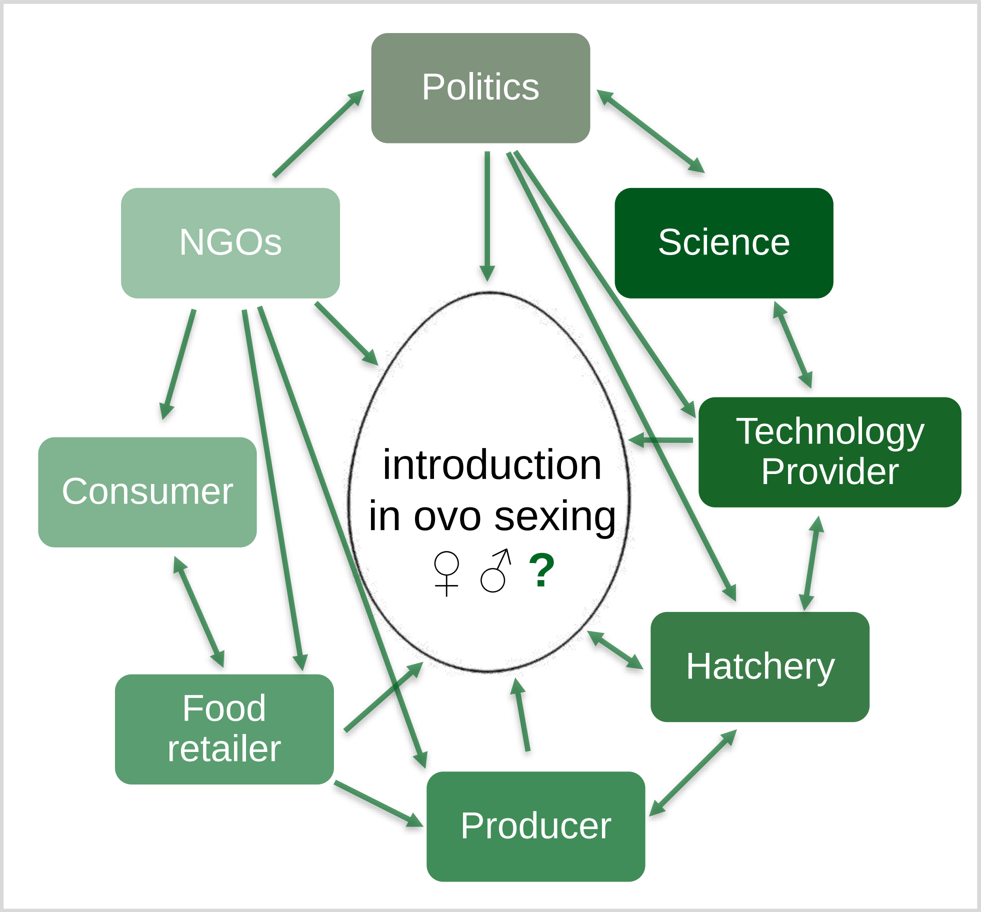
STAKEHOLDERS INFLUENCE THE FUTURE OF IN OVO SEXING

very complex interactions between stakeholders

circumstances are strongly country-specific!



MARKET MODEL IN OVO EUROPE
THEORETICAL FIGURES

Price DOC = 0.80 €

+ extra handling
+ extra hatching eggs (+8 %)
+ rearing of sexing errors

+ AAT royalty per female DOC

= 1.90 – 2.0 € / in ovo sexed DOC

237 - 250 % price increase

250 eggs per hen housed

= 0.4 ct – 0.5 ct / table egg

Per capita consumption ~ 240 eggs

= 0.96 – 1.25 € annual costs for consumer

AAT machines = rental machines → AAT service is needed
Machines are not for sale!

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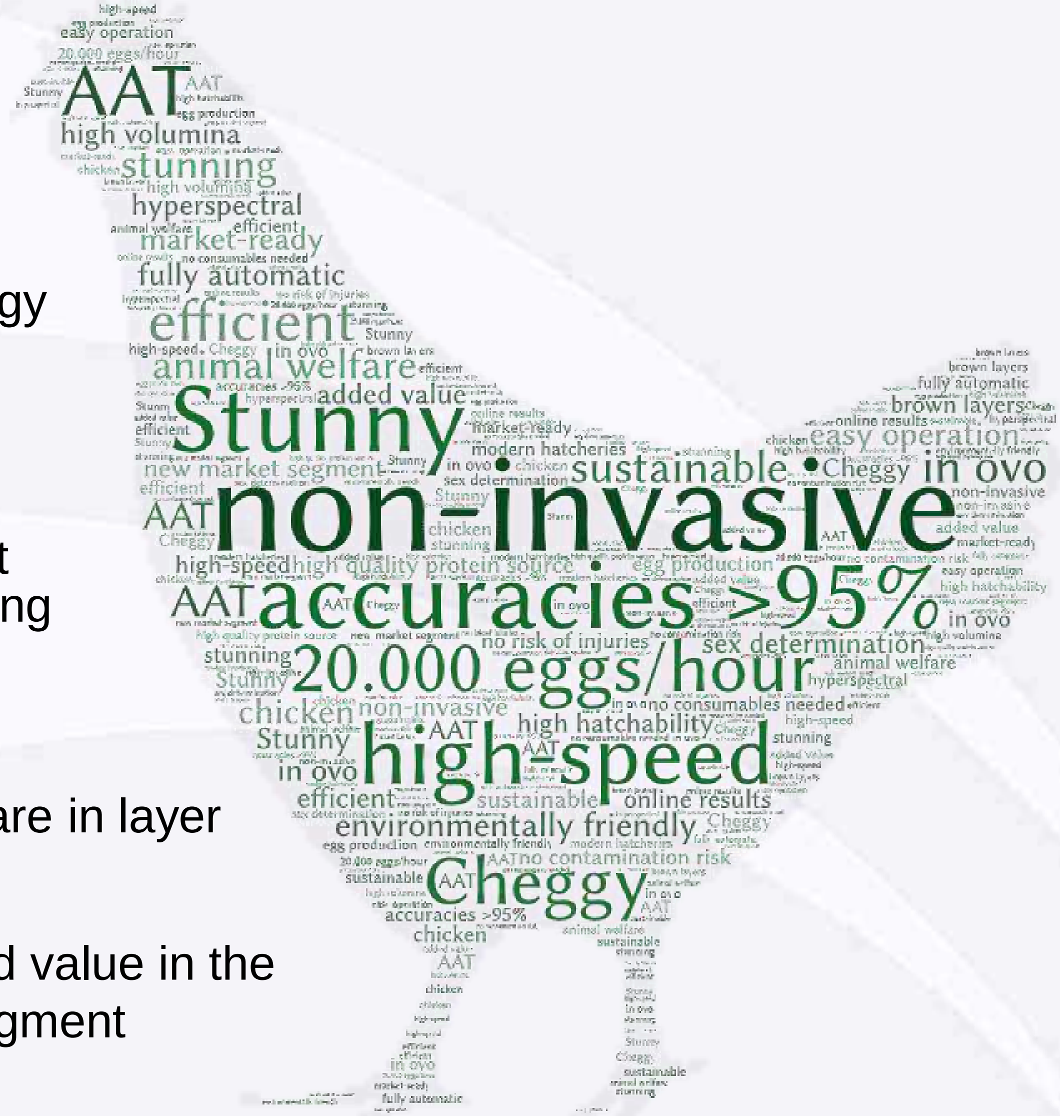
OUTLOOK

- Market-ready in ovo technology for brown layers
- Additional secure stunning of embryos
- Most sustainable and efficient alternative to avoid chick culling

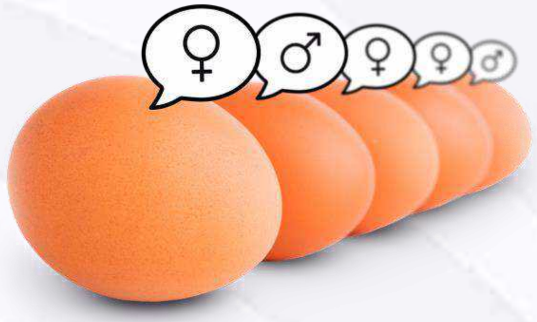


→ Big milestone for animal welfare in layer business

→ Opportunity to increase added value in the supply chain / new market segment

OUTLOOK

All current methods for in ovo sex determination increase the use of resources:



- 100 % accuracy not achievable
- sexing errors need to be reared
- additional hatching eggs
- additional egg handling, process relevant losses
- influence flock age, egg age, egg quality, etc....

→ Without additional price from the market/ willingness to pay of consumers + pressure from legislators, the high-priced in ovo processes could not be established on the market

EW GROUP COMPANY

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Cheggy.de
improve animal welfare · protect environment · save money

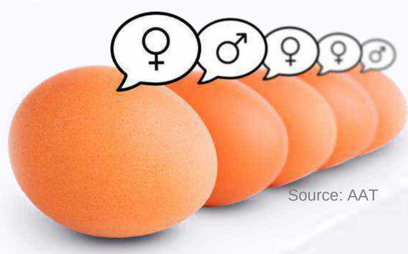
CHANGE IN PLANNING CRITERIA FROM A PRACTICAL POINT OF VIEW

without sex determination
in ovo sex determination

Female hatch
41 %

Hatching eggs / ♀ chicks
~ 2.4

Plannability



- Sorting out female embryos (determination errors, sorting errors)
- Higher mortality of embryos due to procedure
- Non-determinable hatching eggs

- Increased volatility of hatching results
- Compliance with target quantities and husbandry requirements by law

- **Inadequate plannability for the hatchery**
- **Rearing of sexing errors (♂) and surplus (♀)**

INVASIVE METHOD – IN OVO

Currently no production!

- Sampling of allantoic fluid (invasive)
- Analysis of metabolic marker by use of mass spectrometry

Source: IN OVO

Speed	6,500 eggs / h
Weekly capacity[1]	Ca. 60,000 – 70.000 ♀ DOC
Accuracy	~ 93 %
Female hatch	28 % (-13 %)
Female loss[2]	**33 %**
Hatching eggs / ♀ DOC	3.6 → **50 % more hatching eggs!**
Plannability	✕
Sustainability	✕

[1] Assuming 1,800 females/h , 10 h/day and 4 hatches/week
[2] Hatch loss / hatch rate of 41%

Hatchery Het Anker, Netherlands
Planned: Lohmann Deutschland, Germany

INVASIVE METHOD - PLANTEGG

- Sampling of allantoic fluid (invasive)
- DNA analysis through PCR test

Source: PLANTEGG

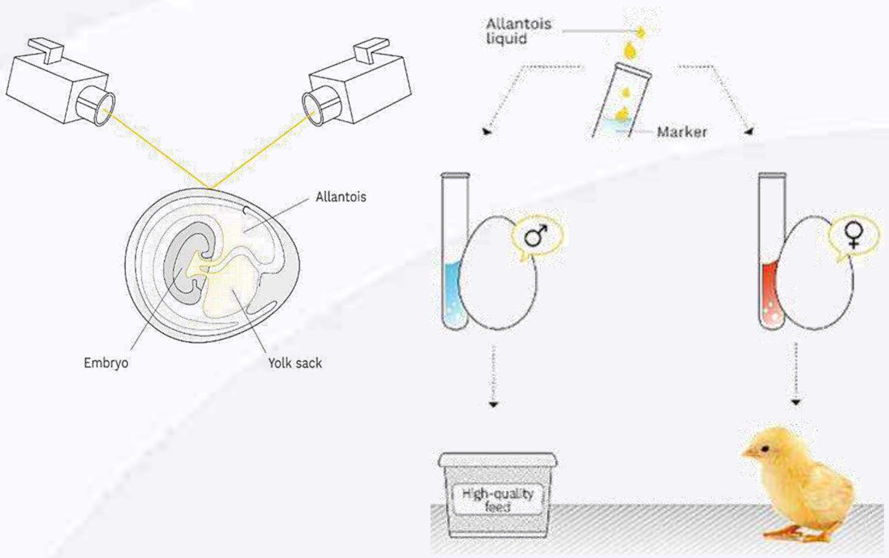
Speed	3,000 eggs / h
Weekly capacity[1]	Ca. 25,000 – 40,000 ♀ DOC
Accuracy	~ 98 %
Female hatch	32 % (-9 %)
Female loss[2]	**22 %**
Hatching eggs / ♀ chick	3.1 → **29 % more hatching eggs!**
Plannability	✕
Sustainability	✕

[1] Assuming 1,000 chicks/h , 10 h/day and 4 hatches/week
[2] Hatch loss / hatch rate of 41%

Hatchery ter Heerdt, Netherlands

INVASIVE METHOD - SELEGGT

- Sampling of allantoic fluid (invasive)
- Endocrinological analysis through ELISA test



Source: SELEGGT

Speed	3,000 eggs / h
Weekly capacity[1]	Ca. 25.000 - 40,000 ♀ DOC
Accuracy	~ 97 %
Female hatch	31 % (-10 %)
Female loss[2]	**24 %**
Hatching eggs / ♀ chick	3.2 → **33 % more hatching eggs!**
Plannability	✕
Sustainability	✕

[1] Assuming 1,000 chicks/h , 10 h/day and 4 hatches/week
[2] Hatch loss / hatch rate of 41%

Hatchery Barneveld, Netherlands

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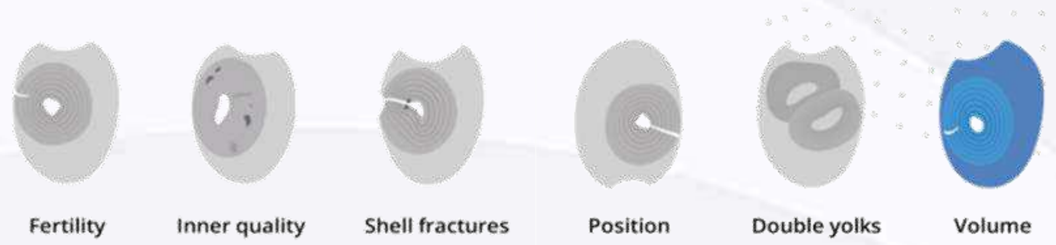
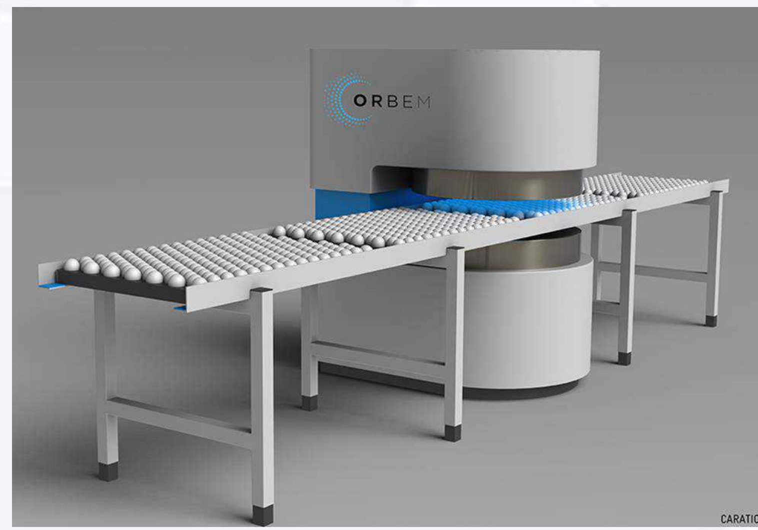
NON-INVASIVE - ORBEM GENUS



- MRI technology and artificial intelligence classification
- Pre-incubation analysis *(pilot phase)*
- Day 14 sexing *(commercially not available, but one unit planned in France with Hendrix)*
- No official results available
- Studies with AAT before day 7 revealed **no sufficient accuracies**



NON-INVASIVE - CHEGGY

- Non-invasive process
  - no risk of contmination
  - no risk of injuries to the embryo
- Hyperspectral measurement of feather color

Speed	20,000 eggs / h
Weekly capacity[1]	300,000 ♀ chicks
Accuracy	~ 96 %
Female hatch	38 % (-3 %)
Female loss[2]	**7 %**
Hatching eggs / ♀ chicks	2.6 → **8% more hatching eggs**
Plannability	✓
Sustainability	✓

[1] Assuming 7,500 chicks/h , 10 h/day and 4 hatches/week
[2] Hatch loss / hatch rate of 41%

Hatcheries in GER, BEL, FR, IT, AUT, ES

Cheggy.de
improve animal welfare · protect environment · save money

AAT GmbH
EW GROUP COMPANY

COMPARISON

	In ovo	Plantegg	Seleggt	AAT / Cheggy	Rearing males
Speed eggs / h	6,500	3,500	3,500	20,000	According to hatchery
Accuracy	~ 93 %	~ 97 %	~ 94 %	~ 96 %	
Female hatch	28 %	32 %	31 %	38 %	41%
Female loss	32 %	22 %	24 %	7 %	0 %
Hatching eggs / ♀ chicks	3.6	3.1	3.2	2.6	2.4
Consumables	Test kits, sampling plates, needles, biomarker			-	Barn, feed, etc.
Plannability	✗	✗	✗	✓	✓
Sustainability	✗	✗	✗	✓	✗
Additional need hatching eggs				1m	-
Additional parents*				~4,347	*Rearing capacities*

Sample calculation on s … *s:*

*Assuming 230 hatching eggs/hen-housed

INTERIM CONCLUSION

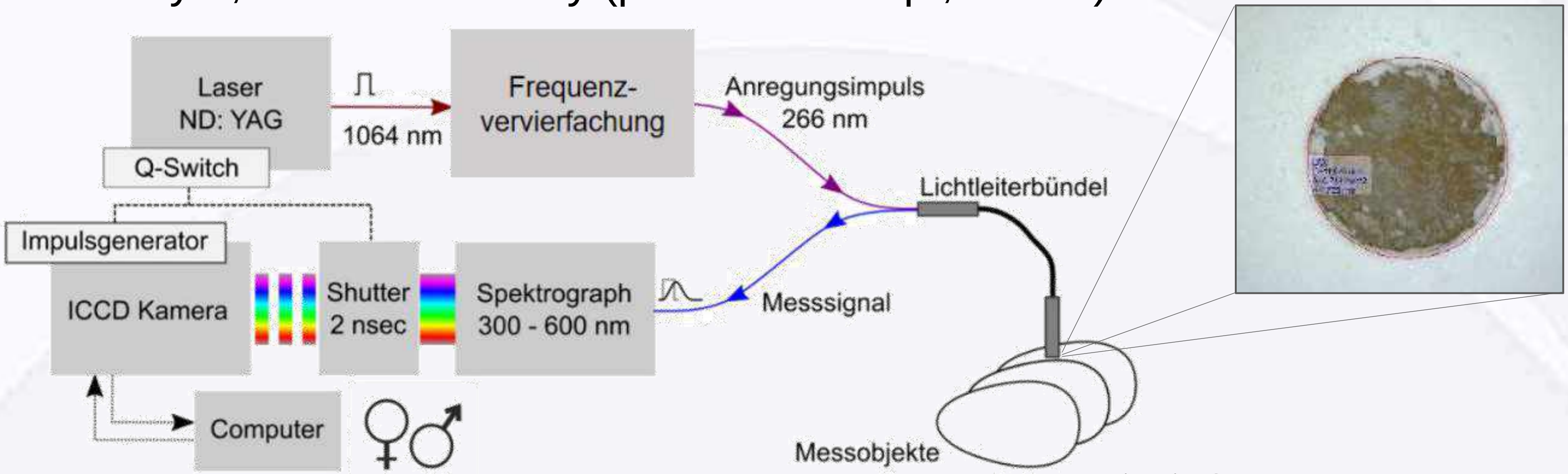
- Liquid-based methods so far allow **insufficient predictability of hatch**, **significantly increase resource consumption** and are **expensive**; problematic due to **high losses of female embryos**
- Cheggy approach is **plannable and sustainable**, although **for browns only** and applicable after down can be detected
- All determination methods fully match actual legislation in the same way
- Rearing of males is **plannable but not sustainable**

→ **All methods are necessary, in order to manage challenges regarding phasing out!**

RESEARCH – APPROACHES BEFORE DAY 7

Fluorescence Spectroscopy

- TH OWL
  - "Minimally invasive" opening of the eggshell (< 2 mm, egg membrane remains intact, no sampling).
  - Time-resolved laser-induced fluorescence spectroscopy of proteins
  - Day 6, > 90 % accuracy (proof of concept, *n* = 31)



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