# GUIDELINES ON PRACTICAL RECOMMENDATIONS FOR THE DEVELOPMENT OF GENEBANKS OF ANIMAL GENETIC RESOURCES

## 1. BACKGROUND

Animal Genetic Resources (AnGR) can be maintained in vivo (in situ and ex situ conservation) and in vitro. Ex situ in vitro conservation requires public and/or private genebanks. Many countries have established national genebank collections of AnGR, as a backup. Cryoconservation is the only contemporary method for the *ex situ* conservation of AnGR.

The most remarkable advantage of cryoconservation of genetic material is the capacity for long storage of reproductive material without deterioration of its quality. Furthermore, genebanks have multiple functions in addition to long-term conservation. Reproductive material from genebanks can be used to support *in vivo* conservation, as a backup in case of genetic or sanitary problems, to develop new lines/breeds, to modify and/or reorient selection or for research purposes.[1]

*Ex situ* in vitro conservation is developed and supported by a wide variety of actors: governments, research institutions, universities, conservation NGOs, and private breeding companies. The most recent effort in the inventory of genebanks was the ERFP's Ad Hoc Action to support the development of EUGENA, to identify candidate EUGENA gene banks, and improve the information about the genebanks in Europe. The AHA identified 125 different ex situ in vitro collections, a small representation of all actors working in the *ex situ* in vitro conservation in Europe. In the case of *ex situ* in vivo conservation, the lack of information is even bigger.

The relevance of genebanks is recognized by the UN in the 2030 Agenda, specifically "Sustainable Development Goal 2" (Zero Hunger, indicator 2.5.1b) which shows the number/proportion of animal genetic resources for food and agriculture secured in either medium- or long-term conservation facilities in each country. In accordance with this indicator (April 2023), only 152 local breeds and 61 transboundary breeds in Europe have sufficient material to allow reconstitution in case of extinction (4.31% of the total local breeds and 8,83% of the total transboundary breeds, respectively).

Various initiatives have focused on strengthening *ex situ* conservation activities across Europe in the past. The IMAGE[2] project underlined relevant gaps in gene bank activities. The most noteworthy of these are the lack of or insufficient collections for many European breeds, and the low transfer of material from gene banks to livestock breeding or breed conservation programs in Europe.

According to a survey by Leroy et al. (2020), many breeds in European countries have either no or insufficient material in genebanks. *Ex situ in vitro* programs should be established, implemented, or strengthened to initiate or expand collections for all breeds, [3] especially local breeds at risk. Complementary genebank collections should be established for all breeds. When it comes to collecting

[1] FAO, 2012. Cryoconservation of Animal Genetic Resources Guidelines. http://www.fao.org/3/i3017e/i3017e.pdf.
[2] Innovative Management of Animal Genetic Resources (IMAGE) project, funded by the European Union´s Horizon 2020 research and innovation program. https://www.imageh2020.eu/.
[3] Leroy et al. 2019. Cryoconservation of Animal Genetic Resources in Europe and Two African Countries: A Gap Analysis. Diversity, 11, 240.

genetic material, critically endangered, local breeds should have priority, although gene banks should also maximize genetic diversity across breeds in their collections. These actions are essential to improve the results of the SDG indicator 2.5.1 (b).

Taking into consideration the previous background, the ERFP Ex situ Conservation Working Group had developed the *Ad Hoc Action "Strengthening national capacities towards the development of a national Gene Bank strategy"*. Its main objective was to assess the current situation, needs and barriers and to define solutions and priorities to support national efforts towards the development of a national cryo-conservation strategy.

The current set of guidelines is based on the information collected and analyzed through this AHA. It aims at training actors to adopt good practices in ex situ conservation and contains practical recommendations for the development of genebanks. It can support relevant activities and initiatives in European countries by providing practical examples of solutions to relevant problems and gaps.

These guidelines are based on actions already implemented and could be therefore directly used in similar cases, as proposed in the recommendations, or by creating initiatives based upon the original models.

## 2. OBJECTIVE

The guidelines aim to provide genebank managers and policymakers with a set of practical recommendations on the base of previous experiences, which allow both to face the drawbacks (previously identified) in the development of the genebanks and/or the policy for the *ex situ* conservation of Animal Genetic Resources.

## 3. PROCEDURE FOLLOWED TO DEVELOP THE GUIDELINES

The guidelines have been designed on the base of the work developed in the Ad Hoc Action "*Strengthening national capacities towards the development of a national Gene Bank strategy*". Experts from 10 different European countries (listed in Annex I) collaborate with the Ad Hoc Action, which was organized in the next three successive steps:

1. First step: Development of a questionnaire to collect information concerning genebanking strategies in Europe, analysis of the data collected and assessment of the situation of participating countries.
2. Second step: Identification of the drawbacks on the *ex situ* conservation, following a "Metaplan" procedure. In this procedure each member of the AHA sent a list of drawbacks identified in their country, the leaders of the AHA summarized the proposals from the countries in a list of 27 final drawbacks. Proposals were then ranked by AHA members (they had to select the 20 most relevant drawbacks and give a score between 1 and 20). The drawbacks were grouped in 4 categories (Funding, Organization, Technical, Policies) and are listed in Annex II.
3. Third step: Exchange of the implemented solutions by participating countries in an on-line Workshop, open to a wider audience. The solutions were analysed, summarized and grouped in the same categories to the ones proposed for drawback. A new classification was incorporated for solutions in the light of the complexity of the measure. To facilitate the use of the newly developed tool, "Boxes" were added, containing a more detailed description of each specific recommendation, edited by the country that developed them.

## 4. HOW TO USE THE GUIDELINESS

These guidelines provide a set of recommendations that can support a national ex situ conservation policy or facilitate the development of individual gene banks. The guidelines must be read as complementary the document for the development of the genebanks, "Innovations in Cryoconservation of Animal Genetic Resources-Technical Guideliness" by FAO (https://www.fao.org/documents/card/en/c/cc3078en). Therefore, in the next parts of the guidelines we indicate the link between the recommendations and chapters in the FAO document**.**

The recommendations are classified in four sections, defined according to the nature of the activities referred in them:

- Policies: recommendations relating to the regulations on the conservation of AnGR and capacity building.
- Funding: recommendations in relation with the sources of financial support and its organization for the development of the *ex-situ in vitro* conservation.
- Organization of the livestock sector: collective measures, which aim to improve the structure and operation of the breeders societies and support individual breeders to participate in and contribute to the *ex situ in vitro* conservation.
- Technical: measures related to all kind of resources for the operation of genebanks, human capacities and procedures to improve the *ex situ in vitro* conservation of AnGR.

There are subdivisions within each section according to the content of the action. Each chapter is linked with the relevant sections of the FAO Guidelines for Cryoconservation.

**The guidelines may be considered ~~as~~ a "tool box" from which each country can select the most suitable solutions to their current situation and development goals.**.

# 5. PRACTICAL GUIDELINESS

## Policies *(FAO Guidelines, Sections 1, 9)*

### Establishment / strengthening of relevant structures

1. Establish a National Advisory Committee for AnGR and a National Focal point for AnGR.

2. Develop a National Plan of Action/ National Strategy for the conservation, development and sustainable use of Animal Genetic Resources, in liaison with the Sustainable Rural Development Policy. The National Plan of Action must highlight the importance of *ex situ* conservation and the further development of existing *ex situ* collections.

3. Underline the global commitments on conservation of the biodiversity (CBD and Agenda 2030) to show the competent authorities their liability in the development of the *ex situ* conservation.

4. Improve data collection on ex situ conservation activities in the country.

**Box 1: Organization and coordination in France in the field of ex situ conservation to enrich the French National Cryobank collections.**

Each breed of ruminants in France is organised in a Selection Organism for the management of the selection or preservation program of this breed. This structure gathers mostly breeders but also other partners such as the AI centres sometimes, which are involved in the production of semen and diffusion of genetic progress. Some breeding programs are using cryopreserved material on a daily basis as for dairy cattle or goat, and, to a lesser extent beef cattle. For the other species, cryopreserved material is not used routinely.
Many specific actions have been coordinated across this last 20 years thanks to different projects to organise collections of cryopreserved material (most of the time semen) for breeds of a wide range of species, including aquatic ones. The coordination of this type of action was facilitated by the creation of the French National Cryobank in 1999. This structure organised all the actions to cryopreserve material for a long-term conservation in livestock species. The Ministry of Agriculture is financing this organism as the genetic resources preservation is a regalian mission. Research organisations (INRAe mostly), technical institutes for livestock species and federations representatives of the breeders are also involved in the French National Cryobank. The advantage of gathering a wide array of different domains representants is that it is quite easy to have the person able to get the information and the good contact for the different actions. The CRB-Anim project was a good example of that synergy as it allowed an important increase in the diversity of the material stored in the French Cryobank whether it is in number of species represented (inclusion of numerous fish species as well as shell and oyster for instance) or in term of diversity inside the species.
To continue the work done as this project is now finished and in order to pursue the enrichment in cryopreserved reproductive material, the Ministry of Agriculture asked to the National Cryobank to write a strategic plan for cryopreservation. The aim of this plan will be to specify how much genetic material is theoretically needed to reconstitute a breed according to the species and underline what is currently missing in the stocks. New collections will then be organised following these priorities, one of the key issue yet to be solved being the funding of the future collections.

Author: Delphine Duclos.

5. Include SDG 2.5.1.b as an indicator to be followed by the National Institute of Statistics.

6. Establish a structure/organization/institution responsible for the organization/regulation of the genebanking of AnGR, connecting all relevant actors with a long-term mandate.

7. A good initial tool could be a mailing group with institutions hosting collections to disseminate information and encourage them to participate in *ex situ* activities or national/international projects (as IMAGE).

**Legal Framework**

1. Set the legal framework for the process of official recognition for breeders´ associations.

2. Set up a legal framework for the regulation of the genebanking of AnGR. The general rules must be adapted/adjusted to the current situation of the country. The legal frameworks should cover the following:
   - Description of the mission of a national genebank.
   - Set a specific recognition and definition for genebanks in the regulation, including a procedure for their recognition.
   - Description of the breeders and breeders' associations rights and obligations.
   - Define the rules on ownership and distribution of the material.
   - Regulation of the participation of the authorities in the process of access to *ex situ* collections.
   - Regulate a national network of genebanks.
   - Define the requirements for MTA /MAA (see: https://www.animalgeneticresources.net/index.php/publications/tehnical-guidelines/)
   - Establishment of a duplicate location.

3. Aligning of the country legislation with the requirements of the Nagoya Protocol (prepare of PIC, MAA and MTA).

4. Develop cooperation with the animal health services to approve exceptions for the genebanking to the animal health law case by case, or general exceptions for all major species in the national animal health legislation for the collection of germinal products intended to be stored in a genebank (i.e collection in farm/on the field, old material).

**Box 2: Exceptions in the animal health regulations for germinal products intended to be stored in genebanks in Spain.**

Spain has a large livestock heritage consisting of 189 breeds, of which 165 are native and 140 are classified as endangered. The activities for the conservation of livestock breeds date back to the last century and, currently, are organized in a national plan of action. *Ex-situ* conservation has been carried out on a large number of breeds, nevertheless the requirements of the EU animal health legislation were a burden while collecting material from endangered breeds, mainly because of their feral behavior, free range breeding and a low availability of means demanded to fulfill the regulation.

Since 2011 and in the last regulatory update (Royal Decree 429/2022)[1] in 2022, EU regulations were maintained as a reference for the collection and storage of germinal products in Spain. However, a derogation is settled down to allow the collection of reproductive material intended to be stored in genebanks without complying with these regulations (mostly to collect semen directly from farms), provided that these activities did not pose a risk to public or animal health.

In order to apply this derogation, procedures have been developed to regulate the collection of reproductive material in the field for the different livestock species. Those procedures have been developed by the units in charge of Animal Health and Animal Genetic Resources in the Ministry of Agriculture, and with the collaboration of experts of the regional governments, which are in charge of the implement of the procedures. The common elements of which are:

- Collection must take place on farms which are classified as officially disease-free under EU rules on intra-Community trade. This condition is only waived in the case of animals which are genetically highly relevant for the conservation of the breed.
- The donor will be subject to the same serological tests as those laid down in the EU's intra-Community trade regulations, and in addition, etiological tests will be carried out to detect pathogens in the semen.
- In the case of serial collections over several days, the donor must remain isolated and samples must be taken at the beginning and end of the collection period.
- The material must be stored separately and await the analytical results (embargo periods), and the straws and package are marked with and specific code.
- If any of the tests are positive, the collected material must be destroyed, except in extreme cases where its storage is justified by a genetic expert. In these cases, strict storage and requirements for use are established.
- Collections may only be carried out by specifically authorized centers/teams under official control.

In April 2023, 15 centers/teams throughout Spain can carry out the collections in the field, which has facilitated the increase in endangered breeds with material conserved in genebanks.

[1] *https://www.boe.es/diario_boe/txt.php?id=BOE-A-2022-9380*.

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## Communication

Communicate widely on advantages of ex situ conservation to authorities, i.e., by technical meetings with the competent authorities to address the key issues and gaps in legislation, publishing articles and technical notes to inform actors about the steps of organization and stress the urgency of the issue.

**Box 3: International cooperation for the implementation of genebanks. Nordgene.**

Nordic Genetic Resource Center (NordGen) has a mission to conserve and promote the sustainable use of genetic diversity among animals, forests and plants that are important for Nordic agriculture and forestry. NordGen Farm Animals contributes to the national conservation schemes of Nordic stakeholders by offering guidance that highlight all the values related to the broad diversity of the farm animals.

Already for 40 years, Nordic countries have collaborated in conservation of animal genetic resources (AnGR). Differences, however, still exist in the management of AnGR. NordFrost networking project (2021-23) has presented a roadmap that aims to further strengthen Nordic collaboration in the conservation of farm animal biodiversity. This roadmap aims to enhance and harmonize gene banking activities as well as to define the best practices and knowledge gaps in management of AnGR among Nordic countries. With the aid of the roadmap, also the pros, and cons of pan-Nordic back-up genebank for AnGR are identified.
NordGen Farm Animals has also a short - and long-term action plan for its role in cryoconservation of the Nordic AnGR that highlight:

- need to follow the achievements of the frontier in gene banking and disseminate the latest technologies, expertise, and services available to the national stakeholders.
- endorse collaboration between authorities and look for new cooperation opportunities.
- harmonize collection policies and protocols based on the common Nordic goals.
- collect and process Nordic metadata.
- explore models for the joint long-term backup genebank for AnGR

On the European level, NordGen has an active role in the *in situ* and *ex situ* working groups of European Regional Focal Point (ERFP) network. NordGen introduces Nordic perspective to ERFP network and implements the state-of-art achievements of the ERFP network in Nordic collaboration. NordGen also collaborates with Food and Agriculture Organization of the United Nations (FAO) to disseminate the most recent achievements in gene banking of AnGR on the global level.

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## Networking and international cooperation

1. Strengthening cross-border, regional and international cooperation on issues of conservation and sustainable use of biological diversity in farm animals, i.e. promote the coordination of the breeding programs of transboundary endangered breeds, avoiding duplicates to save funds. The cooperation must be led by the National Focal Point in each country.

2. Enrolment and active participation in EUGENA and raise the awareness on this network. The enrolment in EUGENA should have the objective to increase awareness on the relevance of *ex situ* conservation at national level.

## **Funding** *(FAO Guidelines Sections 1, 3, 4)*

**Strategic analysis to ensure the long-term commitment of public funding**. The strategy could have the next elements:

1. Analysis on the cost-benefits, technical and practical feasibility of the *ex situ* conservation. Compare these costs with potential losses without ex situ activities in place.

2. Prioritization the use of the funds depending on the conservation values of the breed/population, listing the breeds according to conservation priorities.

3. Re-analysis the FAO targets of material and donors to be stored and adapt the targets to the real situation of breeds in each country.

4. Integration of *ex situ* activities into the national agricultural policy measures and their funding tools, as the Rural Development Programmes of CAP, Operational Groups, specific aids for islands, etc.

5. Use of state aids to livestock sector (Art. 30, Regulation (UE) 2022/2472) to fund the expenses to collect and store germinal products in genebanks as an eligible cost.

6. Establishment of the genebank, storage and collection activities are covered directly by the budget of the departments that hold the collections. Ring-fence funding, not only for the maintenance of the genebank on an ongoing basis, but also for the collection of the material from the relevant herds around the country.

7. Integration of public funds in the long-term funding plan to cover the costs of the information system.

8. A unity of voice of the breeding sector-research institution is necessary for presenting a case to get funding, increase the funding or plan the strategy of funding.

9. Clear distinction between funds for conservation and funds for research.

**Box 4: Funding genebanks in the Rural Development Programme of Portugal.**

Portugal has a national genebank named Bank of Portuguese Animal Germplasm (BPGA, Banco Português de Germoplasma Animal). The National Institute of Agrarian and Veterinarian Research (INIAV) and the Directorate General for Food and Veterinary Medicine (DGAV) manage the BPGA, assisted by a Commission composed by nine representatives of the animal breeding sector (breed associations, technicians and academic). The BPGA funding is obtained through both public and rural development programmes (PDR 2020 and PDR2030) financial support. INIAV and DGAV provide the facilities, qualified technical staff and running costs (budget of the Ministry of Agriculture and Food). PDR 2020 and PDR2030 attribute a fee for Ex situ conservation actions concerning: a) the annual maintenance of genetic material in the BPGA (0.08 € for each dose and year), and b) the collection of genetic material for the BPGA (maximum 10 000€ for each breed and year), attributed to breed associations that delivery cryopreserved genetic material to the BPGA collection, according to published guidelines. This support is not available for all breeds each year. On average, each breed can benefit from this support every 2 years.

Author: Rosa Lino Neto.

## Breeding organizations

1. Involve breeding organizations or commercial breeding organisations in the funding of the genebanks.

2. Start setting up collections without much funding by gathering surplus stocks from AI centers.

3. Support to breeders owning endangered breeds (RDP), to continue breeding activities, increase populations. Support also the option to collect germinal products from these breeds.

4. Breeders' societies of local breeds provide the farmer that sells a male to be trained as a sperm donor, a small grant to make it more attractive for them to contribute.

5. Set-aside system in the procedure to grant subsidies to breeder' societies, provided that part of the funds can only get if the society develops *ex situ* activities, including the duplication of the collections in a national genebank.

6. Free serological/PCR analyses in the public Animal Health Laboratories for samples from donors of germinal products intended to be stored in a genebanks.

**Box 5: Involvement of private and public actors in the genebank development in Norway.**

Norway has a long tradition of using frozen semen in the commercial breeding work. Already in the 1960's the dairy cattle breeding organisations started to freeze semen on floating nitrogen. From the 1980's, frozen semen has also been used in Norwegian breeding for sheep and goats. Today frozen semen is used in all breeding programs for cattle, sheep and goat, in the active breeding populations as well as in the native endangered breeds. The breeding organisations also store semen for long-time storage in their genebanks.
The genebanks are owned by the breeding organizations, so far there is no public cryo genebank for AnGR in Norway. The breeding organizations are co-operatives, that is, they are owned by their members, Norwegian livestock farmers.
The semen is collected at AI-stations. Most of the doses are for commercial sale, and some of the doses are stored for long-time storage. Many of today's native and endangered breeds have storage of frozen semen from the days when the breeds were commercial breeds. This has been a "life-saver» for these breeds.
Important criteria for the males of endangered breeds that are being selected for semen production are well documented kinship, and that they and their mothers are good representatives for the breed. To insure this, the selection is a cooperation between private and public actors; breed societies, the respective breeding organization and Norwegian Genetic Resource Centre. In addition, Norway has implemented a tool for the endangered native cattle breeds to check the relationship to the already existing AI-bulls. In this way we can select bulls that widen the gene pool in the cryo genebank.
The importance of having cryoconserved material in genebanks for longtime storage is highlighted in *National Strategy for the conservation and sustainable use of genetic resources for food and agriculture*[1]*(in English)*, *Strategy Plan for Norwegian Genetic Resource Centre*[2]*(only in Norwegian)*, and *Plan of Action for native endangered farm animals in Norway*[3] *(only in Norwegian)*. Private and public actors in Norway have recognized the importance of maintaining the existing gene banks. There is also an awareness of the lack of a genebank for some species like for example native horse breeds. It has been easier and more obviously to develop genebanks for breeds and species that use frozen semen in the active breeding work. It seems to be a challenge to develop gene banks for species where the breeding work doesn't use frozen semen, and thereby has the possibility to finance gene bank for longtime storage.

[1] *m-0754-e-lmd_strategy_eng_high.pdf (regjeringen.no)*
[2] *NIBIO Brage: Strategiplan for Norsk genressurssenter (unit.no)*
[3] *NIBIO Brage: Handlingsplan for bevaringsverdige husdyrraser i Norge 2021-2025 (unit.no)*

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## Research opportunities

1. Participate in international projects that enable the development and strengthening of national capacities for the implementation of cryo-conservation and the development of genebanks.

2. Exploit the opportunities offered by research projects, with focus on initiating the collections.

3. Promote a better funding from EU research programmes to support projects in relation with AnGR (cryoconservation).

4. Promote public/private partnerships for the funding of genebanks.

## Organization of the livestock sector *(FAO Guidelines Sections 1)*

### Legal framework

1. Organize the livestock sector in breeding societies/cooperatives responsible for the breeding programmes in accordance with the EU regulation, where relevant. Monitoring the populations of local breeds by the authorities and facilitate tools for pedigree registrations.

2. Develop extension services, capacity building for breeders and foster the knowledge transfer from experts to breeders.

3. National Genebanks should prioritize their activities on endangered breeds. Advocate for a cryopreservation step in any conservation programme, which also must foresee the integration between *in situ* and *ex situ* conservation. Take advantage of the process of adaptation to the new EU Breeding regulation (Regulation 1012/2016) to integrate the cryoconservation activities into the breeding programme. Furthermore, the national genebank expert teams also have to contribute to the *ex situ in vitro* collection of material of the mainstream breeds and encourage breeders' societies of those breeds to integrate a back up collection in their breeding programmes.

4. Technical incentives or reduction in fees by breeders societies for breeders collaborating with the *ex situ* collection activities.

### Funding

1. Establisment of long term funding for breeders' associations of local breeds by RDP measures, or national aids (Art. 30, Regulation (UE) 2022/2472).

2. Promotion of ex situ conservation through financial support of breeders' associations.

### Cooperation

1. Enhance the communication between Research Institutes, Departments of Reproduction and Artificial Insemination, Regional Centres for AnGR and Breeders Associations, with the objective to improve cooperation and complementarity of the actors.

2. Develop specific projects to foster the involvement of breeders' societies (mainly those more reluctant) in genebank activities.

3. Increase awareness of Breeders, Breeders Societies and AI companies on the role of *ex situ* conservation by distinguishing cryopreservation for preservation / cryopreservation for industrial dissemination:
   a. Building interest by reporting on case studies showing benefits of *ex situ* (lost variants, health risks) and the links of genebanks with in situ conservation and breeding programmes (limit inbreeding; conserve while fertility is good).

b. Organization of meetings and workshops could be a good choice to increase ~~the~~ awareness. Other channels that can serve the same purpose are: official webpages, articles, press releases, radio interviews, TV reports, etc.

4. Promote the exchange of information, between involved entities, useful for setting priorities.

5. Involvement of the entities conducting the evaluation of utility and breeding value to co-decide about the material stored in genebanks.

6. Direct participation of institutes or public center' experts in animal reproduction in breeding programmes. Breeding programmes (conservation) should have the advice of an expert in genetics.

7. Rare breed umbrella society is on board with the development of a genebank.

## **Technical issues** *(FAO guidelines: Section 3, 5, 6, 7, 8, 10)*

### Capacity building

1. Investment in infrastructures and equipment to establish laboratory facilities and equipment necessary for cryo-conservation.

2. Human capacity building and training on the methods of testing, storage of samples in liquid nitrogen, and the processing of the necessary documentation for their description and identification.

3. Development of a diploma or any official document recognising the skills of AI technicians in cryoconservation for a range of species.

4. Organization of these activities around a plan for the capacity building and training.

5. Support the Universities and Institutes in their tasks in relation with AnGR.

6. Continue to use strategies to overcome differences among different areas of the country.

**Box 7: How to become an inseminator in France?.**

To become an inseminator, there are different training courses depending on the type of animals to be inseminated:
- for ruminants (cattle, sheep or goat), you must pass the certificate of aptitude for insemination technician functions (CAITF)
- for horses, it is a certificate of aptitude for the functions of equine inseminator (insemination licence) or certificate of aptitude for the functions of equine centre manager (insemination centre manager's licence).

To obtain the CAITF, the exam has a theoretical and a practical part. The legislation specify that the future inseminator has to be able to:

- carry out the act of insemination while respecting animal welfare, hygiene and safety rules and regulations
- manage the semen deposit in compliance with regulations and hygiene rules.

Most of the candidates choosing this formation already have a 2-year post-baccalaureate specialisation in animal production and the course is carried out under a professional training contract, when the candidate already has an employer. Two centres in France offer training to prepare for this exam.

Unfortunately, such training courses do not exist for the other species and persons able to make insemination have to learn by themselves or with the help of experienced people.
Author: Delphine Duclos.

## Cooperation

1. Inventory of institutions hosting a collection and their material.

2. Exchange of material between genebanks of transboundary endangered breeds and use EUGENA to facilitate these exchanges.

3. Intensify cooperation with universities and research institutes for the improvement of conservation methods, both at national and international level. Focus these efforts in the improvement of methodologies for cryoconservation in "difficult species" (species in which cryoconservatin of gametes and embryos still requires further research on the improvement of routine techniques).

4. Promotion of research projects and technical development.

**Box 8: EUGENA: the European Genebank Network for Animal Genetic Resources.**

The European countries are highly interdependent, with respect to AnGR. Animal genes, genotypes and populations have spread all over Europe since ancient times. Continuous development and improvement of AnGR has taken place in Europe and AnGR have been systematically exchanged internationally, within Europe and globally. The management of AnGR in the European Region demands an international approach and common organizations.

EUGENA is a network of nationally recognized Member Genebanks in European countries with the aim to support the medium/long term ex situ conservation and sustainable use of AnGR. EUGENA will support the ex situ conservation and sustainable use of AnGR and facilitate the implementation of the UN´s SDG, FAO's GPA and the Nagoya Protocol for ABS in the European Region and the application of the new animal health Regulation 2020/686 in EU countries. EUGENA is governed by ERFP and is working on the basis of Terms of Reference (ToR), as agreed by the General Assembly of ERFP.

EUGENA is the only European network of officially authorised genebanks for AnGR. EUGENA's membership accredits the entities that are effectively working for the conservation and sustainable use of AnGRs and, for instance, EUGENA genebanks can benefit from the new regulations on intra-Community trade in germinal products (Regulation (EU) 2020/686), which allow exceptions to the animal health requirements for reproductive material intended for the conservation and sustainable use of local breeds. In addition, EUGENA is useful for governments to demonstrate compliance with UN Sustainable Development Goal Indicator 2.5.1.b.

However, the main value of EUGENA is the promotion of synergies between genebanks, which is essential to optimize the efforts of conservation and sustainable use of AnGR at pan-European level, especially when dealing with transboundary breeds.
Development of EUGENA was initiated by ERFP in 2016, and since then the network has grown constantly in terms of number of countries, Genebanks members and information about genebank collections. Currently (April 2023), EUGENA is composed 13 genebanks from 9 different countries, which store 1.719.129 samples (semen, embryos, hair, DNA, ovarian tissue, primordial germ cells, ovarian tissue and blood) from 13 different species and 335 breeds.

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## Genetics

1. Proper identification system of animals, herd book establishment and data recording in farms.

2. Promote cryoconservation before a breed reaches a critical status. Support the Universities and Institutes in their tasks in relation with AnGR.

3. Develop molecular tests for pedigree testing.

4. Analysis of demographic, genealogical and performance data kept by Breeders Associations to set priorities.

**Information Systems**

1. Develop and operate a good database for genebanks.

2. Regular collection of information of the material stored in institutions hosting genebanks in the National Database for AnGR.

**Reproduction technology,**

1. In case of lack of technical information on the implementation of semen freezing protocols (e..g. poultry) experts from countries with success on the specific species have been addressed to transfer knowledge.

2. Local breeds benefit from the expertise on *ex situ* conservation applied on commercial breeds (cattle, sheep and goat), in the collection and storage of the material and the storage of information related to the collection.

**Sanitary aspects**

*1.* Create and accredit molecular tests to assess the sanitary status of the material collected instead of that of the entire flock or breed.

2. Organization and distribution in different locations to safeguard the duplicates. The creation of duplicate collections requires well established links between AI stations and breeding centres.

**Box 9: Ex situ in vitro conservation method of the female genome in poultry species.**

Unlike mammals, in avian species the females are the heterogametic. The egg and embryo, which can contain the ***w*** chromosome, cannot be frozen. Most of the reproductive biology techniques developed so far in birds to preserve genetic material have focused on sperm and embryonic cells. If we use only the sperm, the female genome left out from the in vitro gene conservation practice. Although the female gametes are unfreezable due to the huge amount of vitellus as well as their special physico-chemical traits, the first 24 hours after hatching the structure of the ovary allows its cryopreservation, cutting and transplantation of the gonadal tissue. The primary oocytes are located marginally in the ovary and at this age they are in a developmentally dormant state after hatching. This makes the freezing possible, as the biochemical properties of vitellus have not caused a problem yet.

Grafting a gonadal tissue piece into recipient day-old chicks and after sexual maturation artificially inseminating these gonadal chimera hens with the frozen/ thawed sperm of the donor breed, the donor genotype can be regained in 100% in F1 generation.

The method was first developed by Canadian scientists in the early 2000s. The Hungarian Farm Animal Genebank (NBGK-HGI, Godollo) has been conducting research in this field since 2010. The aim was to develop an effective technique for the long-term conservation of the female genome and regain the donor derived offspring of Hungarian indigenous poultry breeds under very simple conditions. These procedures have been introduced into our gene conservation practices from 2020.

There are several benefits of the method:

- The entire female genome can be preserved. Obtaining donor-derived progeny in the F1 generation only takes about half a year. In contrast, if only sperm is used, it takes 6-7 generations and about 3 and half year as well as it never reaches hundred percentages.
- Less sperm doses need to be stored.
- Gonadal chimera chicken can produce donor-derived progeny in her entire life.

Of course, the method has some difficulties too:

- Perfect ovariectomy of newly hatched recipient chicks is difficult, because of the ovary lays on the adrenal gland and there is the abdominal aorta between the two organs, which is vulnerable and interventions can be induced excessive bleeding. Applying electrocautery needs less manual dexterity than the usage of iris scissor and forceps but it is hard to avoid that a small piece of recipient ovary remains beside the donor organ with both techniques and therefore it may happen that offspring can derive from both ovaries.
- Not all genotypes are suitable recipients among the domestic chicken breeds therefore it is suggested to investigate the genetic distance between the donor / recipient combination and the breeds to be preserved can help finding the appropriate pairs. Other species, as duck, guinea fowl seems to be less problematic in this respect.

Among the cryopreservation methods, the vitrification procedure proved to be the best. It is quick, easy and effective as well as doesn't need too much equipment. The organs are collected on acupuncture needle and after a cryoprotectant treatment they are dropped directly into liquid nitrogen (LN). For thawing the organs with needles are immersed into a special warm thawing solution directly from the LN.

Operations can be performed in simple conditions. Only a table in a lab, which was disinfected with ethanol, is enough. Heated room and heated operating table need during the induction of anesthesia and waking up.

Although this procedure is invasive but based on the behavior of the animals, they do not seem to be suffering. The mortality ratio during the intervention and within the first two weeks of the operation is close to zero.

This method is simply, doesn't require too much equipment and effectively applicable if we would like to obtain donor derived progeny and our aim is to preserve the full donor genome.

Though it is approved for use in many countries some still require following a strict authorization process. Consequently, before applying the procedure in a gene conservation program, it may be necessary to acquire country-specific permits from the relevant authorities.

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**ANNEX II. DRAWBACKS IN THE DEVELOPMENT OF GENEBANKS.**