The French National Cryobank

Historical overview of gene bank development in your country

The use of cryopreservation and the setting up of genebanks to secure the genetic diversity of farm breeds has been widely assessed (FAO 1998; ERFP 2003; Blackburn 2004). France was among the first countries to organize a national cryobank in 1999.

The project was co-funded by twelve different organisations that are involved in genetic resources management. All these organizations signed the National Cryobank convention. The main signatory is the French Agricultural Ministry, which is the major financial source for the Cryobank. The other partners involved are the National Agriculture Research Institute (INRA), the Institut français de recherche pour l'exploitation de la mer (IFREMER), the Fondation pour la Recherche en Biodiversité (FRB), five Livestock Institutes that deal with breeding programs in different species (Institut de l'Elevage for ruminants, Institut Technique du Porc – IFIP - for pig breeding, Institut Technique de l'Aviculture – ITAVI - for poultry and rabbit breeding, IFCE for horse and donkey breeding, SYSAAF - for poultry and fish breeding). The other partners are the French federation for domestic breeds associations (Races de France), the federation for Animal Insemination Production Centers (Union Nationale des Coopératives d'Elevage et d'Insémination Animale - UNCEIA) and ultimately a veterinary laboratory whose function will be developed later on (Association pour le Contrôle Sanitaire, l'Etude et le Développement de l'Insémination Artificielle et du Transfert Embryonnaire - ACSEDIATE).

AI was already used routinely for cattle and goats since the 80's, and AI method was also achievable for sheep or horses but rarely used on the field. Then, thanks to the National Cryobank, several research projects were funded or co-funded in the frame of national programmes dedicated to genetic resources, and AI became possible for other species like poultry, duck, goose or fish and shell fish species.

Objective(s) of national cryopreservation programme/policy

SAMPLING AND STORAGE STRATEGIES

Choice of biological material and location of the collections

The choice of the biological material may differ according to conservation aims and available technologies. For instance, in order to reconstruct a breed, embryos are more appropriate than semen. However, semen is often the simplest and cheapest material to sample and/or to re-use, and sometimes it is the only available material. For this reason, the French collections are mainly made of semen, with the notable exceptions of rabbit, due to the ease of collecting embryos in this species, and of the Rambouillet Sheep breed, due to its unique historical status. Because the collections are duplicated and stored on two locations for security reasons, twice the quantity of material theoretically required for a given goal is collected.

Genetic classification of the material to be preserved

From a genetic point of view, three main types of material to be preserved are considered, according to both the threats to the populations and the potential uses of the materials in the future (Table 1).

- "Type I" material 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 where a breed becomes extinct and will need to be recreated. Ollivier and al. (1995) had simulated such a scenario by assuming that a breed is recreated after 5 or 6 generations of outcrossing followed by backcrossing (depending on the species generation interval).

Table 1. Definition of the main three types of material stored in the French National Cryobank and potential uses for these materials in the future (from Verrier et al., 2003).

Type	Origin of the material		Potential uses	
	Population status	Kind of animals	Main use	Other uses
I	Endangered	As diverse as possible	Restoration of the population	Help for *in situ* management
II	Non endangered	Extreme and/or original genotypes	Change of genetic goals	Research
III	Non endangered	Representative	Genetic analysis	Change of genetic goals, research

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 2. The total stock in the National cryobank is twice more important because of the duplication of stocks.

Table 2: Number of semen doses necessary to recreate a breed.

Species	Number of semen doses by male	N= Number of generation of inseminated females
Cattle	400	5
Goat	100	6
Pig	40	6
Poultry	40	6
Rabbit	40	6
Sheep	100	6

In most rare breeds, cryopreservation 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 National Cryobank was created 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 did not want to be part of it.

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

- "Type II" material are samples issued from animals that are "original" for one or several traits, but that are not kept as breeders because their average estimated breeding value (EBV) is too low.

We can organize the type II in three categories:

- Animals that have an extremeEBV for production (milk, meat …) or functional traits (fertility, number of offspring by litters…) either high or low,
- 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 "interesting" genotypes for production (such as a rare allele for casein in milk production), or for research (a letal gene or a defective gene such as scrapie receptiveness in sheep).

- For "Type III" material, the goal 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 defined 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 monitor genetic trends and correlated responses along breeeding programmes, or to change breed's selection objectives,.

OVERVIEW OF THE COLLECTIONS IN THE CRYOBANK

Table 3 shows the partition of the stored materials in the cryobank by species. The French National Cryobank count more than 260 000 doses of genetic material corresponding to more than 350 000 straws. A dose is corresponding to the number of straws necessary to inseminate one female: for cattle one dose of semen is equal to one straw but for other species it can be different, for example for pig one dose is equal to 6 straws on average. On the contrary,for a straw containing several embryos, the number of doses is equal to the number of embryos.
Half the collection consists incattle breeds because of the regular contribution from the AI centers to the Cryobank, as each year a sampling of the main dairy breeds is realised. In the case of the Holstein breed, a recent study showed that substantial amounts of the breed's genetic variability was captured in the French as well as in the Dutch and US cryobanks (Danchin-Burge et al., 2011).

Rabbit have the greatest number of donors because for this specy only embryos are stored, then for each dose we have 2 donors.

Table 3. Proportion of stored material of each type in the French cryobank on 15/04/2012

	Number of breeds	Number of donors	Number of doses	Number of straws
Cattle	18	841	135 570	135 570
Goat	8	85	6 548	6 362
Equine	17	116	7 700	70 609
Sheep	36	821	74 292	73 047
Rabbit	34	1253	12 040	1 134
Duck	15	484	2 748	2748
Pig	10	238	8 523	47 970
Poultry	24	499	14 628	14 628
Total	**162**	**4207**	**262 049**	**352 068**

The project CRB Anim, which will start by the end of 2012, will finance the collection of genetic material for some breeds that are not part of the French National Cryobank yet, particularly for fish, shellfish, poultry, horses and pigs.

Management of the French National Cryobank

A group Council

The 12 partners who signed the National Cryobank convention are represented in the Group Council, that meets about approximately three times a year. It decides the main goal and discusses the issues the Cryobank is facing. The council elects a President and an administrative secretary for a two years term. The current President is Michèle TIXIER-BOICHARD, an INRA's researcher at the Animal Genetics Division. It is also taking all the decisions concerning entrance or exit of genetic material. A scientific committee was set up to provide expertise and advise on sampling strategies, technological options and research needs.

Storage and documentation

For most species, individual data on animals of selected French breeds are managed by centralised information systems under the supervision of stockbreeding organizations . When a sample issued from such an animal is deposited in the Cryobank collection, this information is copied from the database to feed a specific Cryobank's database called Cryobase. The Cryobank's database can be

accessed by the web. Information considered as confidential has a restricted access, but the number of breeds and doses per breed can be consulted freely: www.cryobanque.org .
For each exit or entrance of genetic material, an additional clause is added to the convention between the Cryobank and the depositor to describe precisely the genetic material involved (breed, donors, type of material, number of straws etc)

Gene bank security

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

Sanitary arrangements/regulations

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 good way to manage samples with variable sanitary status, or with sanitary status that are likely to change with time, is to use the CBS^{TM} straw. This straw specificity is to have both ends 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). However, its use may sometimes be difficult for technological reasons and financial reasons. It was nevertheless decided that genetic material of fish and shellfish, will be stored with CBS straws, since the legislation for these species is not stabilised.

Legal issues (related to genetic material and data)

Owners of biological material can deposit their samples in the Cryobank with the agreement from the council. A convention is then signed between the Cryobank's President and the depositor. As for now, the Cryobank is not entitled to own any samples because of its legal 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 agreement of the samples’ depositor for giving access to its biological material: the depositor has a veto power. If both the council and the depositor agree, the samples are given to the applicant.
The applicant has to fulfill another requirement: it has to give back 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 applicant is planning a commercial use of the samples, a financial agreement must be made between the applicant, 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.

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