

The French National Cryobank: Creation of a cryogenic collection for domestic animal species

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SUMMARY

France is one of the leading countries in farm animal breeding programs and by its number of farm animal breeds. Strong concerns arose in the last decade to protect our farm animal biodiversity, not only by preserving our rare breeds from extinction, but also to protect the genetic variability within a breed: more than thirty years of genetic improvement has lowered the genetic variability of some major breeds. It is also important to keep enough genetic variability in a breed so that the selection target can be easily changed if needed.

A way to achieve a long term conservation is by using cryo conservation technology. Biological materials can be safely kept and re used after a very long period of time if they are frozen and kept in liquid nitrogen. Cryo-conservation can therefore be used as a complementary tool for managing live animals and preserving their genetic diversity. A French National Cryobank has been created by eleven different partners, including the French Agriculture Minister, to implement a national collection of frozen biological material from nine different farm animal species. Such collections already existed in some species, but the Cryobank target was to unify the different collections that existed, to secure them, and to trigger new collections for species or breeds that are not or only partly preserved. Lots of questions that were not foreseen have arisen as the project was implemented, which has delayed the germplasm collection growth. As for now, most of the organizations that are dealing with farm animal breeding programs have decided to support this project and we can expect the collection to be well implemented in the coming years.

INTRODUCTION

For the past few years, a growing awareness of the importance of genetic resources went along with an increased number of actions aiming to preserve and enhance them. European and international consultative forums were created within the European Union, European Association for Animal Production (EAAP), and the FAO (Food and Agricultural Organization of the United Nations) to discuss this important topic and trigger specific actions to preserve our genetic resources worldwide.

French Authorities were aware of the importance of these actions but also wary of their dispersed nature and their long-term fragility. As a result, they have decided to develop and implement a national policy on genetic resources. This commitment was enhanced by the ratification by France of the Convention on Biological Diversity in 1992 (UNEP, 1992).

For domestic animal species, two main actions evolved from this commitment. First of all, the "Bureau des Ressources Génétiques", created in 1982, saw its action and means reinforced in 1993. Secondly, the Agriculture Minister asked experts united in a task group called "CNAG Scientifique" to think about a way to show that France was fulfilling its commitment to preserve its biodiversity. As a result, it was decided to focus on long term conservation by using cryo-conservation. The idea was to create a French National Cryobank which will store, in a liquid nitrogen environment, biological samples issued from domestic species. The biological material were to be sampled in order to represent all our genetic variability and to become a patrimonial collection. Yet, it was strongly assessed that this *ex situ* conservation had to be thought as a mean of integration and help of *in situ* conservation programs (BRG, 1999, Danchin-Burge *et al.*, 2000). The French National Cryobank cannot be seen as independent from the classic genetic resources management.

1. THE NATIONAL CRYOBANK ORGANIZATION

This project was co-funded by eleven different organizations that are already involved in genetic resources management.

All these organizations signed in November 1999 the National Cryobank convention that gives the basic management rules for the French Cryobank.

The main signatory is the French Agriculture Minister, which is the major financial source for the Cryobank. The other partners involved are the National Agriculture Research Institute (INRA), the Bureau des Ressources Génétiques (BRG), five Livestock Institutes that deal with breeding programs in different species (Institut de l'Élevage for ruminants, Institut Technique du Porc –ITP- for pig breeding, Institut Technique de l'Aviculture – ITAVI - for poultry and rabbit breeding, Haras Nationaux for horse and donkey breeding, Syndicat des Sélectionneurs Avicoles et Aquacoles Français – SYSAAF - for poultry and fish breeding). The other partners are the French federation for domestic breeds associations (France UPRa Sélection), the federation for Animal Insemination Production Centers (Union Nationale des Coopératives d'Élevage et d'Insémination Animale - UNCEIA) and ultimately a veterinary laboratory whose function will be developed later on (Association pour le Contrôle Sanitaire, l'Étude et le Développement de l'Insémination Artificielle et du Transfert Embryonnaire - ACSEDIATE).

It was soon discovered that an obvious partner was missing: we didn't have a representative for the organizations that run local conservation programs. The problem is that these organizations are not united in a federation, and they are too numerous to join individually the Cryobank. It was decided that from now on, an informal representative for these organizations will sit at all the next proceeding National Cryobank meetings.

All partners are represented in a Group Council that meets about approximately three times a year. It decides the main goal and discusses the problems facing the Cryobank. The council elects a President and an administrative secretary for a two year term. The current President is Bernard BIBE, chief manager for the INRA's Animal Genetic Department.

A smaller group, constitutes a Board which can meet any time a specific problem arises. A Scientific Committee has been set up with thirteen different experts on all the species and general topics that are linked with the Cryobank, such as cryo preservation, population genetics, biodiversity and sanitary rules.

2. CONSERVATION TARGETS

2.1. Species and biological material

The Cryobank's goal is to have a collection of samples that are representative of the Improved Animal Populations' (breeds, strains...) genetic diversity in nine domestic animal species: three species of ruminants (cattle, goat, sheep), equine and donkey breeds, rabbit, pig, fowl and fish breeds. Eventually, all domestic species might be kept in the French national collection.

The main biological material kept in the Cryobank is semen, but we can also find embryos as well as cells (fibroblasts are probably going to be the main type of cells that will be preserved). One of the next tasks of the Cryobank is to set up a DNA collection (on the other hand, cells conservation allows to keep at the same time viable cells and a DNA potential) and a physiological serum collection, in order to be able to check a sample's sanitary status at any time.

Collecting and freezing semen is a routine technique for most domestic species. The process is well known and used on a daily basis for cattle, horse and goat, but the results are more variable for rabbit, sheep and donkey, where fresh semen instead of frozen semen insemination is the customary technology. For pig, cockerel and fish, frozen semen technology has just been improved. In the case of fish and mostly fowl and rabbit species, the creation of the National Cryobank has been a trigger to improve and standardize semen cryo preservation technology.

Collection and freezing processes for embryos are also available for most species. As this technique is relatively newer than the semen technique, it is sometimes not as well implemented. For some species, the creation of the National Cryobank has also been a trigger to improve and standardize the existing processes for embryo collection and freezing.

The implementation of the French National Cryobank is being done with limited financial resources: most of its funds are devoted to the maintenance of the collection, not to collect biological material. It is the main reason why most of the current collection is semen doses, which is the cheapest biological material that can be collected. As technology improves and costs drop, embryo collection will be preferred, in particular for species such as horse or cattle that have a long generation interval.

2.2. Biological material sampling

Experts from the scientific committee have designed procedures to sample a breed's genetic variability. We know how to sample species which use frozen semen as a main or quite normal way of breeding (cattle, goat, horse, sheep). For those species, we can implement a collection for a low cost, but for other species, specific funds will be needed if we want to have a decent sampling. The number of samples that needs to be kept in the collection were multiplied by two for security reasons (see § 3).

In order to preserve the French domestic animal's genetic diversity, three categories of materials are kept in the Cryobank collection.

2.2.1. "Type I" material

"Type I" materials are rare domestic breeds.

The number of samples needed to represent a breed's genetic diversity depends on the species concerned. The goal is to keep enough biological material from a breed in case of the worst possible scenario: we need to be able to recreate a breed if it becomes extinct. Ollivier *and al.* (1995) had simulated such a scenario by assuming that a breed is recreated after 5 or 6 generations of outcrossing

(depending on the species generation interval). For all species, except for the equine, it is assumed that we can recreate a breed just by using semen issued from 25 males that are not related (in order to keep inbreeding at a limited level). The number of semen doses necessary to recreate a breed varies according to the figures shown in Table 1. We have to keep in mind that these figures are twice as many as what we actually need, as the collection is doubled.

Table 1: Number of semen doses necessary to recreate a breed.

Species	Number of semen doses by male	N= Number of generation of inseminated females
Cattle	800	5
Goat	200	6
Pig	80	6
Poultry	80	6
Rabbit	80	6
Sheep	200	6

In most rare breeds, cryo preservation is used as a way to help farmers to have their animals reproduce in pure breed. It is also used to preserve different strains in order to lower the level of inbreeding. The creation of the National Cryobank was post factum to most breeds' cryo-conservation programs. Meetings have been held between Cryobank and conservation programs representatives to see how the existing cryo collections could join the patrimonial collection. Most of the "*in situ*" representatives agreed to join the Cryobank program, yet some breeds representatives don't want to be part of it.

The following type materials ("type II" and "type III") are issued from breeds with a selection program.

2.2.2. "Type II" material

They are samples issued from animals that are "original" for one or several traits, but that are not kept as breeders because their average EBV is too low.

We can organize the type II in three categories:

- Animals that have a very high or very low EBV for production (milk, meat ...) or functional traits (fertility, number of offspring by litters...)
- Animals with a unique pedigree, or coming from a strain which is very narrowly spread.
- Animals with a unique genotype. We want to keep animals with "good" genotypes (such as a rare allele for casein in milk production), but also to keep animals that have a fetal gene or a defective gene (such as scrapie receptiveness in sheep).

2.2.3. "Type III" material

The goal with "Type III" material is to have a "snapshot" of the genetic variability of a selected breed at a specific time.

The animals that are sampled have to be representative of their breed for a certain period of time. Each breed "snapshot" is sampled on a period of time that represents the average breed's generation interval. This type of material will allow us, for example, to measure a breed's genetic improvement, to change a breed's selection target, or to check the correlated responses we have between improved and non selected traits.

3. PATRIMONIAL COLLECTION SET UP AND USE

3.1. Collection storage sites

For security reasons, the collection is doubled. One storage place, called the "primary site", is located at the ACSEDIATE (the eleventh partner of the National Cryobank) and contains a whole set of the collection.

The second set of the collection is dispatched on other "secondary sites" that can be single (pig, sheep...) or multiple (cattle) depending

on the species. When cryo conservation is widely used in a species (cattle and goat), professional production centers have been designed as "secondary sites". Otherwise, a specific Cryobank site is implemented, and a highly secured tank (with automatic nitrogen supply) is used to store the biological material.

3.2. Sanitary rules

For most species (such as ruminants, pigs and horses), French or European laws specify the sanitary requirements to collect and freeze semen.

Yet there are some loopholes: for some species and/or some biological material (embryos and cells mostly) national or European sanitary requirements to collect and freeze biological material don't exist. On the other hand, some endangered breeds don't meet the sanitary requirements for semen collecting and for that reason, they cannot access the only way that can secure their future.

A task group has been designed to work on these questions and see if it is possible to have specific rules in the case of genetic resources conservation.

A good way to manage samples with different sanitary status, or with sanitary status that are likely to change with time will be by using the CBS™ straw. This straw has been designed by Cryobiosystem, and is widely used in France by human tissues bank. This straw specificity is that both ends are sealed (Joly T. *et al.*, 1998, Lavergne Y. *et al.*, 2000). Its main interest is that no exchanges are allowed between the straw contents and its environment, which provides a dual sanitary warranty: the straw content cannot be contaminated by the outside environment, and the environment cannot be contaminated by the straw content (Guérin, 1998). A specific research program financed by the BRG has just started to evaluate the possibilities to use this technology on animal biological material. The answer is not an obvious one for technological reasons (the straws are bigger than the ones commonly used, and it changes the freezing process) and financial reasons: the CBS™ straw costs ten times more than the usual straw and a specific and costly material is needed to fill the straws and mark them.

3.3. Cryobank collection flows management

Biological material owners can deposit their samples in the Cryobank with agreement from the council. A convention is then signed between the Cryobank's President and the depositor. As for now, the Cryobank can't own any samples because of its judicial status. The depositors keep ownership of their samples, but they agree not to use fully their ownership rights, and they entrust the Group Council to manage the national collection.

When an organization wants to use samples issued from the patrimonial collection, it has to submit a request to the Group Council. The Group Council will make a statement and then will ask the samples depositor if he agrees to give its biological material: the depositor has the final decision. If both the council and the depositor agree, the samples are given to the asker.

The supplicant has another requirement: it has to donate to the Cryobank an equivalent amount of samples, after a lapse of time that will depend on the breed's generation interval. This condition is necessary if we want to insure that the Cryobank is not going to deplete. Also, if the supplicant is planning a commercial use of the samples, a financial agreement must be made between the supplicant, the material depositor and the Cryobank.

Depositors have to follow the same general rules if they want to use their own samples. Of course, a request made by a depositor to use its own samples will be processed much faster than a request made by an outsider. The Group Council wishes to keep the Cryobank's management practical.

We can expect the following types of requests to use the Cryobank's biological material:

- a rare breed's organization that wants to re-implant a strain that has disappeared;
- a selected breed's organization that wants to do major change in its breeding program (cf. organizations that switched their breed from a dairy cattle production to a meat cattle production during the eighties);
- a research institute that wants to conduct a specific research on an allele...

One of the major upcoming works of the council is to see if it is necessary to change the Cryobank's juridical status, so that the Cryobank can own at least part of the patrimonial collection. In that case, each depositor would choose if he wants to give up his ownership or not. It is quite clear that when entirely private companies rule breeding programs (such as in the poultry, rabbit and pig species), they are not likely to give up ownership of their biological material: they want to be able to forbid the access of their samples from their main competitors.

3.4. Database

For most species, individual data on animals of selected breeds are managed by stockbreeding organizations pooled information systems. When a sample issued from such an animal is deposited in the Cryobank collection, this information is withdrawn from the database to feed a specific Cryobank's database. As for now, the Cryobank's database can't be accessed from outside, but in the long run some information will be accessible from the web. It is agreed that if the depositor wishes it, access to part of the information can be restricted and some information can be confidential.

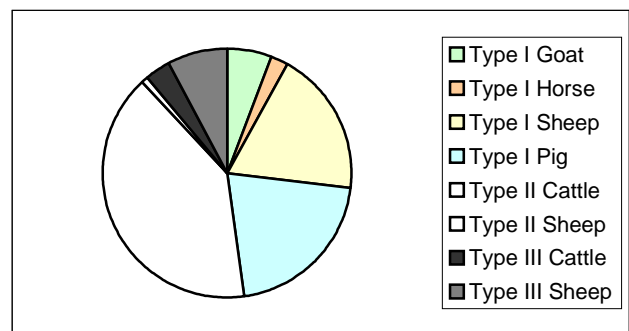
3.5. Cryobank's patrimonial collection current state

Table 2 : State of the Cryobank's collection on 08/01/02

Species	Number of donors	Number of doses	% of doses by species	Number of breeds
Cattle	62	11 414	44%	4
Equine	10	631	2%	3
Goat	17	1 502	6%	2
Pig	73	5 501	21%	5
Sheep	80	7 069	27%	6
Total	242	26 117		20

Cattle species represents the main contributor to the patrimonial collection by the number of doses held in the Cryobank. Yet, only four cattle breeds actually participate in the collection. It is the sheep species that has the most breeds participating in the project. Currently, twelve depositors have signed a convention with the Cryobank. We can expect to double this figure within a year.

Figure 1: Type of material in the Cryobank's collection by the number of doses.



"Type I" material is the main material kept in the Cryobank, followed by "Type II" then "Type III" material. There is currently only two breeds that have "Type III" material in the Cryobank, but this number is likely to increase soon, mostly in cattle breeds.

CONCLUSION

It was quite easy to get an agreement from the French Government and the main organizations that deal with genetic improvement to create a French National Cryobank. Yet, quite a lot of difficult questions arose as the Cryobank was implemented. As an example, we quickly discovered that fundraising was necessary to help collecting biological material for the Cryobank. Also, communication is a key point in this project. It is necessary to meet most of the conservation and breeding program to present them the Cryobank's goal and to convince them to be part of it. It is the main reason why from 1999 till the end of 2001, the Cryobank collection was quite poor, but it is likely to grow quite fast by the end of 2003.

The French National Cryobank is an ambitious and precursor project to protect the French domestic animal biodiversity. Some other countries, such as the Netherlands (Groeneveld *and al.*, 2002), the United States (Blackburn., 2002) and England have just started such programs. As the project evolves, lots of unforeseen problems have arisen. Yet, the solutions that have been found or that are being developed are quite innovative too, and in some cases it will help not only the Cryobank but also other projects.

One of the Cryobank's strong wish was that the collection had to be seen as a tool for *in-situ* breeds management. In some cases, the very existence of the Cryobank has been a mean to find funds to collect frozen biological material not only to reinforce the national collection but also the every day stock which breeders can use on a daily basis. As for now, fortunately, most of the organizations are convinced that this project is theirs. They are now strongly invited to help in the evolution of the French National Cryobank.

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