Examples of existing repositories and national cryopreservation policies (i)

Flavia Pizzi, IBBA CNR, Italy.

Gene Banking and Cryopreservation Training Workshop
1-3 June 2010

England

Table 1. UK and Eire Bovine Semen Bank of Traditional Breeds (status 31 December 1999)

Breeds	Bulls	Straws
30*	583	79644

Breeds represented in the Bank:
* Aberdeen Angus, Ayrshire, Beef Shorthorn, Belted Galloway, Belted Welsh, Blue Albion, British Friesian, British White, Dairy Shorthorn, Devon, Dexter, Galloway, Gloucester, Guernsey, Hereford, Highland, Irish Moiled, Jersey, Kerry, Lincoln Red, Longhorn, Northern Dairy Shorthorn, Red Poll, Shetland, South Devon, Sussex, Traditional Hereford, Vaynol, Welsh Black, White Park. *(data provided by Dr G. L. H. ALDERSON, 30 December 2002).*

Table 2 RBST Bank of porcine semen.

Breed	No. Collections*	No. stored straws
Berkshire	6	85
British Saddleback	4	44
Gloucester Old Spot	5	27
Large Black	3	30
Middle White	4	34
Tamworth	6	59
Total	**28**	**279**

Data supplied by Mr Marcus BATES, (British Pig Association). *Collections do not necessarily equate to separate individuals.

Table 3. Ram semen samples held by The Heritage Gene Bank

Breed	Farms	Individuals	Semen doses*
Portland	1	13	164
Rough Fell	2	20	324
Lister Lonks	1	4	59
Harrison Dalesbred	1	4	112
Church Dalesbred	1	3	38
Herdwick	13	155	2196

Holt W.V., 2003

France and the Netherlands

Table 2: State of cryobanks' collections by December 2002 (France) and June 2002 (the Netherlands): number of donors and breeds/lines

	The Netherlands		France	
Species	Number of donors	Number of breeds/ Lines	Number of donors	Number of breeds/ lines
Cattle	1 712	8	88	9
Equine	18	4	53	7
Goat	-	-	21	3
Pig	315	16	73	5
Sheep	55	5	89	10
Total	**2 100**	**33**	**324**	**34**

Table 3: State of the cryobanks' collection on December 2002 (France) and June 2002 (the Netherlands): number of doses and percentage of doses by species

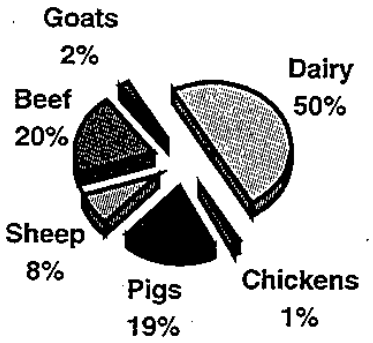
	The Netherlands		France	
Species	Number of doses	*% of doses by species*	Number of doses	*% of doses by species*
Cattle	114 500	*87,10%*	18 614	*49,84%*
Equine	1 110	*0,80%*	1 924	*5,15%*
Goat	-		2 682	*7,18%*
Pig	9 100	*6,90%*	8 629	*23,10%*
Sheep	6 809	*5,20%*	5 501	*14,73%*
Total	**131 519**		**37 350**	

Danchin-Burge C. and Heimstra S.J., 2003

USA

Table 1. Status of collection and live animal activities by the National Animal Germplasm Program.

Species	Collection activities	Planned collection activities
Dairy	Holstein, Jersey, Brown Swiss, Guernsey, Aryshire	Historic germplasm, Milking Shorthorn
Sheep	Targhee, Warhill, Gulf Coast Native	Suffolk, Dorset, Rambouillet, Dorper, Romanov, Texel, Finnsheep, Katahdin, Navajo Churro
Swine	Yorkshire, Hereford, Berkshire, Industry lines	Research lines
Beef	Angus, Hereford, Limousin, Salers, Simmental, Longhorn, Santa Gertrudis, Red Angus, Florida Cracker, Gelbvieh	Historic germplasm
Chickens	Research lines	Research and industry lines
Goats	Angora, Spanish	Tennessee Stiff-legged
Aquaculture		Salmon, Catfish



Blackburn H., 2003

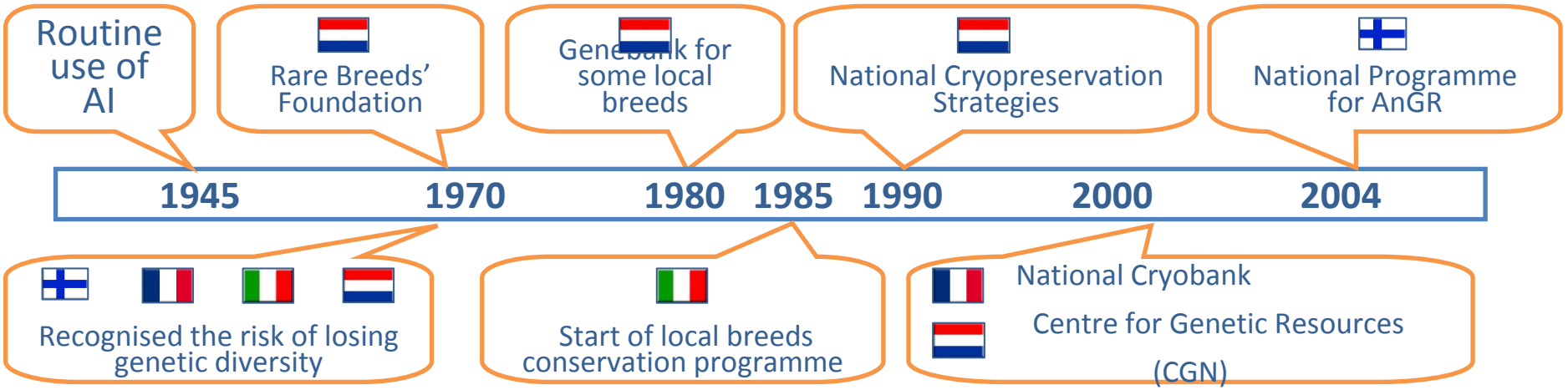
A detailed survey was carried out:

- to **compare cryopreservation activities and policies** in Finland, France, Italy and the Netherlands,
- to **detect similarities and differences** between these 4 countries,
- to **compare the countries' strategies** with the international Guidelines,
- to **formulate recommendations for** initiating or strengthening **cryopreservation programmes**.

In this presentation:

1. Description of **organisational aspects of cryopreservation programmes** in Finland, France, Italy and the Netherlands.
2. Comparison of **sampling strategies** and the **operation** of cryopreservation programmes adopted in the four countries.
3. Identification of **internal and external factors** affecting cryopreservation programmes, to be used in planning conservation policies.

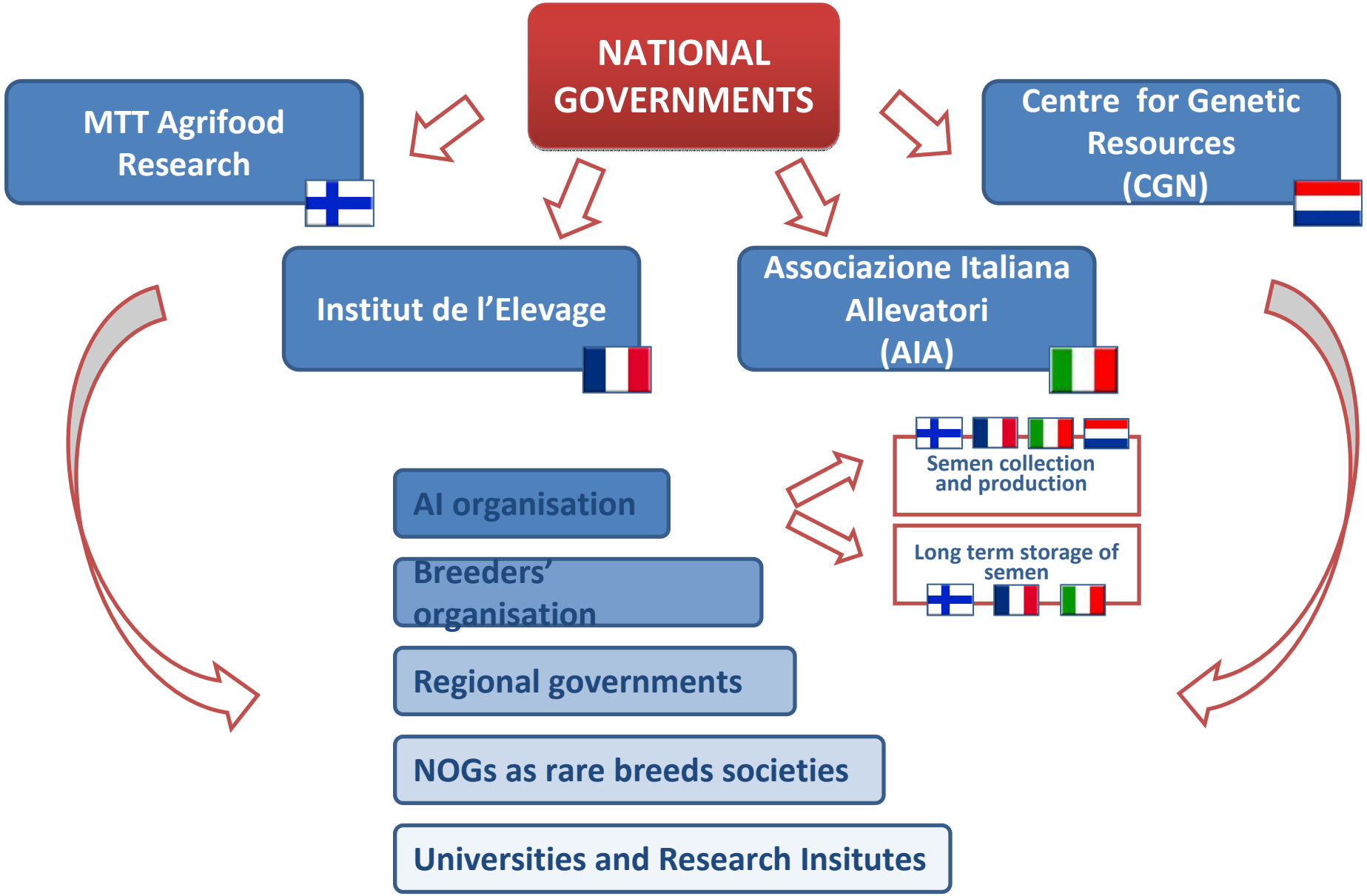
1.1. History and major developments of cryopreservation



1. Organisational aspects of cryopreservation

1.2. Involvement of stakeholders

A variety of stakeholders is involved in the conservation of local breeds.

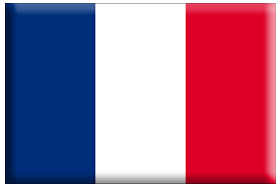


1.3. Responsibility assessment

The definition of ownership and right of access to the cryopreserved material are important aspects of cryopreservation programmes

The **use of stored semen is jointly agreed by the national programme and the AI centre**s. Experts of **FABA Service produce an annual list of bulls** recommended for all the Eastern and Northern Finncattle cows.

In **CGN Gene Bank collection all semen is owned by CGN.** There are **agreements** with the organisations or persons and there is a **general document** describing the conditions for distributing CGN semen.



Local breeds' semen is commercialised directly by AI stations except for the doses stored in the **genetic reserve.** This used only at request of French Livestock Institute and breed societies. **No official agreements** exist between AI stations and other stakeholders.

The organisations (Italian Breeders' Association or regional governments) **that fund semen collection are responsible for the use** of semen in the framework of conservation programmes.

1. Organisational aspects of cryopreservation

1.4. Funding source

National governments

Regional governments

Collection

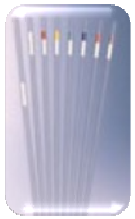
- Genebanks bulls: National funds
  AI bulls: Viking Genetics
- Until ‘90: National funds
  Now: Regional funds
- National and regional funds
- National funds

Storage

- Genebanks bulls: National funds
  AI bulls: Viking Genetics
- AI organisations
- National and regional funds
- Breeding organisations
  Owners of private collections

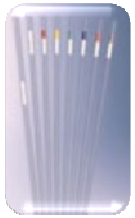
1. Organisational aspects of cryopreservation

Semen packaging



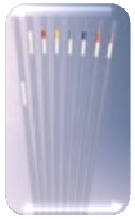
Straws 0,25 ml

100% 98,3% 66 % 31 %



Straws 0,5 ml

69 %

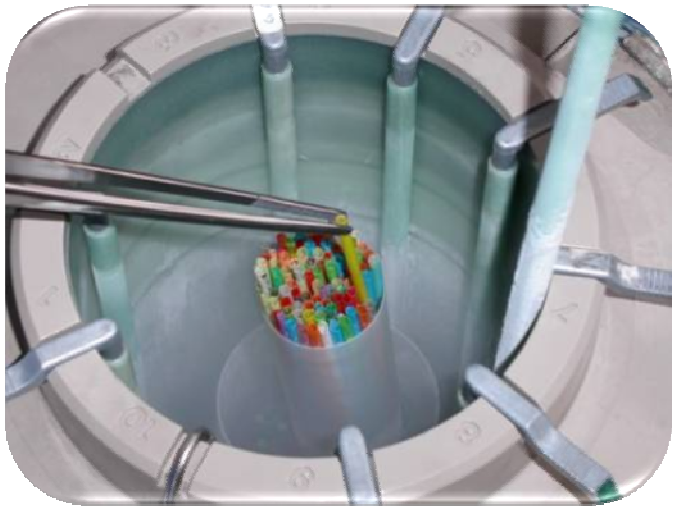


Straws 1 ml

1,5 %

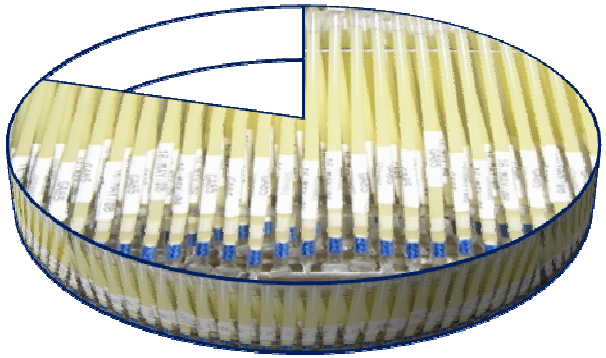
Pellets

34 % 0,2 %

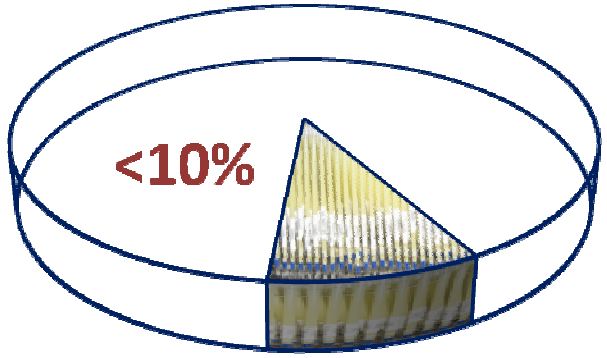


1. Organisational aspects of cryopreservation

1.5 Use of artificial insemination



Mainstream and local breeds



Some local breeds

< 20% of AI in almost 50% of the local breeds of EURECA wide survey among NC of AnGR programmes

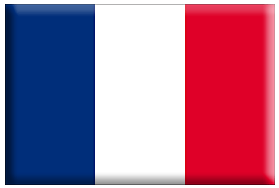
2.1. Aim of storage

Genetic material cryopreserved in gene banks can be used to :
i**) reconstruct a breed** in case of extinction,
ii) **create new lines** or breeds,
iii) as **a back-up** to quickly modify and/or reorient selection programmes,
iv) **support populations** conserved *in situ* in cryo-aided live schemes,
v) as a **genetic resource** for research

The European national gene banks for cattle combine various aims

The main aim is to **preserve genetic material**, semen and embryos, **from all the major family lineages** within the local breeds

National Gene Bank receives **semen from all AI bulls** (100 straws/AI bull to gene bank)and collect additional bulls at AI stations, but also 'on farm'. **Distribute semen to support the conservation** of local breeds "at risk"



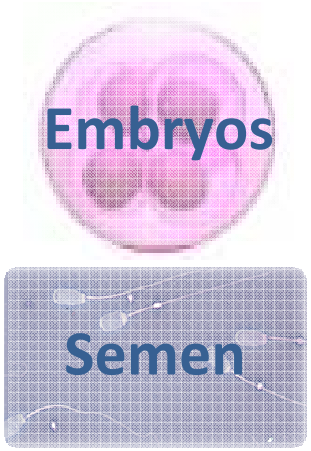
Semen of local breeds **is collected by AI centres for routine** use, **200 doses** per bull are kept for **management of conservation** programmes. Some doses are **also stored in the French National Cryobank as genetic reserve**

National cryobank has not yet been set up, semen **collection** of local breeds is performed **by breeders' associations to support the management** of local breeds. For **each bull at least 50 are kept as a genetic reserve**

2. Sampling strategies

2.2 Type of genetic material stored

Semen and embryos form the most common material for the cryopreservation of farm animal genetic resources



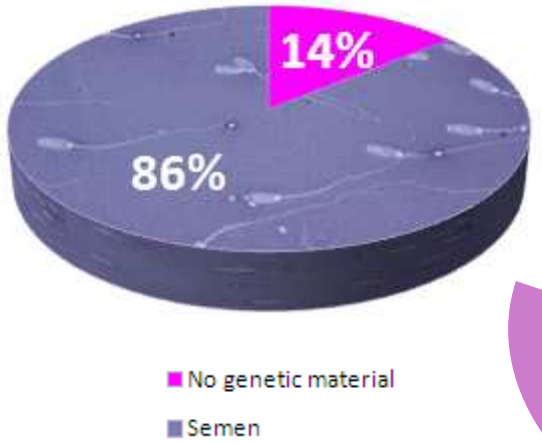
Possibilities	Limitations
• Carry the entire genome including extra-nuclear genetic material •Allow the complete and immediate recovery of the breed in case of extinction	•Freezing not available for all species •High cost •In small population not enough embryos donors
•Freezing technique routinely available in all species •Low cost	•To reconstruct extinct breed several backcrossing and high number of doses are needed •<100% genome recovered, cytoplasmatic effect lost or altered

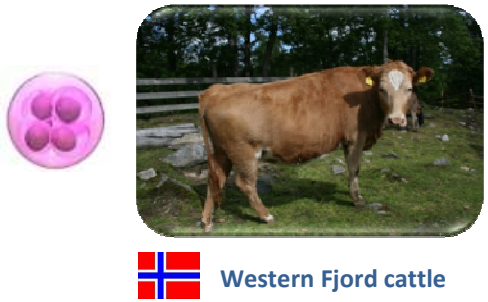
Indication: the storage of both semen and embryos is recommended, the costs for storing embryos plus semen of cattle in gene banks are not significantly different from those for storing only semen.

2. Sampling strategies

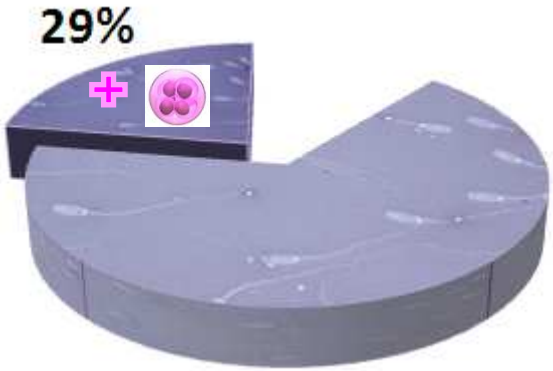
2.2 Type of genetic material stored

The most commonly cryopreserved genetic material of European local breeds is **semen**, as shown in the four countries studied, as well as in the Europe-wide survey carried out within the EURECA project





Western Fjord cattle



2. Sampling strategies

2.3. Selection of donors

The selection of donor animals for cryopreservation can be based on different criteria:

i) random sampling,

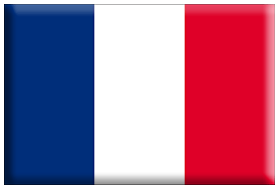
ii) selecting animals carrying specific genotypes/alleles/haplotypes,

iii) maximising genetic variation (e.g. Oldenbroek, 2007).

Sampling strategies vary in the four countries, most of the time **the choice of bulls be collected is based on pedigree information, morphology and performance**

Investigations about the **optimum policy for the management of storage**: the impact of each bull on the genetic variation is considered with the aim of **minimising the average coancestry** among the stored material

Sampling strategy largely **relies on activities of AI organisations**, or on proposals of breed interest groups or herdbooks. National Gene Bank (CGN) invest in depositing **additional semen only after considering coancestry**



Bulls are chosen by the French Livestock Institute in collaboration with breed societies. The **criterion is to have a good representation of the existing genetic variability**, bull morphology and characteristics of the bull's dam are also considered. **Decisions are based on pedigree analysis**

In Italy bull donors are chosen **based on their contribution to increasing genetic variation** of the stored genetic material. Donors are selected **by the Italian Breeders' Association, or research institutes** in specific cases

2. Sampling strategies

For a total of **52 local breeds** from Finland, the Netherlands, France and Italy detailed information on **2,536 bulls** was collected.

Country	Breed classification according to FAO criteria					
	Critical	Endangered	Critical maintained	Endangered maintained	Not at risk	Total
Finland	0	2	0	0	1	3
France	1	0	1	12	4	18
Italy	0	1	1	9	12	23
Netherlands	1	3	0	0	4	8
Total	2	9	4	17	19	52

2. Sampling strategies

2.4. Number of bulls and number of doses in storage

	N° of bulls	N° of doses
Finland	223	351 981
France	563	1 307 166
Italy	1091	264 208
The Netherlands	659	1 095 162

Western Finncattle breed

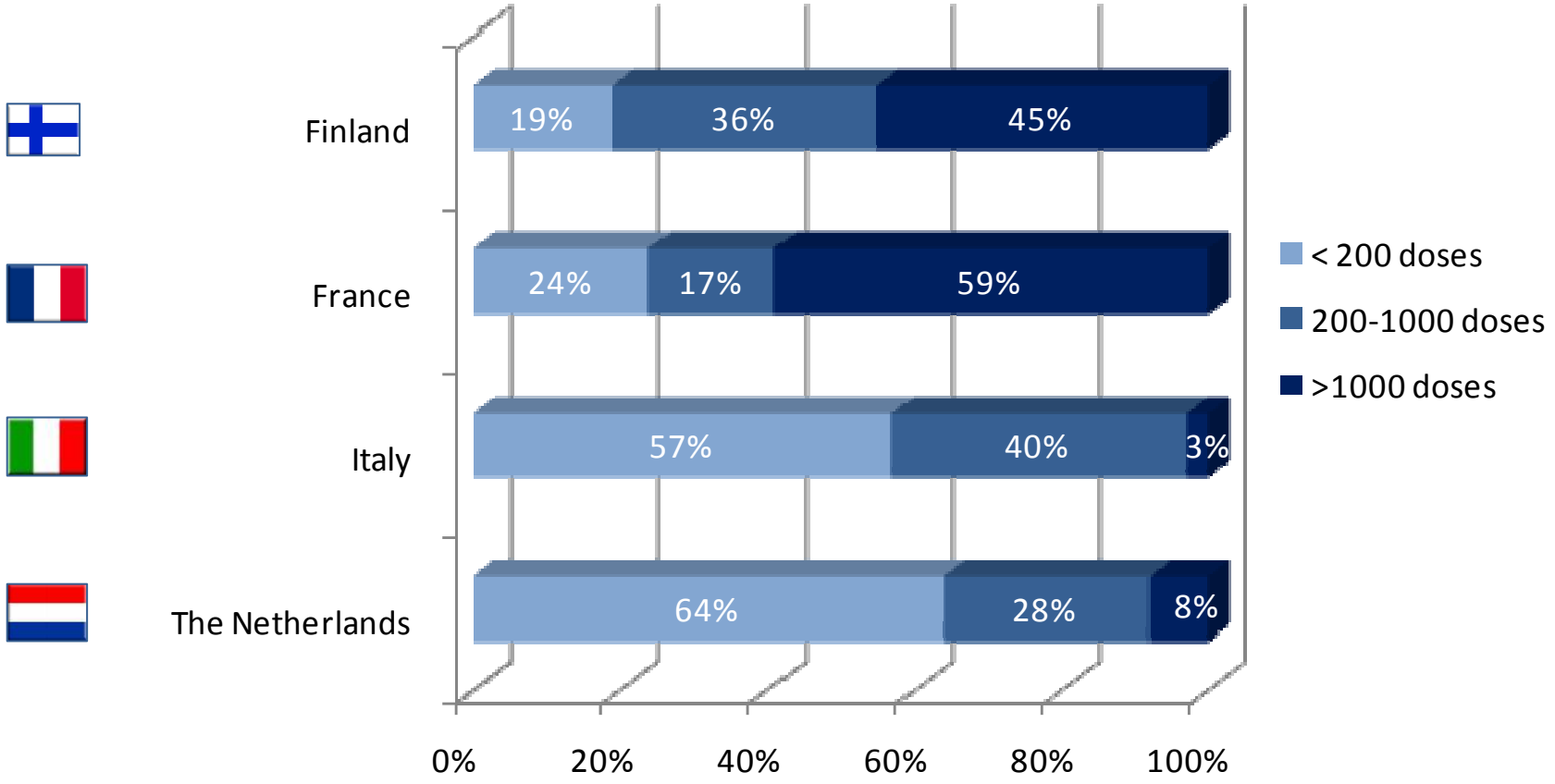
Reggiana

Villard de Lans Breed

Meuse-Rhine-Yssel cattle (MRY)

2. Sampling strategies

2.6. Distribution of semen doses stored per bulls



2. Sampling strategies

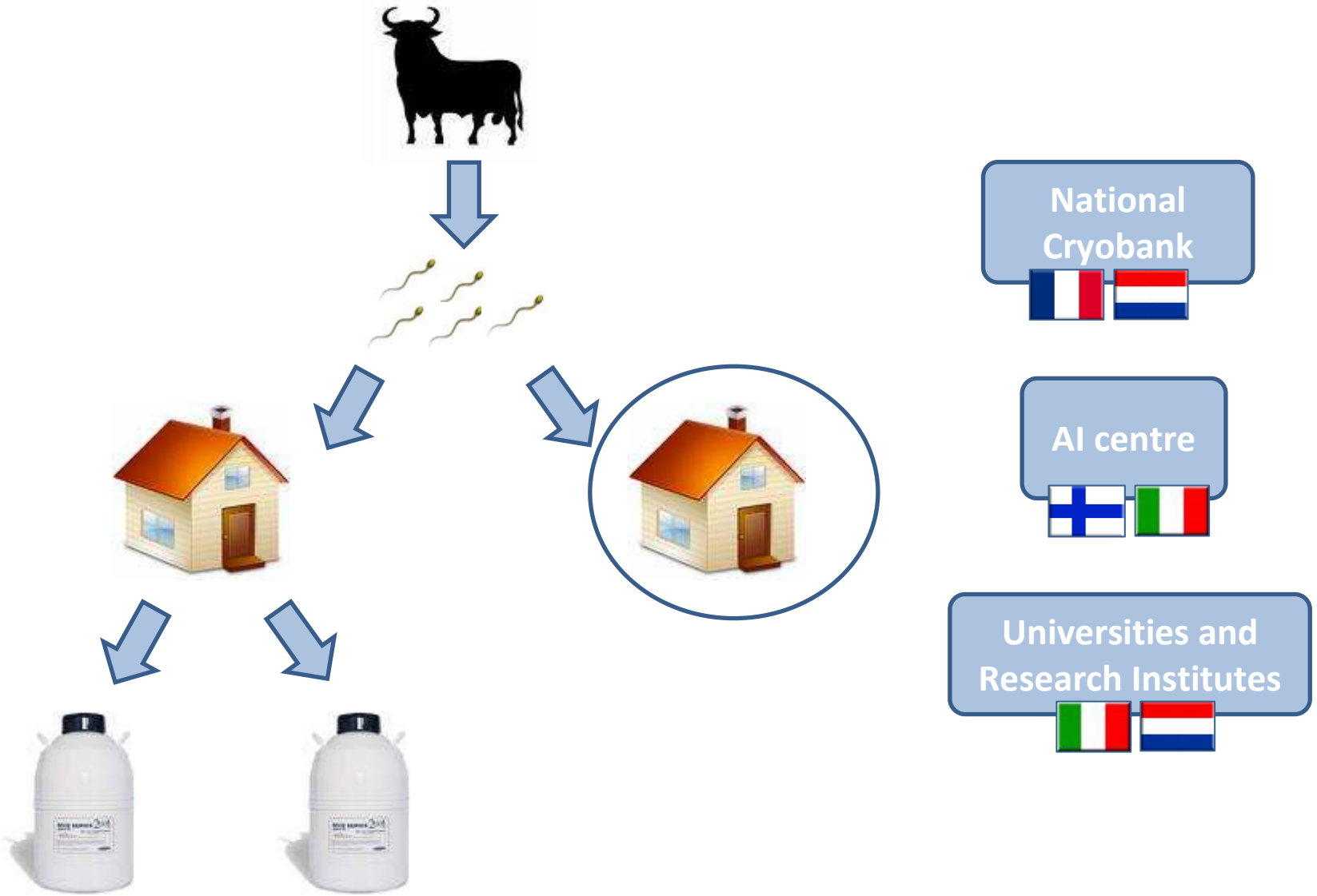
3.1. Collection methods

Biosecurity is an important issue for gene banks because gametes and embryos may carry pathogens capable of surviving cryopreservation.

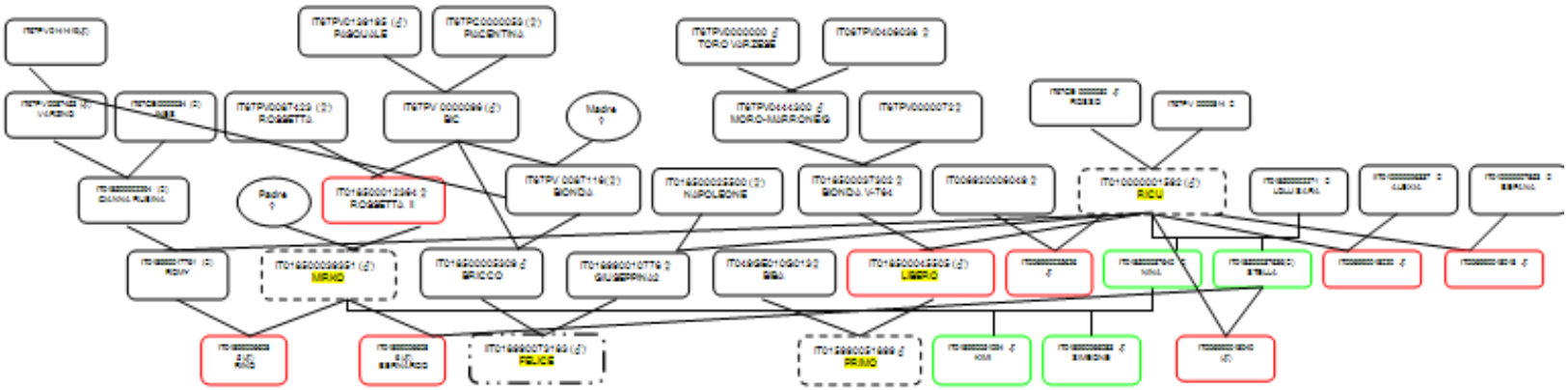
	Collected in EU-certified and non certified AI centres	Collected on farm
Sanitary status	as mainstream bulls	lower
Storage	without special conditions	separate tanks
Export	yes	no
Costs	high for local breeds	lower

Indication : in small populations where few doses per bull are needed for conservation, the storage of semen collected on farm under strict control could be an option.

3.3. Storage sites



3.4. Pedigree information



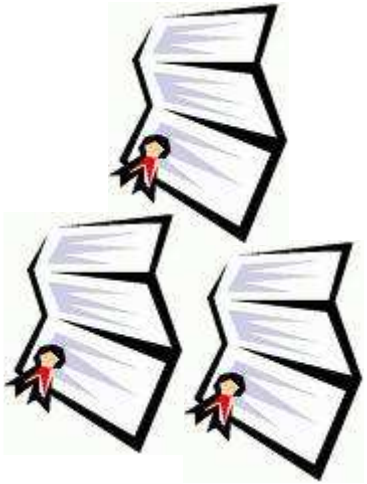
86%

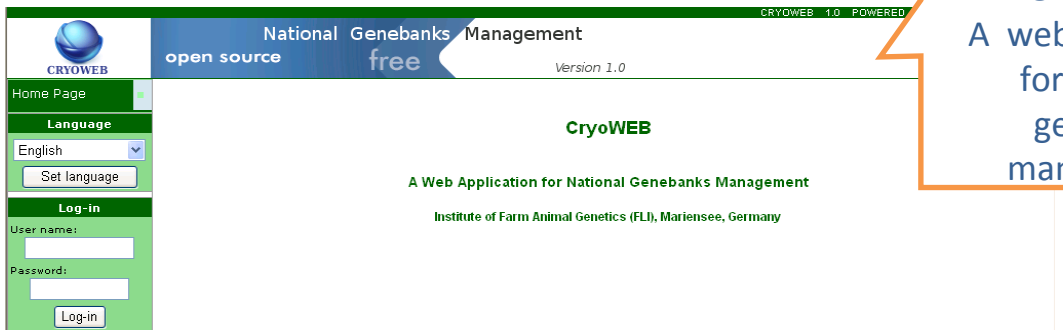
83%

3. Operations of cryopreservation programmes

3.5. Cryobank data management

Cryobanks require comprehensive documentation of their content, donors, freezing procedures.





CryoWEB
A web application for national genebank management

faba

FABA service Database
for the cryo-material of cattle breeds

INSTITUT DE L'ELEVAGE

"Cryobase" Database
all genetic material stored in the French National Cryobank

4. Factors affecting cryopreservation programmes

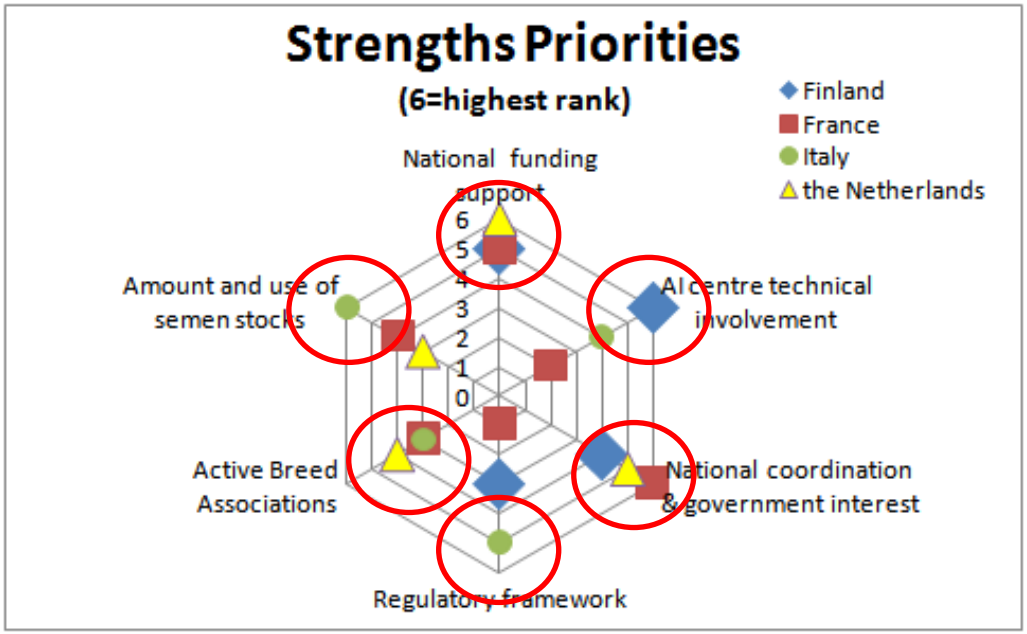
•First**, experts defined the internal and external factors affecting the cryopreservation of AnGR** within their country. **Internal factors** are the **strengths and weaknesses** of their programmes that have affected the past and may compromise or benefit the future of programmes. **External factors** are the **threats and opportunities** of the national framework that could limit or favour the efficient development of AnGR cryopreservation.

•Second, **the experts agreed on some common internal and external factors** that affect the various levels of the cryopreservation programmes in the four countries

Strengths and weaknesses	Opportunities and threats
Funding	Farm AnGR conservation policies
National coordination	Support by breeders' associations
Breeders' association involvement	Infrastructure: AI centre (number, geographical
AI centres' involvement	distribution, etc)
Regulatory framework	Funds for AnGR management
Genetic material stored	Socio-cultural interest in AnGR conservation

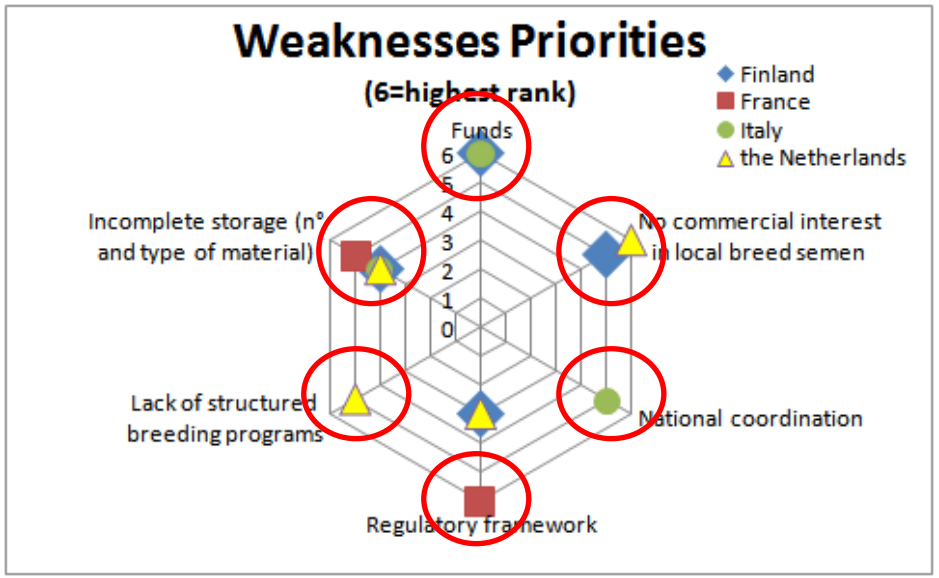
Experts were asked to rank the factors from 1 (least important) to 6 (most important) to quantify their importance.

Ranking of cryopreservation strengths as indicated by national experts



- Finland, France and the Netherlands considered **the financial support from central government** and the **presence of a national programme** as the principal strengths
- **Active breeders' associations** are considered a driving factor in the Netherlands, France and Italy
- **The technical involvement of AI centres** in cryopreservation is recognised as a positive factor for the Finland, French and Italian programmes
- The **presence of stocks of semen** and **their use in supporting local breeds' conservation** is perceived as a highly positive internal factor, mainly in Italy
- The **use of EU common regulations** is considered as a strength for Finland and France
- Italian and French experts underlined the **possibility of some derogations for local cattle to facilitate collection** (e.g. collection *on farm*, complete pedigree not compulsory) as a major strength

Ranking of cryopreservation weaknesses as indicated by national experts



• **Limited availability of funds** is considered as a weakness of cryopreservation programmes. In Finland the **high cost of cryopreservation** and in Italy **limited public financial support**

•The **absence of commercial interest** (value) **in semen of local breeds** is a limiting factor in the Netherlands

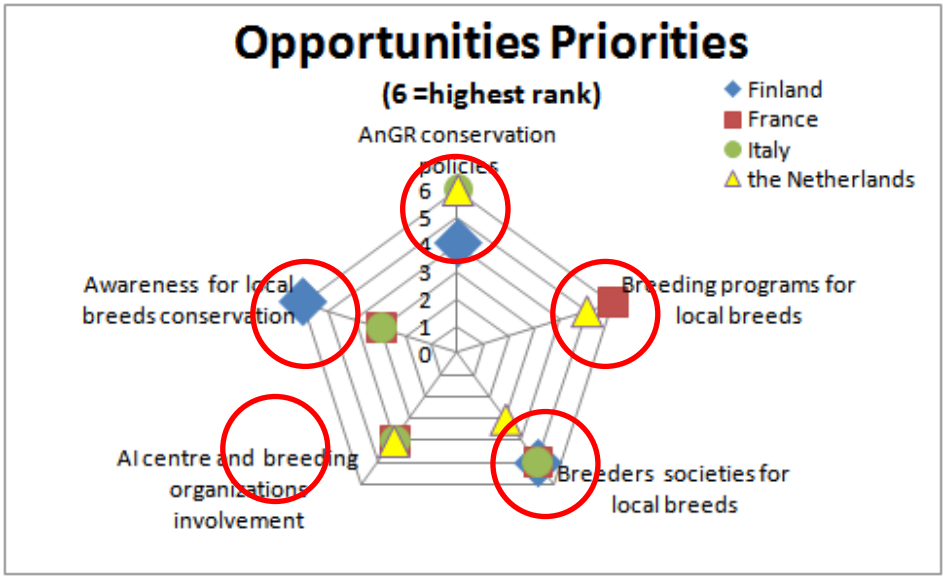
•In all four countries there is concern about the **amount and type of genetic material stored in the cryobanks** (e.g. embryos stored)

•**Lack of regulation for the protection of doses** is perceived by the French experts as a potentially relevant weakness, and the **ambiguous ownership of the genetic material** is perceived as a weakness by the Finnish experts.

•Dutch experts recognise the **lack of structured breeding programmes for local breeds** as a weakness for the cryopreservation of AnGR

• According to the Italian experts, the **cryopreservation activities greatly suffer from the absence of a national coordinated programme**

Ranking of cryopreservation opportunities as indicated by national experts

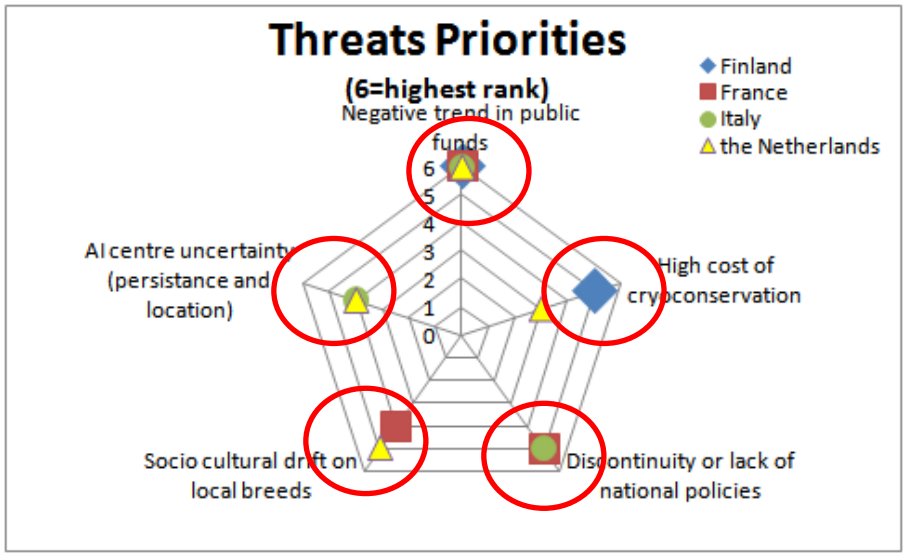


• The **use of *ex situ*** (cryopreservation) **strategies to support the *in situ* conservation programmes** represents the main opportunity for an efficient development of cryopreservation programmes.

• The **presence of local breeders' societies** in all four surveyed countries, and **an increased awareness of the conservation of local breeds**, mainly in Finland, are recognised to have a positive effect.

• The **involvement of breeding companies and AI centres in taking joint responsibility for the long term storage of local breeds** is perceived as an opportunity to facilitate cryopreservation activities in France, Italy and the Netherlands.

Ranking of cryopreservation threats as indicated by national experts



• **Negative trends in public funds**, together with the **high costs of collection and storage of genetic material** (Finland), are recognised as the major threats.

• The **lack of national strategies**, **or their discontinuity**, are also considered as a limiting factor by the Italian and French experts.

• The **uncertainty about support from AI centres** in terms of their continued interest in local breeds (the Netherlands) and about the **availability of AI expertise in the region where local breeds are raised** (Italy), can threaten the cryopreservation.

• **Socio-cultural and demographic factors**, in terms of a **decreasing interest** in local cattle farming (e.g. high farmer age in the Netherlands), are generally perceived as a threat.

Conclusions

• Cryopreservation greatly benefit from **being developed in the framework of a national programme** for AnGR conservation. There is always a **need for national or public or joint funding** for long term objectives.

• **Different ways of organising** cryopreservation, depending on the role and responsibilities of stakeholders. The **close involvement of breeders, breed associations and AI centres** in linking the cryopreservation schemes with routine AI operations, was the **most important factor** for efficient programmes. The collaboration between stakeholders **should be confirmed by contracts**.

• **Freezing semen is a cost-efficient method** to preserve variation; choosing bulls for storage should be preceded by careful planning and optimisation of genetic contributions. **Embryo collections are less common**, while **embryo storage in combination with semen** could be a **(cost-)effective strategy** for re-establishing a breed.

• **Cryobanks require comprehensive documentation** of their content, donors and freezing procedures. A database system for the management of all this information should be implemented within a cryopreservation programme.

• **Long term storage and use of cryopreserved material requires security and sanitary measures**. Secured storage should be guaranteed through **duplicate storage sites and continuous monitoring** of the storage conditions. National **regulatory framework to control the sanitary risks** but at the same time **facilitate flexible collection** (in particular *on farm*) and the **use of genetic material** should develop and implement .




Thank you for your attention