In ovo sexing tools

Sophie Réhault-Godbert (INRAE, France)

Katia Grenier (CNRS, France)

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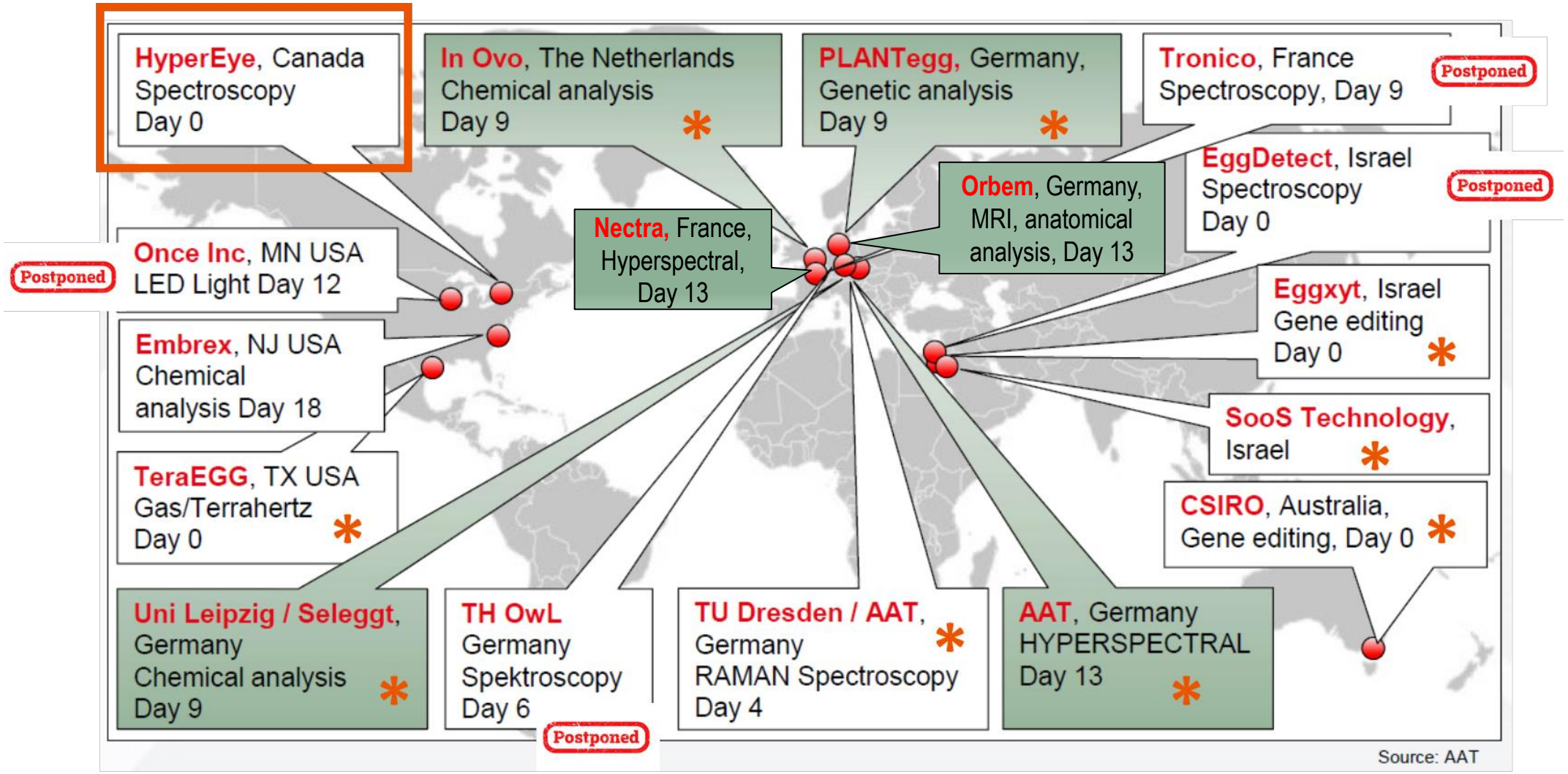
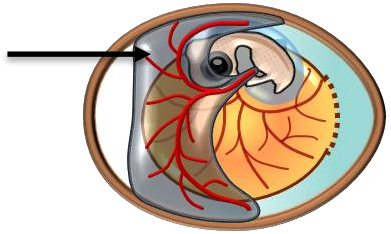
PPILOW – Overview of the methods that have been developed in the last decades



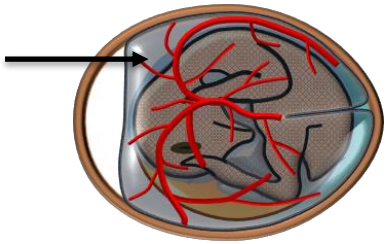
6 marketed techniques (3 semi-invasive / 3 non-invasive)

PPILOW – Semi-invasive methods with *in ovo* sampling

≈ 7 days



≈ 15 days

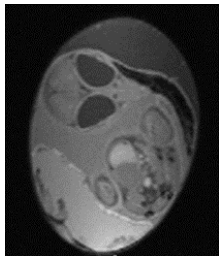


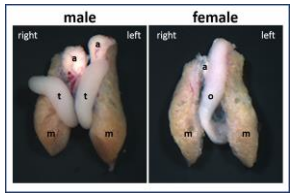
	Who ?	Country	Method	Strain Specificity	Incubation day	Precision	Speed
Invasive (saniatiray risk and chick mortality)	PLANTegg	Germany	Dosage (chromosome)	All chicken strains	9	>95%	Low (3,000 to 6,000 eggs/hour)
	SELEGGT responsible solutions	Germany	Dosage (hormone)	All chicken strains	9	>95%	Low (3,000 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)

PPILOW – Non-invasive methods

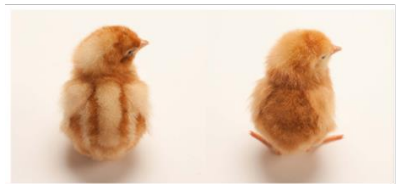
Magnetic resonance imaging (MRI) : gonad development





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

♀ ♂

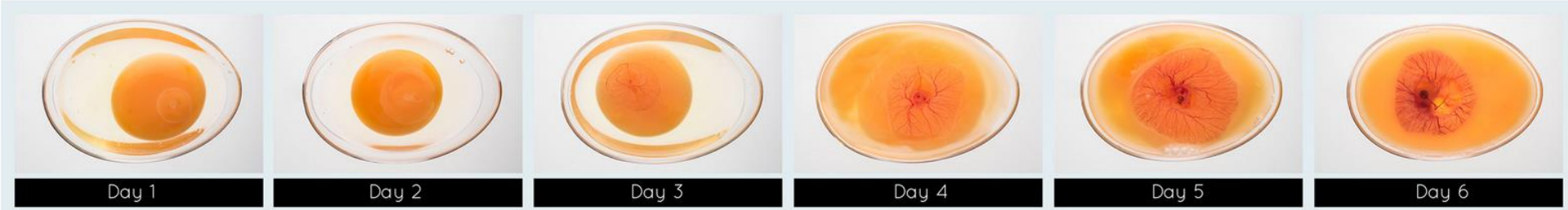


	Society	Country	Method	Strain specificity	Incubation day	Precision	Speed
Non invasive	ORBEM	Germany	MRI	All chicken strains	13	>95%	Low (3,000 eggs/hour)
	AAT EW GROUP COMPANY	Germany	Hyperspectral spectroscopy	Strains with feather colour sexing	13	96%	18-20,000 eggs/hour
	NECTRA	France	Hyperspectral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

Day 13 (of 21)

WP5.3 Development of new ovosexing methods : early non-invasive tools (1/5)

Subtask 1: identification of early biomarkers of sex in ovo (≤ 8 days)
Focus on molecular markers = detectable from the start of the incubation as a result from the activation of the genome and embryonic metabolism



Invasive step : sampling of the different structures of the egg; identification of the sex of the embryo (from embryonic or extra-embryonic structures) by PCR; analyse the molecular profile of samples, and associate the profile to the sex of the embryo

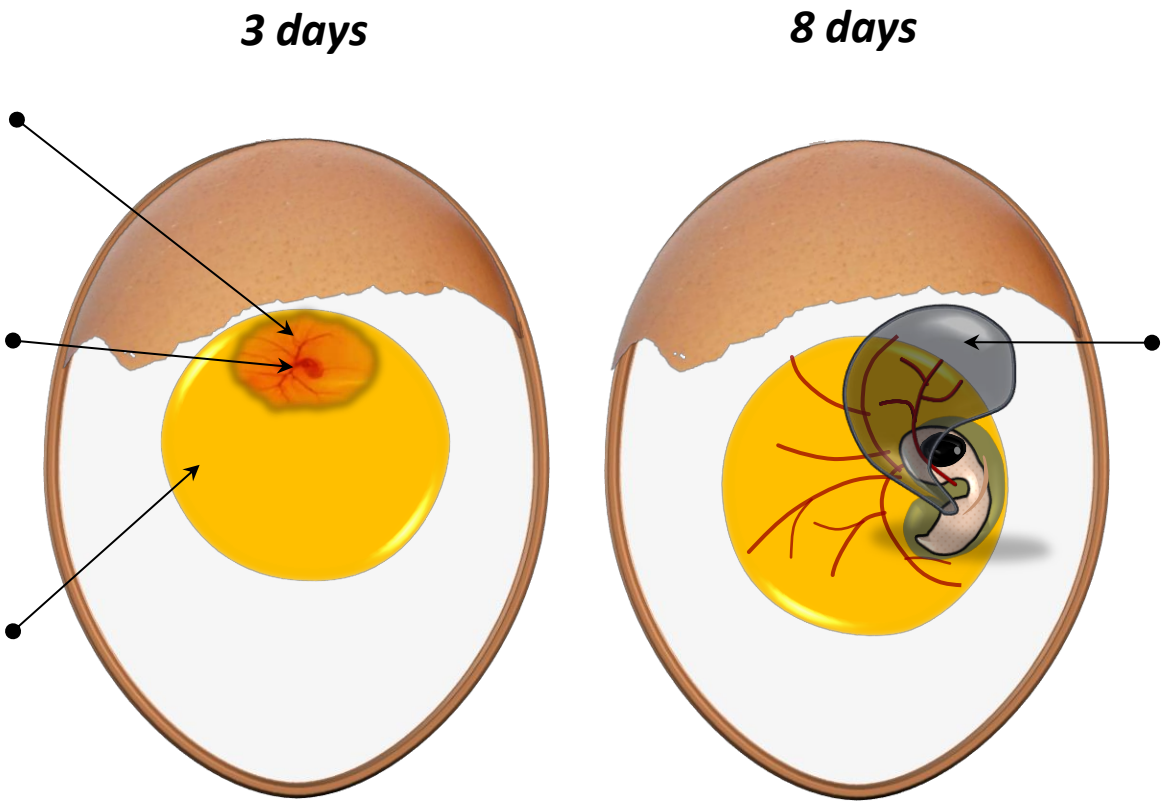
WP5.3 Development of new ovosexing methods : early non-invasive tools (2/5)

Incubation

1. **Yolk sac** (comparison ♂/ ♀):
> 1000 differentially expressed **genes**

2. Embryo (comparison ♂/ ♀):
>30 differentially abundant **proteins**

3. Yolk (comparison ♂/ ♀):
-4 **lipids** with differential abundance
-12 **metabolites** exhibiting differential abundance
-no difference in hormones (ten steroids analysed)

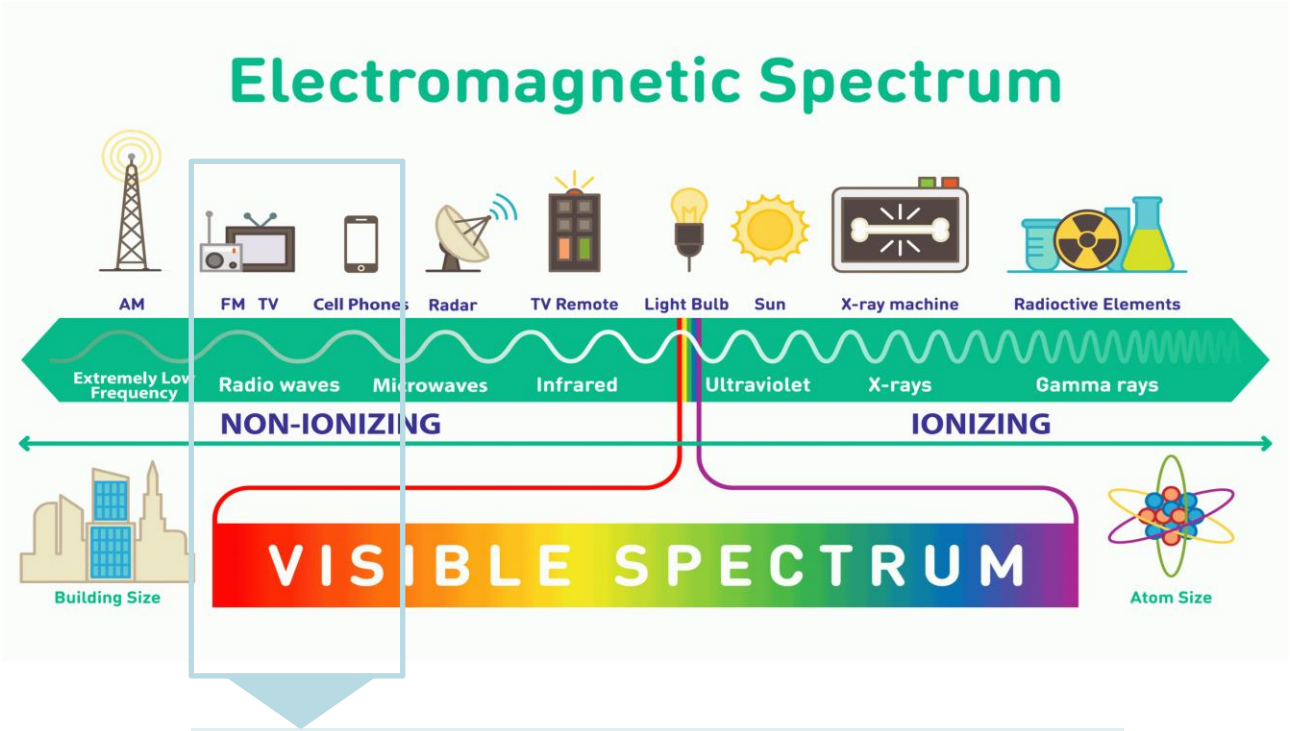


Chorioallantoic membrane: (comparison ♂/ ♀):
>100 differentially abundant **proteins**

The embryo and extra-embryonic membranes exhibit most of the differences between male and female « eggs » at early stages of development

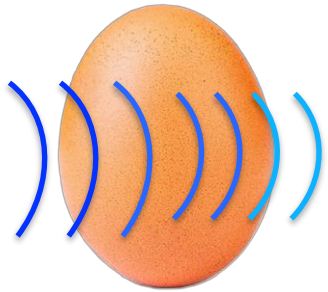
WP5.3 Development of new ovosexing methods : early non-invasive tools (3/5)

Subtask 2: Development of a non-invasive ovosexing technique using radiofrequency (RF)



-frequency range = non-ionising, not harmful
-expected to be of low cost

RF dielectric spectroscopy



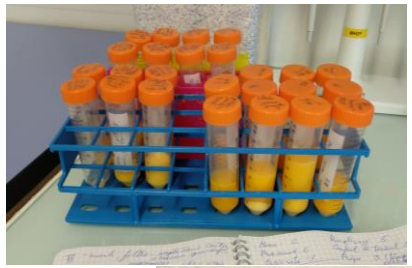
- Label-free
- Non-invasive
- Quick
- Global response of the structure

WP5.3 Development of new ovo sexing methods : early non-invasive tools (4/5)

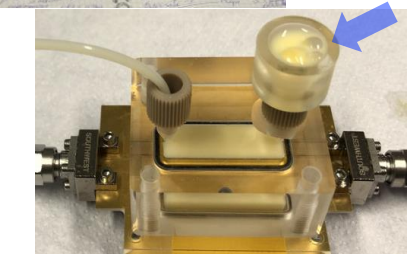
1. Which dielectric source of sex discrimination in eggs

For yolk and albumen

➔ RF millifluidic sensor

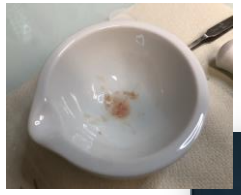


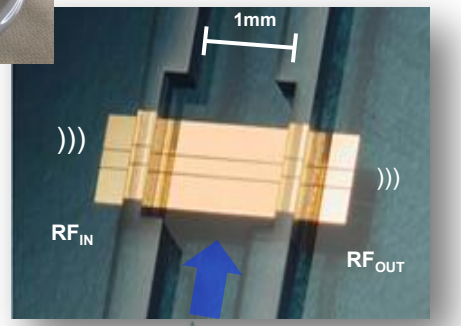
mL samples



For embryo and yolk sac

➔ RF microfluidic sensor

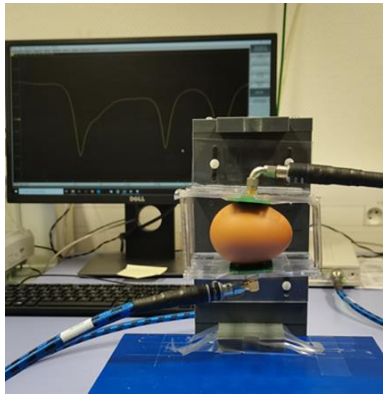




µL samples

2. Chick egg RF sexing RF

- Development of a specific test setup
- Implementation of Artificial Intelligence techniques for RF data treatment



➔ Tens of preliminary assays + design of the various sensors

➔ 5 independent testing campaigns on hundreds of egg constituents, whole eggs, or both

WP5.3 Development of new ovo sexing methods : early non-invasive tools (5/5)

➜ All tested egg constituents include the dielectric sex information (albumen, yolk and embryo with yolk sac)

➜ Sexing rate increases with incubation time:

➜ Yolk :	70% at 7 days	to	83% at 8 and 9 days
➜ Albumen :	50-60% at 7 days	to	66-90 % at 8 and 9 days
➜ Embryo + yolk sac :	80% at 7 days	to	90 % at 8 days

} Egg constituents

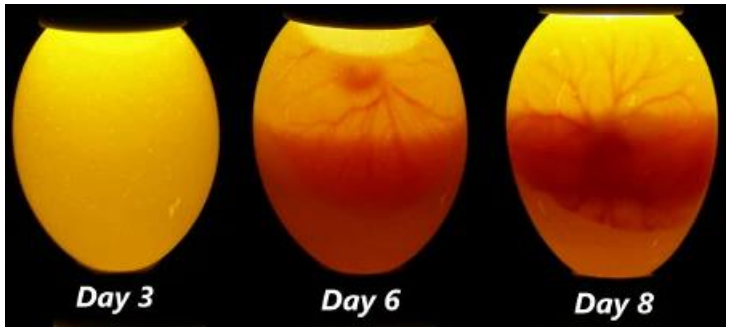
➜ Whole eggs : 70% at 6 and 7 days, 80 - 83 % at 8 days of incubation

PPILOW – Take-home messages

1

→ Identification of thousands of early sex biomarkers

→ The embryo and the extra-embryonic structures are the most discriminant between males and females at early stages = promising targets for the development of early ovosexing tools



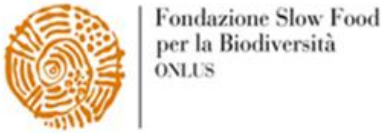
2

→ First demonstration of non-invasive ovo sexing with RF spectroscopy

→ Dielectric sex information in all egg constituents, with a dominance in the embryo + yolk sac, then yolk and finally albumen

→ Sexing rate up to 83% at 8 days of incubation for whole eggs

PPILOW **PARTNERS**



Thank you for your attention

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