

Taiwan, June 2013

# Animal germplasm banking in France

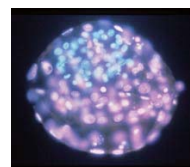
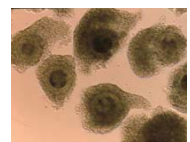
Pascal Mermillod



ALIMENTATION  
AGRICULTURE  
ENVIRONNEMENT



## Contents



- General presentation
- National cryobank for domestic species
- Wildlife preservation
- Birds specificities

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## Resources and organisation

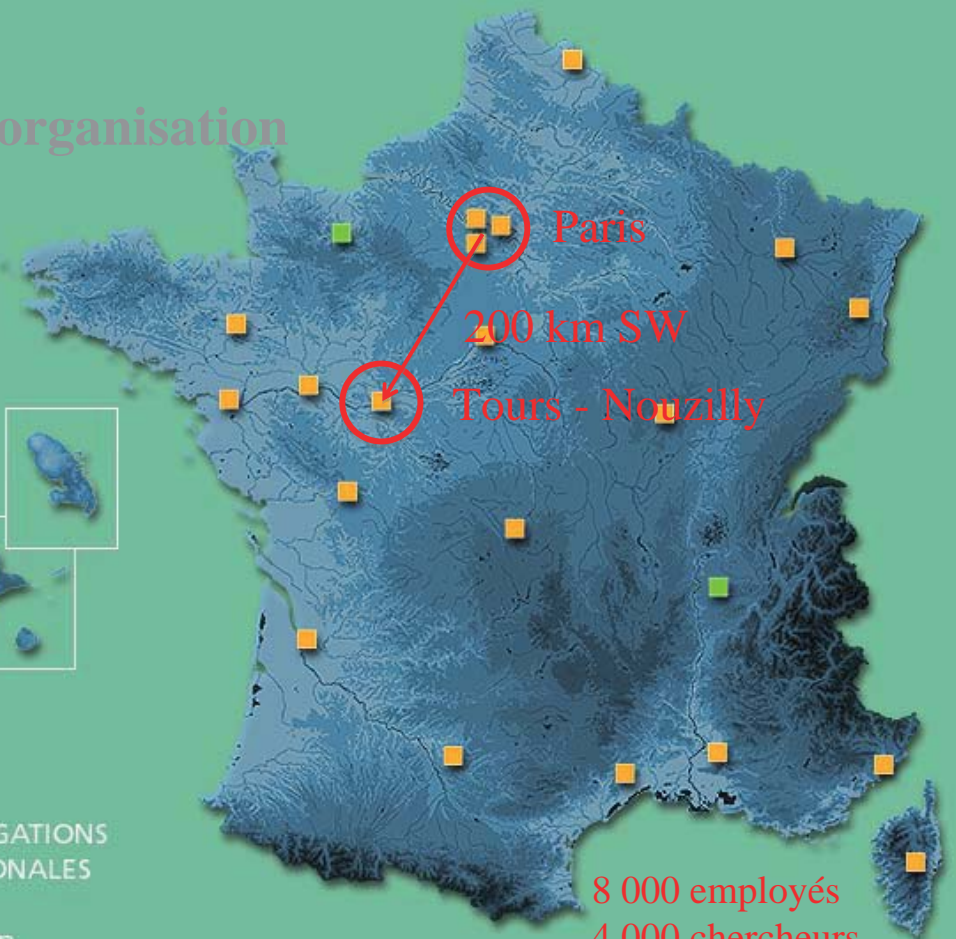
### CENTRES

- > Angers
- > Avignon
- > Bordeaux-Aquitaine
- > Clermont-Ferrand-Theix
- > Colmar
- > Corse
- > Dijon
- > Jouy-en-Josas
- > Lille
- > Montpellier
- > Nancy
- > Nantes
- > Orléans
- > Paris
- > Poitou-Charentes
- > Rennes
- > Sophia-Antipolis
- > Toulouse
- > Tours
- > Versailles-Grignon
- > Antilles-Guyane



### DELEGATIONS REGIONALES

- > Caen
- > Lyon



8 000 employés  
4 000 chercheurs  
4 000 techniciens



## Unité de Physiologie de la Reproduction et des Comportements (PRC)





UMR Physiologie de la Reproduction et des Comportements

<http://www.tours.inra.fr/prc/>

- 150 permanent staff
- 10 research teams
- 50 students (thesis, post-doc,...)
- Cell and molecular biology equipments
- Large animal facilities (cattle, sheep, goat, horse, pig, poultry and rodents)

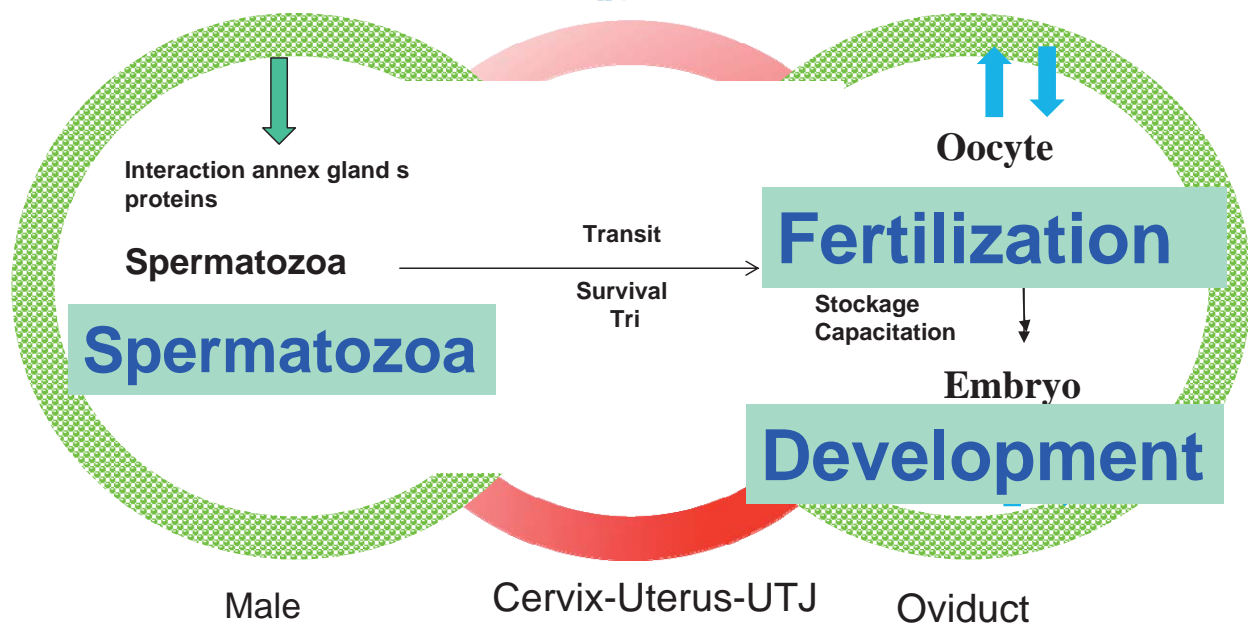
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**INRA**

## Cell Interactions and Fertility

- P. Mermillod – X. Druart
  - A. Fatet
  - N. Gérard
  - G. Goudet
  - F. Guignot
  - Y. Locatelli
  - C. Perreau
  - E. Corbin
  - C. Douet
  - G. Tsikis
  - M. Bertholdo (Australia)
  - P. Khonke (Australia)
  - A. Cordova (Peru)
  - J. Souza (Brazil)
  - C. Soleilhavout (France)

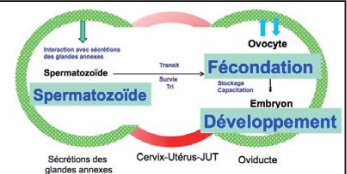
# Topics



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# Objectives



- Study of the dialogue between gametes/embryos and somatic cells
- Study the functional consequences of these interactions on fertilization and early development
- Use this knowledge to improve assisted reproductive techniques
  - Markers of sperms quality
  - Artificial Insémination (AI)
  - Embryo production
  - Genetic selection and conservation

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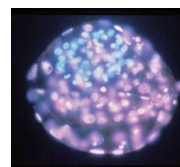
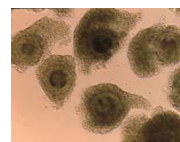


# Activities

- Sperm selection and transport
- Fertilization regulation
- Early development regulation
- Embryo quality and cryopreservation
- Sperm Conservation
- In vitro embryo production
- Transgenesis

Cryobanking

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## The French Cryobank of biological material as safe ex-situ conservation



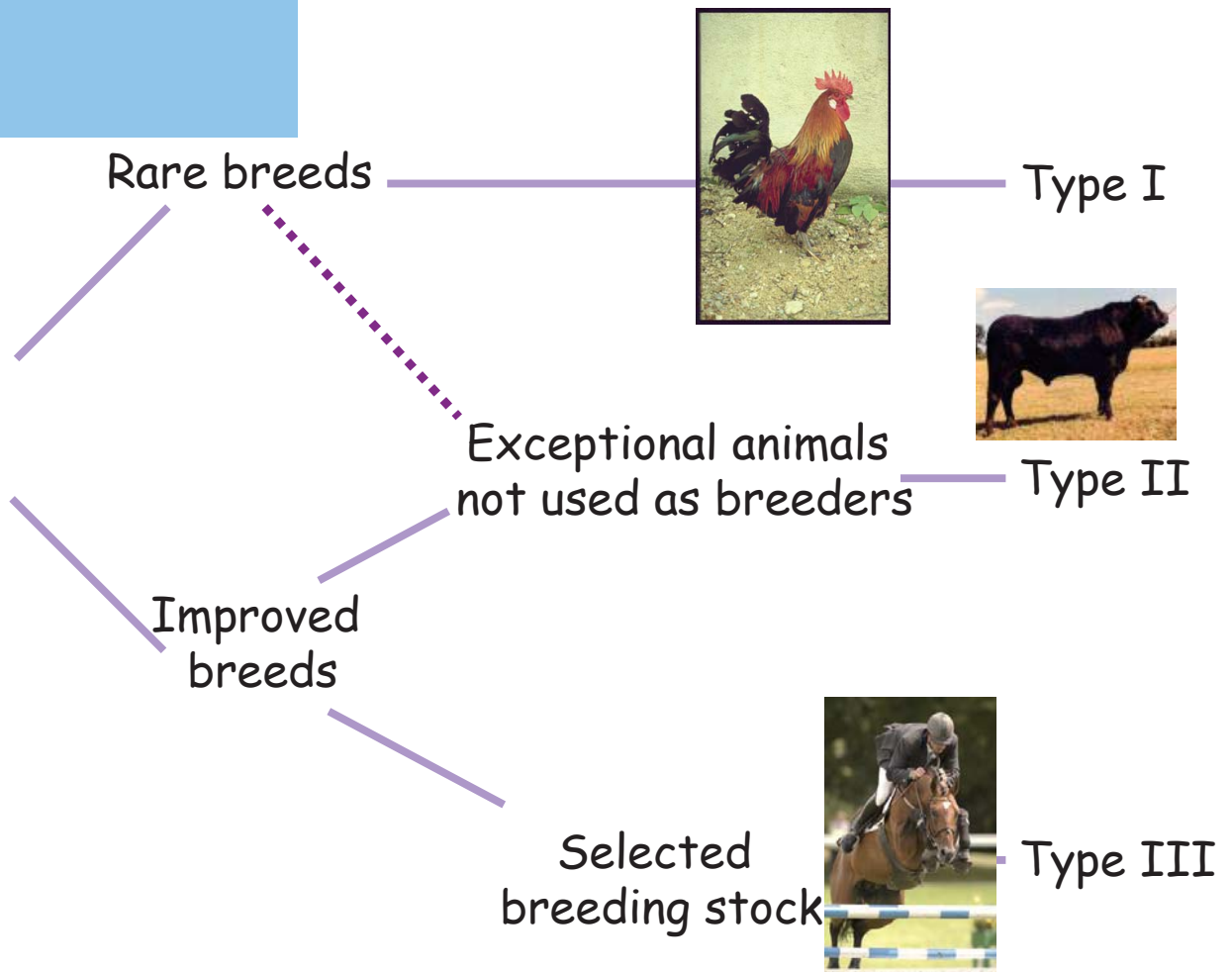
C. Danchin-Burge  
Institut de l'Elevage

## French National Cryobank

- Created in 2003
- Ministry of Agriculture
- Breeding associations
- Administrative council
  - Create the rules of use
  - Make decisions
- Scientific council
  - Edit the standards of cryopreservation
  - Emerging technologies and tissues to preserve

# What can you find in the collection ?

- 12 **livestock** species
- **Type of Biological Material**
  - ✓ Semen (++++)
  - ✓ Embryos (++)
  - ✓ Cells (+)
- **Improved Animal Populations (breeds, strains...)**
- Categorized in **three types** of genetic variability



## "Type I" material

- **Aim :**

To be able to reconstruct a breed (extinction or loss of a substantial number of animals)

- **Constraints :**

- Technical : success rate of AI / ET
- Effective size: minimum number of animals to recreate the breed



**25 unrelated males**

Example **cattle**: 800 doses / male

Or 600 embryos

Ollivier et Renard, 1995

## "Type II" material

- Animal **exceptional** by one or several traits but not used as a breeder
  - Exceptional by a production / functional trait
  - Exceptional by its pedigree
  - Exceptional by its genotype

## "Type III" material

- A breed "snapshot"
  - Sampled on its average generation interval
  - Allows a "live" measure of a breed genetic improvement
  - Allows a "live" measure of correlated traits that are selected (*against or positively*) with an improved trait
  - Allows to come back after selection bias

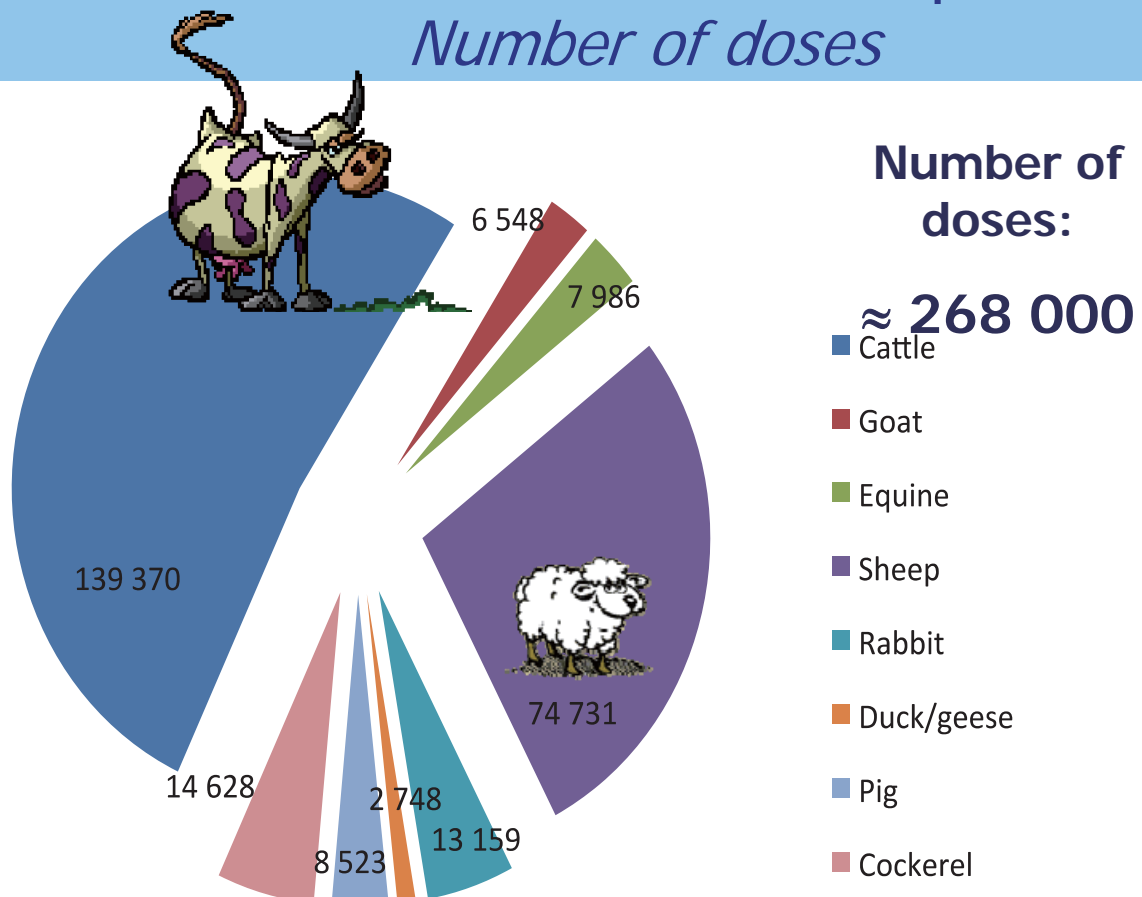
## What animals are sampled by type ?

- |          |   |                                                                                                                       |
|----------|---|-----------------------------------------------------------------------------------------------------------------------|
| Type I   | — | Animals as diverse as possible<br><i>Goal: preserve the maximum genetic variability possible of a breed</i>           |
| Type II  | — | Extreme and/or original genotypes<br><i>Goal: preserve rare genotypes within/between breeds</i>                       |
| Type III | — | Representative of the population<br><i>Goal: preserve an accurate "picture" of a breed for a given period of time</i> |

## What are the potential uses of the material?

- Type I — 1. Help for in situ management  
2. Recreation of an extinct population
- Type II — 1. Change of genetic goals  
2. Research (functional genomics)
- Type III — 1. "In situ" analysis of the genetic improvement of a population  
2. Change of genetic goals  
3. Research

## Collections - 15/10/12 update *Number of doses*

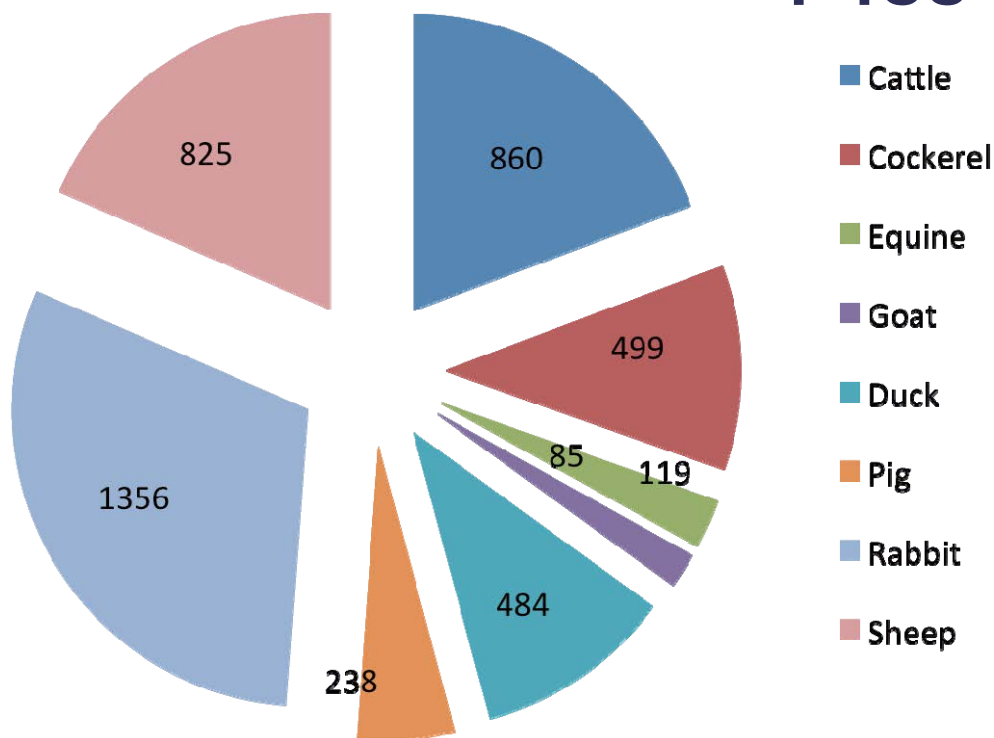


## Collections 15/09/12 update

### Number of donors

CRYOBANQUE NATIONALE

4 466 donors

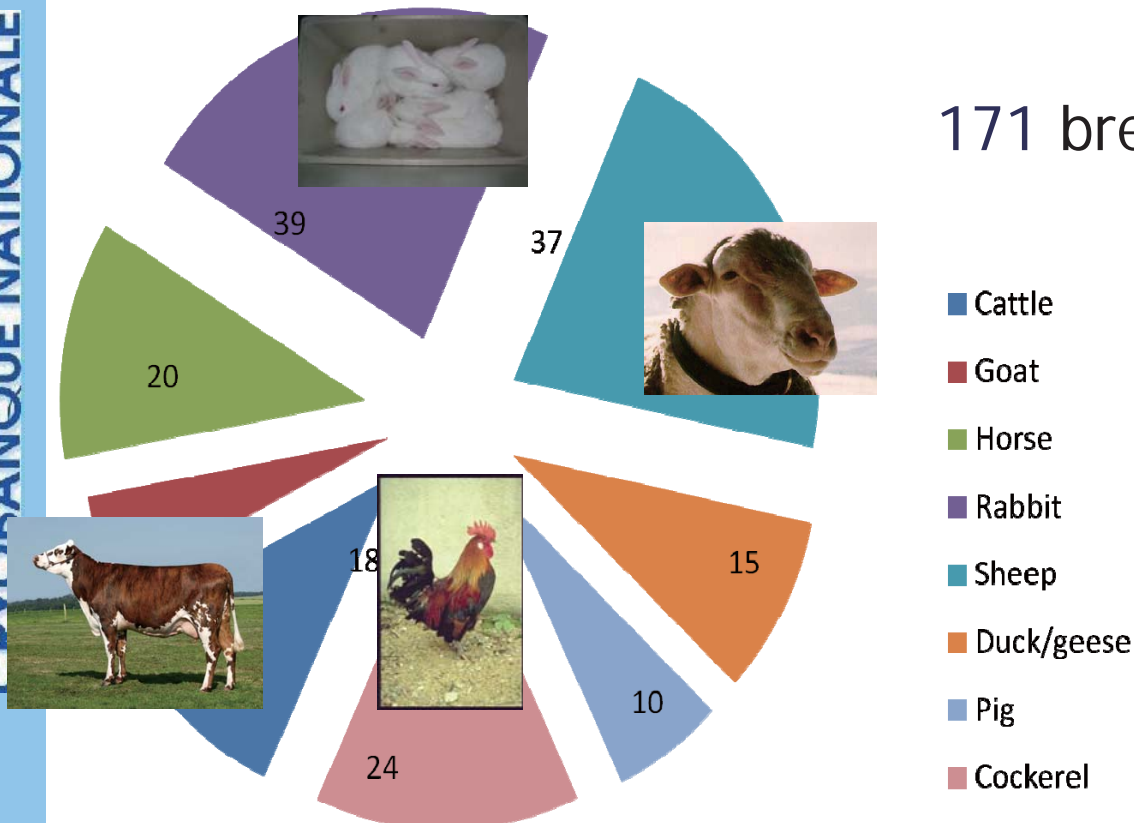


## Collections - 15/10/12 update

### Number of breeds

CRYOBANQUE NATIONALE

171 breeds



# Collections 15/10/11 update

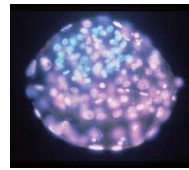
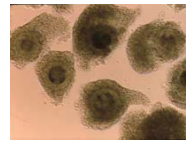
## *Type of biological material*

Type of biological material	NO of doses	Species
Cell	1 007	Goat, Sheep
Embryo	14 274	Goat, Sheep, Rabbit, cattle
Semen	251 638	All but Rabbit

## Is there any material that was ever used from the Cryobank ?

- First requests in 2005, always done by the depositors themselves
- Total of 5 requests:
  - Pig (2rare breeds) and Sheep (1 rare breed), to restore genetic diversity
  - Rabbit and goat: new experiments on extinct lines
- Restoration goals were always achieved, however the number of doses taken out of the Cryobank was not always compensated

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Réserve de la Haute-Touche is one of the biggest zoo in France. In middle of 500 ha forest more than 1200 animals from 130 species and 5 continents are presented to visitors



Yann Locatelli





## Muséum National d'Histoire Naturelle RESERVE DE LA HAUTE TOUCHE

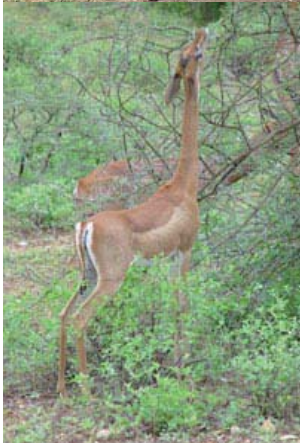


Research is one of the mission from the zoo that belongs to MNHN.

- ✓ Reproductive physiology and behavior (mainly on wild ungulates).
- ✓ Assisted Reproductive Technology (focusing in cryopreservation).



## *Sixth extinction crisis*



Extinction rate is currently 100 to 1000 times superior to what observed 100 years ago ...

### Artiodactyla (ordre)

Famille: Antilocapridae	2
Bovidae	137
Camelidae	4
Cervidae	34
Hippopotamidae	5
Moschidae	1
Suidae	12
Tayassuidae	1
Tragulidae	1

### Carnivora

Famille: Felidae	41
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# Transfer of IVP embryos

- Cervidae (200 species, 40 threatened)



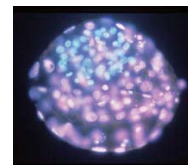
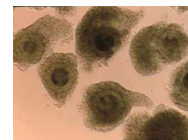
**Elaphus kids in 2004**

**Sika kids in 2006  
(interspecific transfer)**



**Recipient hind  
cervus elaphus (common**

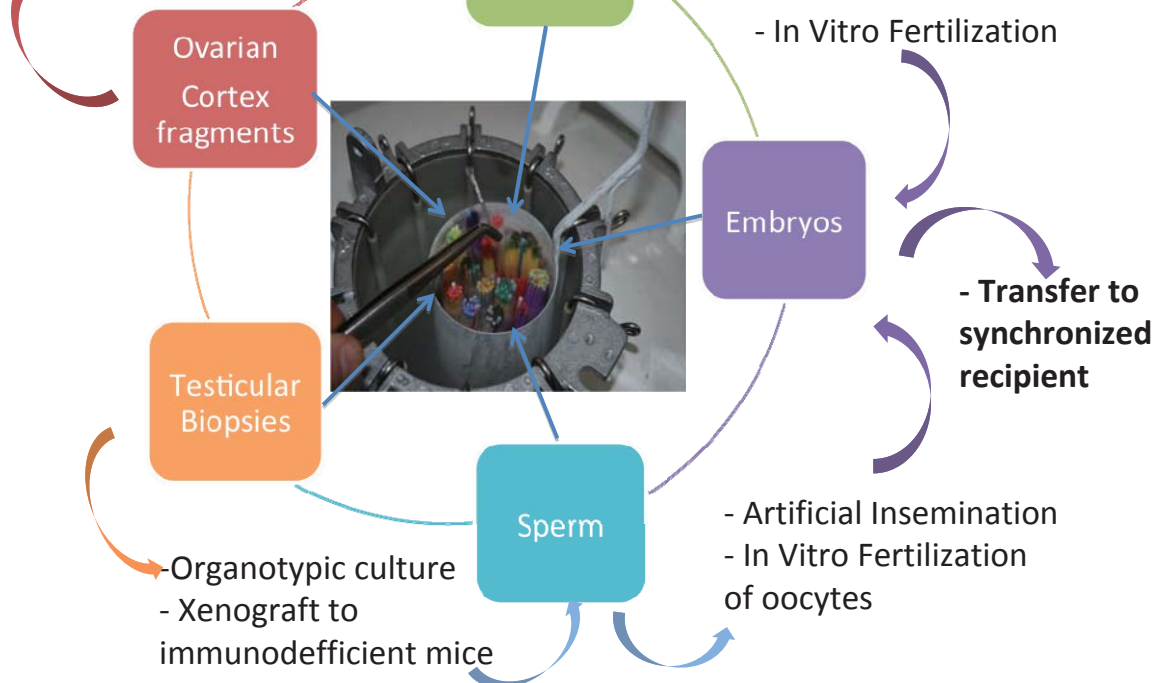
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## Which kind of samples can be cryopreserved?

- Organotypic / isolated follicles culture
- Xenograft to immunodeficient mice



## Limited technologies

Tissue	Cattle	Sheep	Goat	Pig	Horse	Rabbit	Chicken
Ovary	-	+/-	+/-	-	-	+/-	-
Oocytes	+/-	+/-	+/-	-	-	-	-
Sperm	+++	+++	+++	+++	+++	+/-	+
Embryo	++	++	++	+	+/-	+++	-
Cells	+	+	+	+	+	+	+

Ovarian tissue and oocytes preservation and use

Some specific problems to be solved

New techniques for chicken

# A challenge for the sustainable production of human food resources

Decrease of genetic diversity in poultry  
(rural restructuring, selection,  
commercial needs)

Gene diversity is conserved in small  
populations exposed to  
management failures and inbreeding

Muir et al., 2008

High risks of total disappearance  
in case of health  
problem

**Management of reproductive cells to ensure the sustainability of poultry genetic diversity.**

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## Development of Avian Reproductive Biotechnologies for the management of genetic diversity

### National Cryobank, Avian ressources

2 sites of storage



**Primary site of french  
germplasm cryobank**

**Avian site, INRA Tours  
Research center of Tours**

>10 years experience in semen cryobanking

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*Blesbois et al., 2006; 2007; 2010*



## Cryobank current poultry stock

Espèces	Populations	Males	Staws
Gallus (individuel)	19	512	15816
Gallus (collectif)	4	32 (8 x 4coqs)	500
Total Gallus	23	544	16316
Pekin Duck	4	155	1031
Barbary duck	9	325	1330
Goose	1	17 familles	367
<b>4 species</b>	<b>37</b>	<b>977</b>	<b>19044</b>

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### Examples of fertility rates with frozen sperm of domestic birds

	Chicken	Turkey	Goose
Average fertility (F/I) (min-max)	60 [10-90]	30 [5-65]	60 [30-70]
Average number of chicks per ejaculate	2	1	1.5

F/I: fertile eggs / Incubated



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## Birds: limitations

- Low number of inseminations per ejaculate
- Low fertility of frozen sperm (except gallus)
- No embryo transfer
- No cloning
- Need for research

## Birds: perspectives

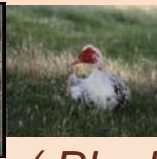
- Improve the fertility of frozen sperm
  - Adapt techniques for each species (breed?)
  - Freezing speed, cryoprotectant
  - Sperm metabolism, membranes integrity
- New germplasm conservation strategies
  - Primordial germ cells, embryonic cells transfer, cell dedifferentiation, chimerism (Bertrand Pain)

# Sperm Cryobanking Development

- Strong expertise in avian sperm biology and cryoconservation



10  $\mu$ m

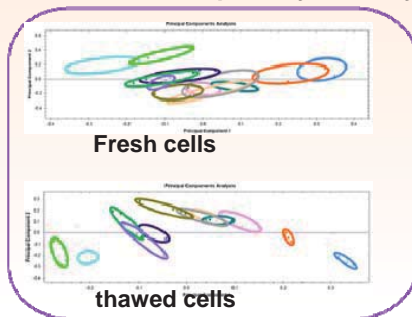


( *Blesbois et al*)

- Elaboration of freezing-thawing protocols for new species



- Evaluation of quality of cryopreserved spermatozoa



Differential proteomic study of peptide profiles of fresh and thawed cells:

## Development of Avian resources

### Target cells for cryobanking

- Isolated gametes
- Gonad tissues
- Blastodermal cells
- Primordial germ cells
- Somatic cells reprogrammed in germ cells

### Three major critical points

- 1) Reproductive potential of cryoconserved cells
- 2) Genetic, epigenetic and phenotypic conformity of the progeny
- 3) Conditions of biosecurity of conservation

# CRYOBANKING OF EMBRYONIC GERM CELLS

## Primordial Germ Cells for endangered species

(collaboration with B.Pain)

Objective: To establish sex specific PGC culture



local breed “la poule noire de Berry”

(the Berry black chicken)



Barbara  
Ambruosi  
(Italy)

Michael  
Bertoldo  
(Australia)  
(lucky guy !!!)

Amanda  
Cordova  
(Peru)

Joanna  
Souza  
(Brazil)



**Thank you for your  
attention**

Joanna Souza (work on goat IVP procedure)  
44 oocytes – 38 cleaved – 33 blastocysts (86%)

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**Thank you Herbie !!!!!**



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## 18<sup>th</sup> International Congress of Animal Reproduction

**ICAR 2016**  
July 17th – 21st  
**Tours**  
**France**

