Challenges for an integrated strategy of gene banking for farm animals in Europe

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Outline

➢ From gene banks to biological resource centers
  an infrastructure approach, CRB-Anim in France

➢ Challenges for gene banks as experienced in CRB-Anim
  ➢ Addressing a range of species
  ➢ Coupling genomic and reproductive material
  ➢ Facilitating access to gene banks

➢ European initiatives
An infrastructure for gene banking

Genetic diversity: a heritage and a resource for the future to meet societal challenges

Genomics
 Genome an archive of population history & the basis of phenotype prediction

Cryobanks (ex situ/in vitro) complementary to in situ management

Documented biological samples

Progress in reproductive biotechnologies

Connect reproductive and genomic biobanks → Biological Resource Center
From a gene bank to a BRC

✓ Objectives of gene banks
  breed preservation/back up/reconstruction
  combined *ex situ* / *in situ* management of genetic resources
  monitoring genetic changes,
  creating new breeds
  research

✓ Requirements
  provide traceability, safety, comply with sanitary regulations
  improve practices and technologies, propose standards,
  disseminate information, facilitate distribution with clear rules (IP)

// core missions of a Biological Resource Center:
Collect/Characterize/Secure/Distribute biological samples
CRB-Anim
An integrated infrastructure of French gene banks for domestic animals

- WP1: coordination, scientific committee, stakeholders committee, access rules and fares, communication
- WP2: technological developments, genomics, reproductive biotechnologies
- WP3: collections enrichment
  8900 donor animals, 22 species, semen, embryos, cells, DNA; ~350 000 samples
- WP4: information system, Web portal, quality certification
- WP5: characterization of collections
- WP6: training (academic, professional, continuous,)
- WP7: economic exploitation: support to livestock sector, genetic models and diagnostic tests, biotech process

6 partners
11 M€
Challenge 1: addressing a range of species

- **Species-specific issues**
  - Biology of reproduction → sampling and storing procedures
  - Selection history → sampling rules
  - Legal organisation → sanitary regulations, property issues
  - Breeders’ attitude → willingness to contribute to gene banking

- **Main difficulties encountered**
  - Local breeds cannot generally comply with regulations aimed at securing exports of reproductive material made for transboundary breeds
  - Cost of using gene bank material: logistics, know-how
  - Very variable fertility rate obtained from gene bank material

⇒ The endangered breeds are often the main motivation for gene banking but they encounter the most technical difficulties to benefit from gene banks
Variability of fertility and consequences for sampling: exemple in chickens

Blesbois et al., 2007

Number of straws to restore the line (n=40) to 97-98% identity after backcrossing

473  973  492  536
To answer these issues: improve methods to use cells with reproductive potential from gene banks

Main critical points:

- Reproductive potential of cryopreserved cells
- Safety conditions (storage media)
- Epigenome integrity following cryopreservation

Cells/tissues concerned: (from oyster to horses, The « difficult » cases)

- Mature gametes (semen)
- Embryos and larvae
- Diploïd germ cells (isolated or in gonadic tissus)
- Somatic cells and tissues

To be reprogrammed in germ cells
Current approaches to improve predictability of fertility results of frozen semen for chickens

Find a fast, reliable and simple method for fertility screening

- **Pilot study with proteomics**: Labas et al., 2014
  - Intact cell MALDI-TOF mass spectrometry (ICM-MS) = fast, reliable and relatively inexpensive tool to **phenotype male chicken fertility**
  - Molecular basis of infertility

1- Use of ICM-MS in a larger rooster population including different genetic backgrounds
   ⇒ **reproductive phenotyping**
2- Identify and characterize biomarkers
   Linked to fertility ⇒ **fertility molecular signatures**
Challenge 2: Coupling genomic and reproductive material

DNA banks and reproductive gene banks are poorly connected

➢ Many advantages
  - Enhance knowledge about gene bank collections
  - Assess their representativness
  - Enhance the scientific value of collections
  - Including the characterization of genetic defects
  - Marker-assisted sampling/marketer-assisted use
  - Refine the choice of animals on the basis of genotypes

➢ Major difficulties
  - Genotyping cost (lower cost/marker)
  - Data sharing policy
  - Database interoperability, standard descriptors
Assessing Representativeness

Ex: NJ tree on wild Gallus

57K SNP chip

Choosing animals for further study &/or sampling

Domesticchick project
Enhancing the scientific value of gene bank collections

Screening a DNA bank to identify an IBD region for a genetic defect

Candidate region from linkage analysis

Mb scale

- Genotyping or partial sequencing of the candidate region
- In carriers animals

Find a common region

Scan non carrier populations

Final interval: kb scale
Trace the history of a recessive mutation

Carrier bull of a genetic defect

Storing DNA and semen in a BRC

Candidate mutation

Produce carriers to study the mode of action

Develop a genetic test

Check other gene banks’ collections

Modify the population management

French National Observatory for cattle genetic defects
Coupling gene banks: agree on common descriptors

- Contact
  Biological Resource Center
- Species/breed/line
- ABS information
  Specific regulatory issues
- Sanitary information
- Associated publication(s)
- Associated project(s)
- Animal
- Sample
- Conditions of access
- Phenotypic data
- Environmental data
- Genetic data
  (genealogy, selection history)
- Molecular data
- Other associated data
  (physiological stage at sampling)
- Material type
  (tissue, sampling protocol)
- Quality control
- Storage information

+ To agree on thesaurii ontologies

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Challenge 3: Facilitating access to gene banks

- Provide easy access to useful information: [web portal] to search for desired samples
  - define options/criteria with users’ groups
  - Main request will rely on species/aim/type of material

- Provide clear rules for entry/distribution of samples
  - Rely on a quality management system

- A demonstration project of CRB-Anim on local pig breeds
Flow of samples

- **Technological R&D**
  - Omics-technologies
- **Genomic BRCs**
- **Reproductive BRCs**
- **Technological R&D**
  - Cryobiology

**Coupling of collections**

- **Web portal**
- **Breeders organizations**
  - Nat Vet Schools
  - hospitals
  - Biotech companies
- **Samples**: DNA, blood, tissues
- **Research laboratories**
- **Samples**: Semen, embryos, cell

**Technological R&D**

- **Genomic BRCs**
- **Reproductive BRCs**
- **Technological R&D**
  - Cryobiology

**Flow of samples**
What is Quality? Why do we need it in a gene bank?

Quality system

- Guarantee traceability
- Transparency of procedures
- Satisfaction of user and of BRC staff

Customers requests

Products and services

Satisfy requirements of customers
BRC quality management system

Management process
- Management
- Continual improvement: internal audit, monitoring and measurement

Realization process
- Sampling
- Reception Registration
- Treatment Conditioning Storage
- Distribution Delivery

Support process
- IT support Web site
- Material resources: Equipments, finances, biosecurity, documentation
- Human resources Training
- Dissemination Exploitation

Requirements

Satisfaction
Using a gene bank: a demonstration project with French pig local breeds
Trends in inbreeding

Storage of frozen semen
Current results

- Using ‘ancient’ frozen semen
  - **Cul Noir Limousin**, 4 boars
    - Round 1: 42% of IA were successful
    - Round 2: 62%
    - 7.2 born alive / litter, 4.7 weaned
  - **Gascon** (3 boars)
    - Round 1: 33%
    - Round 2: 50%
    - 5 born alive / litter, 2.7 weaned

- New entry of frozen semen in the gene bank
  - **Cul Noir Limousin**: 2 boars, 162 doses
  - **Gascon**: 2 boars (on-going)
From a national network of Gene banks to a European Network

**CRB-Anim**: 2 BRC for reproductive material of different species (farm/dogs)

4 BRC for genomic material with some species in common

mostly ‘specialized BRC’

sharing procedures, information, defining a common portal: effort!

impact on distributing samples: not yet!

**European level**: **EUGENA initiative of the European Focal Point**

a European gene banks network

😊 same range of species, same biological and genetic issues

😊 same interest in coupling reproductive/genomic gene banks

😊 in sharing technical solutions, miror collections

😊 **complex governance**: national policy / autonomy of decision

😊 **legal issues**, different property rules, different funding rules
Goals and objectives of the European project IMAGE (submitted)

→ to **upgrade animal gene bank management through genomics and bio-informatics**

to demonstrate the benefits brought by gene banks to the development of more sustainable livestock production systems, by:

• **Enhancing the usefulness of existing genetic collections** to allow the livestock sector to respond to new environmental constraints and market needs while minimising genetic accidents such as abnormalities or loss of genetic variability

• **Optimising complementarity between ex situ and in situ conservation** to maximise resources for the future.

**28 partners** 3 SMEs, 3 NGOs, FAO, 9 research institutions, 11 higher education and research, INRA Transfert.
13 EU countries + Switzerland + Argentina, Columbia, Egypt, Morocco
IMAGE: breakdown of activities into 8 main WP

- WP1: Multi-actor approach
- WP2: Gene bank functioning
- WP3: Reproductive technologies
- WP4: Genomic characterisation
- WP5: Information system
- WP6: Use of genetic collections
- WP7: Outreach
- WP8: Management

Stakeholders:
- CRB-Anim WP4
- CRB-Anim WP2.2
- CRB-Anim WP5,
  ++ Bio-Info
- CRB-Anim WP6
  ++ Methods
- CRB-Anim WP7

EUGENA
Conclusions

➢ Promote a dynamic management of gene banks
  o Developing complementarity between *in vitro*/*in situ* conservation is more valuable than « storing without using »
  o Reintroducing diversity in selected populations, to monitor inbreeding is currently more cost-effective than breed restoration because of limited or unpredictable efficiency of reproductive technologies in many species
  o Innovative uses: combine old and new genotypes for new needs?

➢ To achieve this: cooperation is needed between gene banks
  ➢ Develop standard procedures, explain rules of access
  ➢ Share data and metadata thanks to web tools
  ➢ Funding needed: H2020, genetic resources focus group discussion
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