Secondary
Guidelines

for Development of National Farm Animal Genetic Resources Management Plans

Management of small populations at risk

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▶   Foreword

▶   Use of these Guidelines

In recognition of the importance of animal genetic resources (AnGR), and of the sizeable portion that is currently at risk of loss, and in keeping with FAO's mandate and the Convention on Biological Diversity (CBD) a special action programme for the Global Management of Farm Animal Genetic Resources was launched by FAO in 1992.

One of the objectives of this Programme is the development of Guidelines for country use. The Primary Guideline Document (FAO, 1996), mainly targeted towards policy makers, is designed to help countries get started to identify the main elements and objectives of an animal genetic resources management plan, and to outline the strategic policy directions required to fulfil these objectives. The Primary document is complemented and supported by four secondary documents targeted mainly at those that implement policy, administratively and technically, covering the following issues: characterization, livestock production systems description, active breed use and development, and managing populations at risk to provide guidance for the management of areas identified in the primary document. These Guidelines looks at the specific aspects, options and techniques for the management of populations at risk.

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The Guidelines take the form of a step-by-step manual: the first step being to evaluate the present situation by population censuses and surveys; the second step, to choose between the various conservation options; the third step, to make a technical design for the chosen conservation option; the fourth step is to construct a thorough organization, communication and training plan for the project.

The Guidelines form a manual on how to set about conserving small animal populations at risk. The Guidelines will be periodically reviewed incorporating the information and experience that accumulates on the conservation of AnGR.

▶ ## 1.   Introduction

■ *The importance of livestock*

■ *Reasons for loss of animal diversity*

■ *The Global Strategy for the Management of Animal Genetic Resources*

■ *Objectives for conservation*

■ *Conservation strategies*

■ *The goal and structure of the Guidelines*

▶· ## 1.1   The importance of livestock

Why consider conservation?

Domestication of farm animal species was initiated some 12,000 years ago when people began maintaining animals for work power, food, fibre, and other agricultural uses. Today about 40 mammalian and avian species have been domesticated, and are all important for food and agriculture. But, the majority of the world's livestock production is derived from only 14 species which comprise some 5,000 breeds.

Approximately 1.96 billion people, 40 per cent of the world population, depend on livestock directly to meet part or all of their daily needs. An estimated 12 per cent of all people depend almost entirely on products obtained from ruminant livestock - cattle, yaks, sheep and goats. Livestock transform forage and crop residues, which are inedible to humans, into nutritionally important food products. Approximately 40 per cent of the total land available in developing countries can be used only for some form of livestock forage production. Animals account for 19 per cent of the world's food directly. They also provide draught power and fertilizer for crop production, bringing their overall contribution up to 25 per cent, and thus are essential components in achieving sustainable food security. In addition, livestock serves as a very important cash reserve in many of the mixed farming and pastoral

systems, thereby providing an important form of risk reduction. In total, animals meet an estimated 30 per cent of the value of human requirements for food and agriculture. Due to rapid population growth, consumption of food and agricultural products is increasing. Hence, animals are a crucial element in meeting future global requirements, and are likely to improve the quality of life of many rural communities.

Many breeds of domestic livestock are threatened or endangered. Based on a world wide survey, the World Watch List for Domestic Animal Diversity (WWL-DAD:2, FAO/UNEP 1995) classified 27% (390/1433) of breeds as threatened or endangered (to be defined more precisely in Chapter 2). Extrapolation to the 5,000 breeds believed to exist, indicates that there are between 1,200 and 1,600 threatened breeds on the planet. Globally, it is estimated that about 50 or more breeds are lost per year, which is approximately one breed per week. While many breeds are down to numbers which make the future of these breeds very insecure without action to conserve them, others are in imminent danger of extinction if nothing is done.

▶·   1.2   Reasons for loss of animal diversity

What are the forces at work?

There are several factors which place breeds at risk of loss and threaten domestic animal diversity. By far the greatest cause for genetic erosion is the growing trend to global reliance on a very limited number of modern breeds suited for the high input-output needs of industrial agriculture. This trend is of paramount concern as about 50% of the total variation at the quantitative level is between breeds, the remainder being common to all breeds. Hence moving to a few breeds would eliminate a considerable amount of variation in the species, in addition to jeopardizing readily available gene combinations in other remaining unique gene resources. This has been further amplified by the possibility to access germplasm worldwide and the development and easy movement of highly selected breeds.

Reasons for loss of farm animal genetic resources

- Introduction of exotic germplasm

- Poor agricultural policies

- Restriction of development to a few breeds

- Changing market requirements

- Degradation of ecosystems

- Natural disasters

- Political unrest and instability

In developed countries progress in advanced breeding and reproductive technology have lead to substantial increases in agricultural production in some production systems. The basis for this success was the possibility to develop and apply these technologies, and to access many diverse breeding populations harbouring desired genes or gene combinations. This was further amplified by the possibility to access germplasm worldwide and the development and easy movement of highly selected breeds. What was successful on one hand was deleterious on the other, since improvement programmes this century have only concentrated on a few breeds in each species, using high level inputs, and also upon just one or two traits with the improvement activity being carried out in comparatively benign environments. Proliferation was amplified through application of reproductive technologies, mainly through artificial insemination. Other modern biotechnologies, such as embryo transfer and cloning once it becomes more efficient, may further aggravate the problem if adequate precautions are not taken. The result to date is that a large number of breeds and strains which were highly adapted to very specific environmental and feeding conditions are now threatened, or extinct.

During the history of domestic livestock breeding, all over the world, there have already been a very large number of breeds created, many of which have become extinct., Provided the rate of creation of new breed populations parallels the rate of extinction there is no cause for concern.

However, for the past 100 years this has not been the case: there has been a high increase in the rate of extinction of breeds and varieties which has far outstripped the rate of creation. This represents a dramatic loss of genetic variation within the global pool of domestic stocks. In Europe alone, 60 breeds of livestock have become extinct this century and a further 200 are considered to be endangered (Maijala *et al.*, 1984). In many other countries undergoing rapid agricultural development and change there has been a tendency to focus livestock breeding programmes on relatively few breeds without fully identifying, evaluating and taking steps to conserve the wide range of local stocks available (Hodges, 1990).

For the developing world, there are several primary factors responsible for diminishing animal genetic diversity:

- The introduction of exotic germplasm whereby exotic or other, often non-adapted, breeds have been introduced followed by rapid spread through indiscriminate crossbreeding. This has frequently arisen through wrong advice, often given in externally funded projects, and this has been exacerbated in many cases by flawed and misleading comparisons being made between the indigenous breed and the exotic germplasm. The net result has been that some indigenous breeds or landraces have been lost or displaced;

- Changes in breeders= preferences to other breeds have occurred because of short-term socio-economic influences. These influences may arise from poor agricultural policies which promote quick fixes that are not sustainable in the long term, or from changing (possibly transient) market requirements for the products;

- The complete ecosystem in which the breed was developed may be under threat and the decline is a symptom of some wider forces at work;

- Natural disasters such as drought and diseases;

- Wars and other forms of political unrest and instability.

The locally adapted indigenous breeds in developing countries often have low absolute production figures while productivity itself is often remarkably high, when the production environment and the level of input are taken into consideration. Indigenous breeds produce and reproduce despite the sometimes very harsh environmental conditions, and are considered an important asset since they have developed over time valuable adaptive traits. This productivity in harsh environments is critically important since the vast majority of the world cannot sustain high input/output systems.

The Meishan pig.

This breed originates from China and is renowned for its high litter size. The breed has been used to produce commercial lines with high reproductive rates by international pig breeding companies. These developments have also uncovered a gene that has a large effect on litter size.

There is a little doubt that the 160 or so developing countries harbour the majority of the world's animal genetic resources, much of which will be of short and longer term interest to other countries. Ready access to this gene pool will also benefit developed countries.

▶· **1.3 Global Strategy for the Management of Farm Animal Genetic Resources**

What efforts are being made to address the problem?

In recognition of the importance of animal genetic resources (AnGR), and of the sizeable portion which are currently at risk of loss, and in keeping with FAO's mandate and the Convention on Biological Diversity (CBD) a special action programme for the Global Management of Farm Animal Genetic Resources was launched by FAO in 1992.

This Programme has as its objective to establish practical mechanisms and set of key actions by countries that aim, in particular, at:

- developing and making better use of animal genetic resources adapted to the world's major medium-input and low-input production environments, so as to enable their agricultural systems to intensify sustainably; and

- overcoming the serious threat of genetic erosion amongst the remaining 5,000 or so remaining breed resources of the 14 main farm animal species.

The framework of the Programme comprises four basic components:

- An inter-governmental mechanism whereby governments can directly guide international policy development, within the Commission on Genetic Resources for Food and Agriculture;

- A global, country-based structure with three elements: (i) focal points and networks, including the provision of a National Focal Point responsible for implementing and maintaining the networks within the country, and for undertaking technical exchanges with FAO on the Global AnGR programme; (ii) a stakeholders mechanism to properly involve the broad range

of parties; and (iii) use of the country-secure Domestic Animal Diversity Information System (DAD-IS; see the Internet http://www.fao.org/dad-is);

- A programme of technical activities, of six elements: (i) characterization; (ii) in-situ utilization and conservation; (iii) *in-situ* and *ex-situ* conservation; (iv) guidelines and action planning; (v) the development of a communications and information system, and relevant training; and (vi) co-ordination;

- Expert cadres to guide development of the strategy, and maximise the cost-effectiveness of country participation.

As mentioned above, one of the objectives of this Programme is the development of Guidelines for country use. The Primary Guideline Document (FAO, 1996), mainly targeted towards policy makers, is designed to help countries get started to identify the main elements and objectives of an animal genetic resources management plan, and to outline the strategic policy directions required to fulfil these objectives. The Primary document is complemented and supported by four secondary documents targeted mainly at those that implement policy, administratively and technically, covering the following issues: characterization, livestock production systems description, active breed use and development, and managing populations at risk to provide guidance for the management of areas identified in the primary document. This Guideline looks at the specific aspects, options and technics for the management of populations at risk.

► · 1.4   Objectives for conservation

How will conservation help?

The objectives for AnGR conservation includes economic, social and cultural, environmental, risk reduction, research and training.

These objectives have been summarised elsewhere (Hodges, 1987; Henson, 1992).

- animal diversity should be maintained for its economic potential in allowing to respond quickly and swiftly to changes in market conditions, consumer preferences or environmental conditions.

- animal diversity has an important social and cultural role. Animals are integral part of ceremonies and habits of social ethnic groups. In modern societies they provide recreation possibilities. Farm parks can serve as teaching aid for the urban. The tourism industry may be important in many countries, and may rely on the specific environment of which the local domestic breeds are an integral part.

- animal diversity is an integral part of an agro eco-system. The loss of this diversity would contribute to higher risk in the production system, reduced ability to respond to change, degradation of the environment in question and could ultimately lead to its destruction. Marginal areas and low to medium input production systems, and increased integration of livestock into agricultural production will be important for food production in the developing world. Maintenance and development of adapted breeds are of critical importance to ensure this can be achieved sustainably without adverse environmental impact.

- domestic animal diversity is important insurance to enable response to possible, but yet unknown, requirements in the future. It is risky to rely on only a few breeds: a concentration on a small number of breeds results in losses of genes and gene combinations which are not relevant at present, but which could become relevant in the future. Conserving domestic animal diversity is reducing the risk and enhancing food security. At issue is not only the possible accentuated loss of diversity but the lack of readily available gene combinations, particularly for adaptive fitness to specific environments when AnGR are lost.

- animal diversity should be conserved for research and training. This may include basic biological research in immunology, nutrition, reproduction, genetics and adaptation to climatic and other environmental changes. Genetically distant breeds are needed for research into disease resistance and susceptibility helping to a better understanding of the underlying mechanisms and to the development of better treatments or management of the disease. The conservation activity serves as training for all stakeholders and this in turn leads to greater awareness, knowledge and reduced risk.

Objectives for conservation

- Economic potential

- Social and cultural considerations

- Environmental considerations

- Risk reduction

- Research and training

► · 1.5 Conservation strategies

What does conservation means in practice?

Conservation strategies can be categorized as either conserving animals *in-situ*, in the environment in which they were developed, or *ex-situ*, all other cases. The latter can be further divided into ex-*situ in vivo* conservation and cryogenic storage.

The CBD in its Article 8 gives clear priority to *in-situ* conservation and refers to it as the recovery and maintenance of species or breeds in the environment in which they have developed. This strategy is also the most preferred as the animals continue to evolve in their original habitat.

Article 9 considers *ex-situ* conservation as: (i) the maintenance of small, closely managed populations outside their adapted environment in artificial or semi artificial settings; and (ii) freezing (cryoconservation) of genetic material such as semen, embryos, DNA, cells or ova. *Ex-situ* cryoconservation does not allow for further evolutionary progress of breeds which they would have undergone in their natural environment.

In-situ and *ex-situ* conservation is complementary, not mutually exclusive. The decision will depend on a thorough evaluation of the situation and the possibilities to use one or the other strategy. For example, it is important to note that frozen germplasm can play an important role in support of *in vivo* animal conservation strategies.

▶·   1.6   The goal and structure of these Guidelines

How is the rest of this document going to help?

The objective of these guidelines is to provide technical arguments and a decision aid between the available various options as well as to provide guidelines on how to design and establish animal conservation programmes and genome banks. The considerations and reflections are intended to be relevant to all species of domestic livestock, and where appropriate (for example in cryoconservation) species-specific guidance is given.

This document is designed to provide the necessary technical background for people wanting to set up, implement and monitor conservation programmes. Chapter 2 provides the information on how to evaluate the state of animal genetic resources in the country. These results are used in Chapter 3 to decide between the various options for conservation. Chapter 4 describes the design of *in vivo* conservation plans, and Chapter 5 describes cryoconservation plans. The techniques available for cryoconservation are often complex and precise and this necessitates a more detailed description, but it is not intended to emphasise this option more than the *in vivo* conservation plans, which in

contrast are more diverse in form. In fact the *in vivo* conservation plans are considered more important. Chapter 6 outlines the management of conservation plans, with special emphasis on ownership, personnel and training. Chapter 7 contains concluding remarks. The structure of the Guidelines is shown diagrammatically in Fig. 1.1.

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FAO/UNEP (1995). World watch list for domestic animal diversity, 2nd edition. B. Scherf (ed.). Food and Agriculture organisation of the United Nations, Rome.

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Figure 1.1: The structure of the guidelines
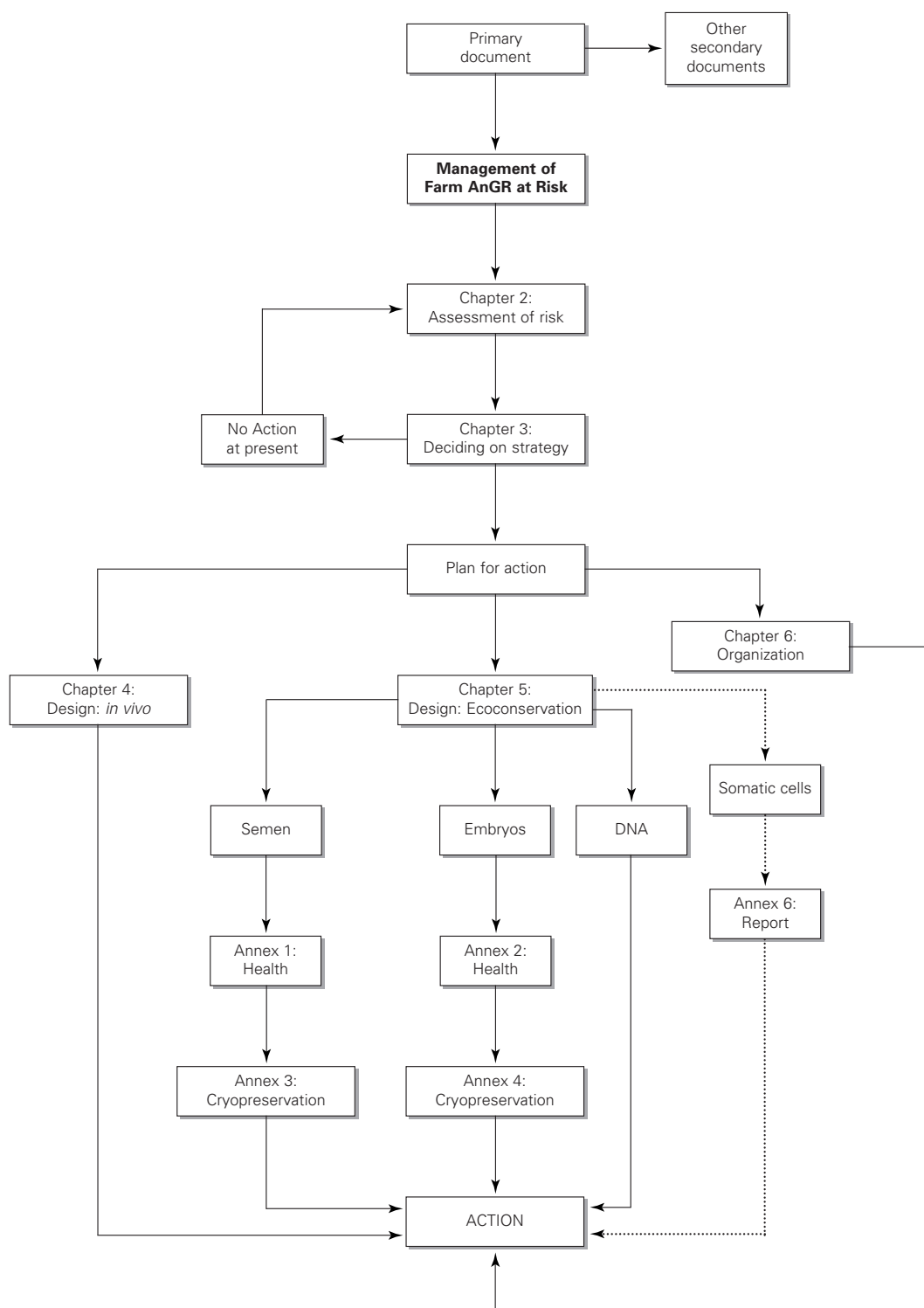
2.   Survey and Analysis of Present Position

- *Understanding the diversity*
- *Sources of information on a breed*
- *Design and conduct of a census*
- *Analysis of census data*
- *Organizing breeders to monitor performance*
- *Conduct of a survey*
- *Designing a survey questionnaire*
- *Categorizing the risk status*

The first step in conservation is to be aware of the animal genetic resources that exist within a country. This information can be obtained initially from written or oral records. Conservation projects will nevertheless require the current state of resources to be ascertained to a high degree of certainty. This involves setting up a partial or complete census to obtain the basic information on what breeds exist within a country and how numerous each breed is. This information will begin to pinpoint conservation needs.

However these needs can only be taken further if we can learn more about the population dynamics of the breeds (are they increasing or decreasing in numbers) and what each breed is valued for and how it is managed. This information is obtained through regular surveys conducted in collaboration with organizations of owners of the breeds. The results of the survey enable it to be categorized according to its risk of extinction, which is an initial consideration in giving priority for active conservation, and can give insight into the possible reasons for the endangerment. The process described in this Chapter is summarized diagrammatically in Fig. 2.1.

Figure 2.1: Assessing the current position
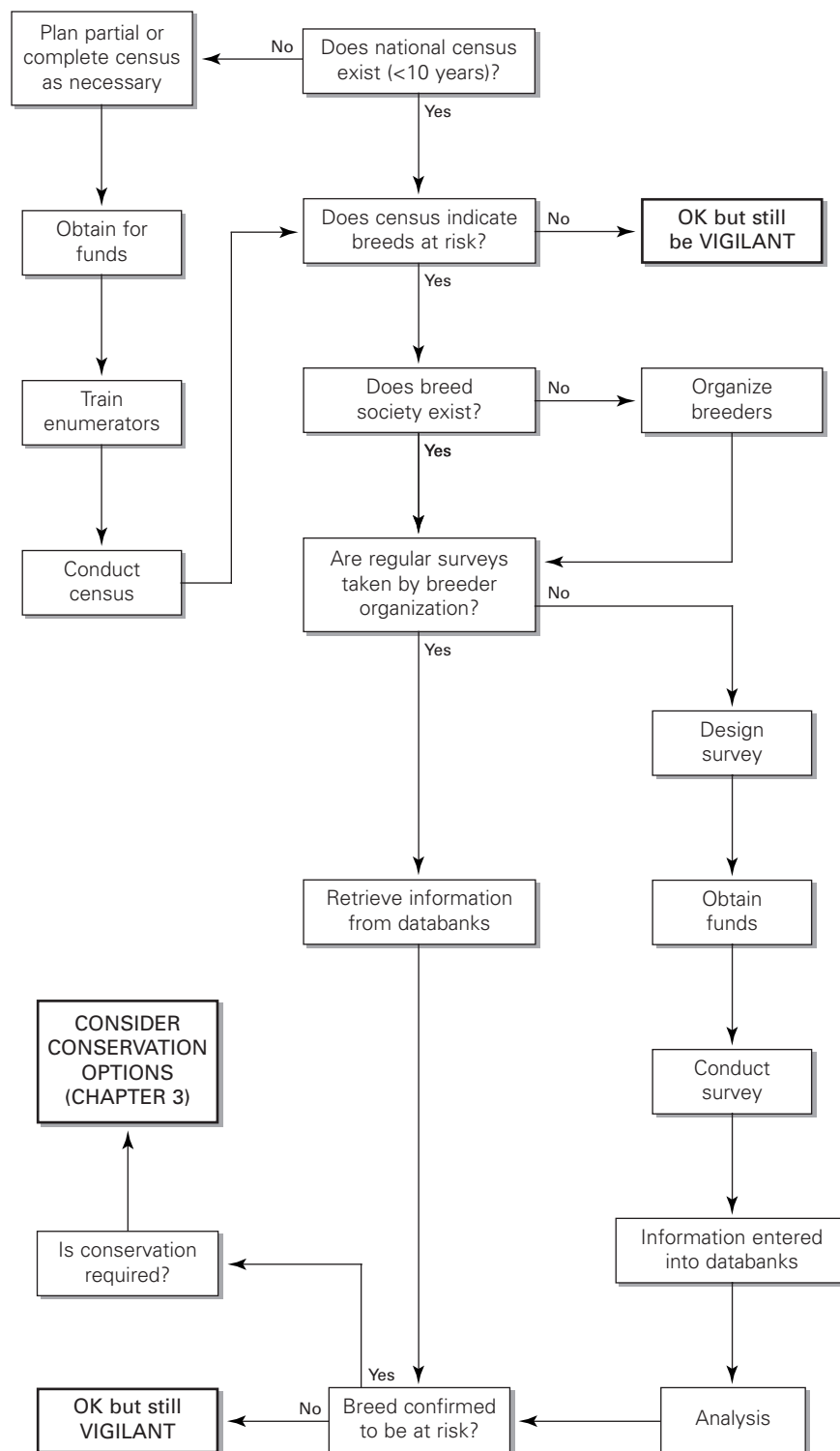
▶·   2.1   Understanding the Diversity

What is a breed and its role in diversity?

The present day diversity within farm animal species may be viewed as the result of a long history of human practice with geographical, sociological and economic influences. For each breed, the primary influences contributing to the final population were complex and included founder effects, migration, mutation, natural selection and selection by man. Even today, one or another of these influences can help to create new animal populations, just as they have contributed, since the beginning, to the loss of many populations.

In order for man to impose his own selection pressure on his animals, he must first control some of the principal components of mortality rates determined by natural selection. He is able to control population size to ensure there is sufficient food for his selected stock, and is able to conserve fodder for periods of the year when forage is not available. He is able to protect his stock from predators and to supply housing or shelter from climatic extremes and is also able to protect livestock from parasites and diseases. The weakening of natural selection pressures has an immediate effect on the survivability (or fitness) of individuals within his flocks and herds and enables him to impose his own selection criteria (meat, milk, wool, draught, work, etc.). The final result of these combined selection pressures, man and nature, in their myriad of forms, is the vast range of distinct populations or breeds that exist today and have existed in the past.

The term most commonly used to describe livestock populations or varieties is 'breeds'. A number of different technical definitions of 'breed' have been published. One example is:

> "a group of animals that has been selected by man to possess a uniform appearance that is inheritable and distinguishes it from

other groups of animals within the same species. It is a product of artificial choice of characters that are not necessarily strategies for survival but are favoured by man for economic, aesthetic, or ritual reasons, or because they increase the social status of the owner of the animals". (Clutton-Brock, 1981)

Pedigree recording has enhanced this definition by supplying parentage and relationship information for many of the "developed" breeds. The concept of breed, however, encompasses any population which falls within definable parameters. In essence it may apply to any group of animals which are located in a geographical area, have some phenotypic characteristics in common, and are recognized by local people as local type. This wider concept has led to the acceptance of "breed" (as used in the Convention on Biological Diversity, by FAO, and in these Guidelines) as a cultural term.

Hall and Ruane (1993) considered that in the Old World the number of breeds are correlated with human population and land area, implying that in the past conditions favouring growth in human population also favoured the diversification of breeds. The same authors state that peripheral and remote countries have the highest ratio of breeds per million people, implying that remoteness can also promote diversification (see also Darwin, 1859).

In an evolutionary classification a primary population characterizes the most important grouping of breeds, occupying the position (the missing link) between the wild species and the standardized (or fixed) breed (Lauvergne, 1982). Whilst this aspect is not directly the subject of these guidelines, it is very important to understand these primary populations because they are the first effort made by men towards the domestication of farm animals and have preceded the standardized breeds which they have derived from them. By encouraging inventories and appropriate conservation of breeds, those breeds closer in evolutionary terms to the primary populations may be identified and these may help to clarify the process of evolution after domestication. As a result genes that have been lost in the more specialized and standardized breeds may be recovered. Such perspectives, developed further in the secondary Guideline for

Characterization of AnGR aid in the establishment of priorities for conservation of the myriad of genetically diverse breeds that we have inherited

▶· 2.2 Sources of Existing Information on a Breed

Where does information on a breed exist?

The first step for conservation of the domesticated animal genetic resources in a country is to make an inventory of all the existing documentation on this subject. The history of livestock populations can be very rich and may have been written down in different documents.

National or regional works on livestock population or agricultural development report a large number of different breed names all referring to one basic population. Conversely, several distinct strains may share a single breed name. This is why the bibliographical work is an essential point for knowing the initial populations of a country and its history.

There have been many meetings of groups to discuss genetic resources in many different regions of the world, and these have produced many documents, publications, theses, reports and plans. Although the operational actions stemming from these discussions have often been disappointing, all of them have given a good description of many diverse breeds. Beyond the proceedings of these groups there are many important individual contributions, such as: veterinary dissertations, ethnological literature, and reports of interviews with specialists, old farmers, chroniclers, etc. Some general summaries of this information may exist (e.g. Epstein, 1971).

However the extent of information available will vary according to the different breeds. For breeds from developed countries the information may be detailed and extensive, including pictures of the animals considered worthy examples. For developing countries the extent of the information may well be very limited (especially in written form), and it

is only possible to localize, and to characterize an indigenous breed only if its existence was recognized as a breed! It may prove worthwhile to consult the bibliographies of other countries about indigenous breeds, especially those countries which have had historical links with the area in question. When information is limited, an important pool of information that may prove valuable is interviews with farmers, the older farmers in particular.

With the CBD accepting country sovereignty over genetic resources, nations are responsible for describing and documenting their own resources. To assist countries obtain, collate, record and base decisions on this information FAO is developing the Domestic Animal Diversity Information System (DAD-IS) for country use.

With the history in mind and the knowledge of how different breeds may relate to each other it is now feasible to consider identifying more complete information on the current position relevant to the task in hand.

▶·   ## 2.3   Design and Conduct of a Census

What is involved in planning for a census?

A snapshot of the current distribution of the AnGR within a country, or a region, is essential so that future steps may be planned with accurate information. A census is a good way of achieving this objective and in its simplest form is a counting of head; but with careful design and analysis it can yield much more information (see 2.4) with only a little extra effort.

The census information is obtained in two stages: (i) a partial census to identify breeds that may be at risk; (ii) a complete census (or as near complete as is feasible) of those breeds identified in the partial census as possibly being at risk.

Both partial and complete censuses must be well planned with respect to their precise objectives, data required, experimental and questionnaire design, training required, collation and analysis of data, storage of data, and reporting of results.

In our context a census is where surveyors (enumerators) meet the farmer on one occasion to obtain straightforward information on numbers of head and some desirable ancillary information. A complete census is therefore very expensive, and it may be that a partial census is an appropriate first step. There are international funds available for the purpose of conducting a well-planned census (contact FAO, UNEP, World Bank), since it is recognized that good conservation is based upon good and reliable information.

In the Guidelines a minimal set of questions are suggested to ask each farmer, and these need to be addressed in an easily understandable form:

- the numbers of different breeds on the farm

- the number of animal for each breed

- the demographic structure by sex and by age group for each breed

- the primary uses of each breed

- sources of breeding replacements and crossbreeding activities

- the geographical location

- the owners age

- some information about the owners family (e.g. number of children, education, farmer=s status, etc.).

A census must be carried out in the field within a short period to avoid climatic or commercial effects. For example, in seasonal systems of production the numbers of animals will vary dramatically according to whether the count is before or after lambing or calving, or before or after the typical time for culling. Ignoring these factors will introduce serious

biases into the data making it uninterpretable, thus wasting the investment. Therefore a decision needs to be taken on when, and over what period of time, the census will be taken.

Statistical help in the design is strongly recommended. There are various issues this guidance should address: what information will be required to achieve the objectives of the census in statistical terms (and if the original objectives are not achievable, what are achievable objectives?); allocation of tasks to enumerators to avoid potential confounding; stratification of the census by region, especially if the period of the census is unavoidably long in relation to the production or the seasonal cycle; information to be recorded for use in subsequent analysis; methods of recording; storage of enumerators' records for future reference; the form of database that is available to store the records for analysis; the transfer of information from the enumerators' records to the database; the form of the analysis; and the analysis itself.

Many enumerators must be recruited; it is important to consider how much one enumerator may achieve in one day. Adequate training must be given to the enumerators to ensure that, among other tasks: they ask the questions in the right way to obtain the required information; they fully understand the purpose of their questions, so that they can answer any questions the farmer might ask on what is required; they can interpret correctly what will be shown or told them by the farmer.

This preparation, together with conduct and the analysis of data combine to make the high price of a census. Many developing countries have not yet conducted a complete census of their animal population. Data from a partial census is only an estimate, and if these estimates are based on small or poorly designed samples the picture of the animal genetic resources will be vague at best.

For small populations at risk, when they are very localized, when no migration is present, it may be sufficient to make a census only within the region concerned. The cost will be lower and the work less. In this case, we must accept that the snapshot provided by the census has a clear

centre, but has vague margins, since the relationships between the different breeds cannot be studied.

In the developed countries, where many economical changes can affect the effective population size and, sometimes, its localization, it is possible to recommend a new complete census every ten years. In the developing countries, which represents most of the animal genetic resources available, cost restricts the regular complete census but a partial census of some breeds may be possible more often. Geographical constraints may rule out a complete census altogether. It is to be hoped that the funds for a global census can be found as soon as possible.

►·   2.4   Analysis of census data.

What information can a census provide?

In all cases statistical advice should be used to help summarize the vast amount of information that arises from the census to obtain sound interpretation of the data obtained.

The results of a well-planned census will show for each breed:

- the number of animals

- the demographic structure by sex and by age group

- the number of herds keeping the breed

- the distribution of herd size for the breed

- the distribution of total herd size where the breed is kept

- the geographical location of the breed

- the age distribution of the owners

- some information about owner=s family

- the associations with agricultural or commercial uses

- the associations with the keeping of other breeds

- some sociological aspects

- some description of the production system in which the breed is kept

Summaries of the total national population will be derived from pooling the information over the breeds.

Further information can then be developed by using information available from other databases. Thus combining the census data with external information such as climatic data, sanitary data, sociological and economic data, it may be possible to explain some of the correlations observed between breed numbers, geographical locations, family size of the owner and herd size.

All this information is important for planning the following surveys and having a first view of the relationship between breeds and their environmental conditions. It transforms the overview of traditional breeds from the bibliographic search (2.2) into what is going on today. In particular:

- a census is a good tool to identify urgent actions for conservation projects by identifying those breeds which are at low numbers.

- a census can act as an early warning of future conservation needs. For example, it is not the same thing if a small population is in the hands of old or young farmers: in the former case, it indicates that this breed is kept today only for sentimental reasons; in the second case, some economical aspect may be in play.

A census is more than statistical data. Its conduct and the communication of its results raises the profile of the conservation principles and the value attached to the genetic resources. It is the starting point for a continuous surveillance in the form of monitoring programs. If some breeding programs already exist, a census will

consolidate them. If there are no breeding programs in existence and no data is available, a census is a good precursor to initiating them.

▶· ### 2.5   Organizing breeders to monitor performance

How can more detailed information be obtained?

To go beyond the information contained in the census it is best to operate through organizations of owners who are interested in conserving and promoting their breed (hereafter termed Breed Associations). It may be that such organizations do not exist and if so efforts should be made to encourage their formation. For a breed with a small census size, the numbers of keepers may be small, and the formation of a Breed Association may be helped in its early stages by the provision of some services either by Government (directly, or indirectly through research institutes) or a non-governmental organization (NGO).

The formation of a Breed Association is in the interests of parties interested in conserving the breed, since its conservation cannot move forward unless enough owners of the breed support the process and participate. This is particularly the case where it is hoped to conserve the breed *in-situ* (see 1.5) as favoured by the Convention, since the keepers of the breed, i.e. the indigenous farmers exert considerable influence over both the breed and the environment in which the breed was developed. Given these constraints it is desirable to tackle this problem as early in the process as possible.

The formation of a Breed Association is in the interests of the farmers since it provides a means to market and promote the breed, and generally to be pro-active in support of the breed.

There are many possible roles for a Breed Association, but two roles central to conservation are developing a registered Herd Book and organizing performance recording among its members, perhaps in collaboration with other agencies such as Research Institutes, NGOs or

other parties to the conservation process (see 6.1.2). The continuous monitoring by the Breed Association of numbers and performance will have a financial overhead, but the Convention recognizes this process as an important part of conserving biodiversity. Therefore funding bodies will consider well argued proposals for support, and a Breed Association would be an essential component of a fully-fledged conservation proposal. The form the Breed Association may take will vary from breed to breed, country to country and no typical model is proposed.

A Breed Association will most likely form a biased sample of the owners, in terms of herd size or quality of management. However the degree of bias can be partly assessed by comparison of the herds in the Breed Association with the census results.

An established Breed Association thus forms an important basis for the regular monitoring of the breed by means of surveys to summarize the information collected by the Society.

►· 2.6 Conduct of a Survey

How does a survey differ from a census?

In a census there is no dynamic data; for example is a breed increasing or decreasing in numbers? Furthermore, there is no information that might be used to develop a conservation plan that is sustainable: how does the breed perform, how is it managed? So, it is very important for future decisions to have a profile of the breeds' evolution, not just a snapshot. Only a regular survey providing continual monitoring of performance can give this dynamic information.

The survey will likely be organized centrally, through the Ministry of Agriculture or its agent. The survey is conducted by asking the Breed Associations that represent the breed to provide information on various aspects of the breed=s performance. The design of the questionnaire is discussed in the next section. To obtain the required information the

organizer of the survey need not contact every farmer, as in the census, but need only communicate with one contact person for each breed involved in the survey. If well organized, surveys on several breeds can be carried out simultaneously.

The regularity of the survey questionnaire will depend on how good the Breed Association is: if it is well organized and has a good infrastructure of associated support (Herd Books for registration, help from scientific or technical institutions to aid the collection of data) then every 3 years may be acceptable. Otherwise it is recommended that the survey is conducted annually. This helps to promote interest and to improve the communication within the Breed Association.

In all the steps that follow the value of good communication and good publicity cannot be underestimated. The results of the survey, the classification of the risk status, and the conservation initiatives that follow the survey must be made available to the Breed Associations and the owners if interest is to be maintained and fostered. Good publicity to encourage interest in the breeds in all risk categories will be invaluable and may arrest decline in numbers and increase the sense of ownership of the breed.

At the national level, all information about animal genetic resources coming from different origins (databases or surveys), must be collected and stored in the same database which may be termed the National AnGR Database. It must include all breeds of a species, not only the endangered ones. We must not forget that under certain conditions even large populations can decrease rapidly and reach an endangered status within a short term.

The information on the database can be used to work on scientific or non-scientific problems of other institutions. For example, analysis of survey information should be used to encourage detailed evaluation of endangered breeds by research institutes taking care that lifetime, holistic assessments are made.

It is anticipated that the surveys for all breeds will be co-ordinated through the National Focal Point. In all cases the results should be made available to the National Focal Point for inclusion in the Global AnGR programme and communication through networks within the country. There may be benefits from communicating the results to other stakeholders and parties involved in conservation not yet included in the networks. Most importantly, these results should be acted upon.

▶· 2.7   Designing the Survey Questionnaire

What questions should be asked in a survey?

A major difficulty is the need for getting objective information, which is crucial for any comparison of data from different breeds and areas. Subjective information is much easier to get but is much less useful. FAO have gained much in understanding many of the limitations and strengths on the current (i.e. 1997) set of variables recommended for collecting data on each breed; this is in terms of interpretation, the ease/difficulty of obtaining the particular items in some countries, use of the data items and the formatting of questionnaires. It is anticipated that a major review will be conducted, lead by FAO and involving countries, which will cover the definition of relevant data variables, and the methods of collection and analysis. This review will lead to an upgrade of the databanking system and, in turn, will form a major component of the third stage of DAD-IS.

The staged development of the databanks will involve: (i) the progression from collection of the basic data (e.g. breed name, visual description, uses, numbers of animals) to more advanced data (performance characteristics, description of the prediction environment, management definition); and (ii) the evolution of AnGR databanks within each country, whilst protecting compatibility with a global databank (i.e. permitting countries to extract particular data items stored locally, and to pass this information for international collation and interpretation that is both meaningful and consistent across countries).

With this in mind, detailed questions will not be recommended here, but instead some perspectives on relevant topics are presented. Given this staged development, it may be that some of these perspectives will not be addressed either in the current FAO recommendations, or the revised recommendations (because of the difficulties of definition, or in anticipation of difficulties in data collection). Readers are referred to WWL-DAD:2 for the list of items that comprise the current FAO recommendations.

At the beginning of 1997 (Jan 15th), the National Focal Point for France in the FAO Strategy, has started an AnGR survey to update the database. 51 questionnaires were sent to the 51 correspondents for the 51 cattle breeds in France. After two months, only 20% had been returned. After a second call (March 20th), the rate had risen to 40% by 80 days after the start, and to 75% at 100 days after the start.

This experience gives reason to think, that for a satisfactory response in an AnGR survey it is important to consider carefully, how much time must be reserved for each phase (collection, data entry, analysis, result) and how strictly the deadlines can be set. Too short times may impair the quality and inhibit co-operation, and too demanding requirements may retard the work seriously. Thus, a suitable compromise has to be found!

One of the advantages of the staged development of survey information is that many of the questions will remain the same, and after two or three years, it is possible to facilitate the work by pre-filling parts of the questionnaire with information from the previous survey and asking only for its verification or modification. A compromise will need to be found between the ease of filling in the forms and getting exact and comparable information between breeds. For many descriptive parts it is best to encourage the person replying to use his own language, so that replies can be obtained more easily, rapidly, and more accurately; however this is at a cost of extra difficulties in processing the data collected. The use of scientific terms instead of popular ones can avoid confusion. All the terms must be clear. The development of a training manual for the correspondents may be useful.

In many countries, the motivation for filling in the questionnaire decreases because of: (i) lack of useful feedback; or (ii) lack of reward for the extra work. To address the first point it is very important to transmit all the information concerning all the national breeds to the Breeders Organizations, and that the information is further communicated to the individual owners. For the second point, a suitable system of reward should be developed.

Present and future perspectives on information obtained from surveys.

General information Besides the local name of a breed, it is important to include all synonyms, as this is a help in identifying a breed, and aids future collation across countries. The region within a country where the breed is located is of interest since it allows other important information on climate, prevalent diseases and general environment to be accessed from other databanks. The primary and all secondary uses of the breed should be identified. A description of the physical appearance of the breed is necessary, such as size and weight, colouring, horns, and other visible characteristics that may be used to define the breed etc. The organization which prepared the reply to the questionnaire (name, address) is vital for communications concerning the breed.

Breed development. Information on the population numbers and trends (breeding females, males in service, males in AI service) is important for the evaluation of the endangerment status of the breeds (see 2.8). Useful information on population structure includes the age distribution of breeding males and females (used for calculating generation intervals, see 4.2). Data on the distribution of breed numbers between juveniles capable of breeding in the future and adults should also be obtained where this can be clearly defined, since the juveniles are important to the immediate future of the breed. The proportion of females that are straight bred allows the threat to the breed from crossbreeding to be evaluated. Where appropriate, additional information on the relative age of straight-bred females (e.g. are first offspring always purebred or always crossbred) may help define the crossbreeding system and refine the calculation of generation interval. Information on the cryoconservation of germplasm (numbers of semen doses, embryos) can help indicate the stage of development of the breeds and their conservation activities.

Management conditions. Obtaining information upon management conditions is particularly important since, (i) as described in 1.2, indigenous breeds are very often under threat from misleading comparisons with other breeds and crossbreds; and (ii) they are an important aspect of the sustainability of the production system. A primary cause of the poor design of comparisons is the preferential treatment of particular breed groups. It would be helpful for survey questions to move towards providing some clues as to the extent of any preferential treatment and the different environmental roles played by the breed groups on a farm. Some items of relevance are: type of management (stationary, transhumant, nomadic; who is in charge of day-to-day management (e.g. the owner or a hired hand); the housing of the animals, when and for how long; the use of by-products from other farm outputs as a significant part of the feed; the need for supplementary feedstuffs grown outside the farm; endemic diseases, and which diseases are controlled by vaccination. Whilst the survey may

usefully seek answers to questions as to whether a breed is more harshly treated than another, the responses are still subjective and cannot be used as a substitute for a properly designed scientific comparison (see also 3.2.1).

Breed performance and special qualities. This is necessary for characterizing the breed, and should attempt to assess *overall* lifetime economic performance (which will depend very much on performance measures such as age at first breeding, re-breeding interval, litter size and longevity) and not solely upon the ability to produce the commercial products (such as milk yield, number of eggs per year) which form the main use of the breed. Proper breed comparisons can *only* be made in a properly designed study, but the supposed relative performance may help to interpret population dynamics. The breed may be kept for some special qualities, not covered in detail by the survey, and it is important to ensure that there is a clear opportunity for such qualities (e.g. lack of water supply, resistance to a disease) to be stated by the corresponding organization. Breed performance is only useful if associated with clear information on the stressors that constrain the production environment, and the level of feed and other inputs.

Additional information. Different links with other databases coming from breeders organizations or scientific institutions may be usefully established. Results from studies of genetic distances may be relevant. An opportunity to present results from conservation programmes may be given. Other information relevant to good management of AnGR may be requested e.g. size of herd by age of owner (as mentioned previously in 2.4).

►· ⠀⠀ 2.8 ⠀ Categorization of Risk Status

⠀⠀⠀⠀ How is the risk of extinction determined?

From a conservation standpoint the most important outcome of the census and the survey is the categorization of risk status for the extinction of the breed. The census may give a preliminary indication of risk but the survey should be able to refine the risk analysis by divining underlying trends and causes.

Breeds may be classified in seven categories: extinct, critical, critical maintained, endangered, maintained, endangered maintained, not at risk and unknown. The categorization is based on overall population size, number of breeding females and the trend in population size, i.e. whether the population size is increasing, decreasing or stable. This is why it is important to organize a regular survey to enable informed categorization and the appropriate prioritization.

▶   Extinct

A breed is categorized as extinct if it is no longer possible to easily recreate the breed population. This situation becomes absolute when there are both no breeding males (semen) and breeding females (oocytes) nor embryos remaining. In reality extinction may be realized well before the loss of the last animal, gamete or embryo.

▶   Critical

A breed is categorized as critical if: The total number of breeding females is less than 100 or the total number of breeding males is less than or equal to 5;
or
the overall population size is close to, but slightly above 100 and decreasing and the percentage of females being bred pure is below 80%.

▶   Critical maintained

As for Critical, but for which active conservation programmes are in place or populations are maintained by commercial companies or research institutes.

▶   Endangered

A breed is categorized as endangered if: the total number of breeding females is between 100 and 1000 or the total number of breeding males is less than or equal to 20 and greater than 5;
or
the overall population size is close to, but slightly below 100 and increasing and the percentage of females being bred pure is above 80%;
or
the overall population size is close to, but slightly below 1000 and decreasing and the percentage of females being bred pure is below 80%.

▶   Endangered maintained

As for Endangered, but for which active conservation programmes are in place or populations are maintained by commercial companies or research institutes.

▶   Not at risk

A breeds is categorized as not at risk if: the total number of breeding females and males are greater than 1000 and 20, respectively,
or
If the population size approaches 1000 and the percentage of females being bred pure is close to 100%, and the overall population size is increasing.

▶   Unknown

Self explanatory, but also a call to action: find out!

If categorization of a particular breed is borderline, further consideration should be given to factors such as:

- degree of crossbreeding in the population

- reproductive rate and generation interval of the population. Populations with low reproductive rates are at relatively greater risk than populations of high reproductive capacity of comparable size.

- special peculiarities and characteristics of the production system (intensive, extensive, nomadic etc.)

- historic and current rates of decline in population numbers

- geographic isolation of the population or its concentration in one or a few locations that would place it at risk as a result of climatic, economic or political changes or disease outbreak.

The number of animals actively used in artificial insemination, and/or the amount of semen and number of embryos stored, and/or the number of herds (or flock) as collected in the other parts of the questionnaire will have to be considered as the use of AI is an indicator that the effective population size may be smaller than that indicated by the number of breeding males alone and so the category would increase in priority. Existing storage of suitably sampled germplasm could reduce the priority for conservation (see Chapter 5).

As a result of the census and surveys the risk status of the national breeds can be assessed and priorities for action can be identified. Populations deemed at additional risk with respect to the above considerations would be placed in the next higher category. The next step is to determine what action is required.

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▶ ## 3.   Considering the Options

■ *Attracting support for projects*

■ *Shaping National Action Plans*

■ *Opportunities for improved economic performance*

■ *Choosing the conservation strategy*

The survey and its analysis will identify the needs for conservation of breeds of farm AnGR in a country. However it will generally be the case that the endangerment of breeds has been brought about by economic and cultural forces and that these trends will continue unless they are addressed, otherwise the conservation activity will not be sustainable in the long term.

Therefore the widest possible base of support should be pursued and this can best be achieved by placing the livestock project into a wider context. By this means support can be harnessed from governments, non-governmental organizations, farmers and the public. In doing so the opportunities for sustainable conservation, and the relative priorities they may have, will become more clear. Finally, general objectives can be formulated that have a good chance of success in obtaining both political and financial support, and are achievable. After these foundations the more detailed technical design of the programme can be considered and the associated operational and financial plans can be developed. Fig. 3.1 summarizes the decision process described in Chapter 3.

Figure 3.1: Considering the options

```
                    ┌──────────────┐
                    │ Breeds known │
                    │ to be at risk│
                    └──────┬───────┘
                           │
                           ▼
                    ┌──────────────┐   No   ┌──────────────┐
                    │ Farm AnGR in ├───────▶│ Ensure they  │
                    │national action│       │ are included │
                    │    plan?     │        └──────┬───────┘
                    └──────┬───────┘               │
                        Yes│                        │
                           ▼                        │
                    ┌──────────────┐                │
                    │Develop network│◀──────────────┘
                    │of stakeholders│
                    └──────┬───────┘
                           │
                           ▼
                    ┌──────────────┐
                    │Establish value│
                    │and potential  │
                    │   of breed    │
                    └──────┬───────┘
                           │
                           ▼
          ┌──────────────┐   Yes  ┌──────────────┐          ┌──────────┐
          │Is network and├───────▶│  Chapter 4   ├─────────▶│  ACTION  │
          │potential      │       │Design: in vivo│         └──────────┘
          │sufficient for │       └──────────────┘
          │an in situ     │             ▲
          │strategy?      │             │
          └──────┬───────┘              │
              No │                       │
                 ▼                       │
          ┌──────────────┐  Yes          │
          │Is finance     ├──────────────┘
          │available to   │
          │an ex situ live│    ┌──────────────┐
          │strategy?      │    │  Chapter 5   │
          └──────┬───────┘     │   Design     │
              No │             │cryoconservation│
                 ▼             └──────┬───────┘
          ┌──────────────┐            │
          │Is finance and │           ▼
          │expertise      │  No ┌──────────────┐      ┌──────────┐
          │available for  │◀────┤Is finance     │      │   DNA    │
          │cryopreservation│    │available for  │      │extraction│
          └──────┬───────┘      │semen collection│     └──────────┘
                 │              └──────┬───────┘            ▲
                 ▼                  Yes│                     │
          ┌──────────────┐            ▼                      │
          │Do nothing and │   ┌──────────────┐               │
          │continue       │   │Semen collection:│            │
          │monitoring     │   │ Annexes 1 & 3  │             │
          └──────────────┘    └──────┬───────┘               │
                                     │                        │
          ┌──────────────┐           ▼                        │
          │Are somatic   │◀──  ┌──────────────┐  No           │
          │cells          │     │Can a strong case├───────────▶
          │recommended?   │     │for future      │
          └──────┬───────┘ No   │re-establishment│
              Yes│              │be made?        │
                 ▼              └──────┬───────┘
          ┌──────────────┐         Yes│
          │Cell collection│           ▼
          │   Annex 6     │   ┌──────────────┐
          └──────────────┘    │Embryo collection:│
                              │ Annexes 2 & 4  │
                              └──────────────┘
```

▶· 3.1   Attracting support for projects

How might funding bodies be persuaded to support
National Livestock Projects?

The endangerment of a local breed means that there are selective forces
against it, since its numbers are declining. Primarily these will be
economic, poor policy, unrest or droughts, either directly in that the
breed has been unfavourably compared to other breeds or species, or
indirectly in that it is part of an ecosystem that is under economic
pressure and consequently threatened with destruction. Therefore it may
be assumed with reasonable confidence that a conservation project will
need to attract support, and most probably funds, from a range of
national and international bodies.

Conservation projects may be justified in that they increase livestock
production and productivity in a sustainable manner and such projects
are a component of the Convention on Biological Diversity. Irrespective
of whether the primary justification is for increasing livestock production
or protecting an environment, funding bodies (e.g. the Global
Environment Facility, UNEP, World Bank, FAO, EU) will require an
environmental assessment of the project. This is most effectively
achieved in the wider context of a National Action Plan, which ratifiers
of the Convention are bound to develop. This will cover biodiversity of
both flora and fauna, domestic and wild. The priorities for funding of a
project, by national governments and co-operating international bodies,
will increase if it can be demonstrated to be of relevance to multiple
aspects of government policy, such as agricultural, environmental,
cultural, social and, where draught animals are involved, energy and
transport.

Documenting the wider importance of a local breed raises it from being
more than a commodity, subject to market-driven economic forces, and
allows it to be valued according to the principles of the Convention on

Biological Diversity. The work carried out in 2.1 is relevant to this. The participants in the projects, ranging from those that provide finance to those that contribute in kind through services rendered, may then extend beyond those international and national agencies concerned with agriculture and domestic livestock, to those concerned with environmental issues and indigenous cultures. Heightened awareness among the general public, who are increasingly urban in lifestyle, of problems that concern the rural community can also play an important role in influencing decisions on funding.

Funding from international agencies

There are two key features that *in vivo* animal conservation projects should aim to provide in order to attract funding from international agencies:

- The project needs to be part of a national strategy for conservation of the whole environment taking account of the ecosystem including plants and forests, since animals cannot be viewed in isolation from their environment.

- The project supports indigenous communities who wish to continue conventional lifestyles. The needs of indigenous people has growing international recognition because it is now acknowledged that indigenous people have been practising sustainable lifestyles for millennia, hence projects targeted at encouraging use and conservation of traditional breeds are likely to be viewed favourably by aid agencies.

It is difficult to get long-term funding from international aid agencies. Therefore, there should be financial commitment by governments to continue the conservation projects, and the projects should also develop conservation plans for breeds at risk in conjunction with their continued use.

▶ ·   3.2   Shaping National Action Plans

What are the principles involved?

The Convention on Biological Diversity defines biodiversity as "genetic resources, organisms or parts thereof, populations, or any other biotic component of ecosystems with actual or potential use of value for humanity". Such a statement places domestic livestock at the heart of such plans, since their products have clear and demonstrable use to humanity, but they cannot be viewed in isolation from their environment including plants, insects and other wild vertebrates.

In order to develop plans that may attract funding and wider support there must be clear relevance to the Convention which implies relationships of the domestic livestock to: (i) conservation of biological diversity; (ii) sustainable use; and, (iii) equitable sharing of benefits from use.

The arguments for conservation of biological diversity will arise from the survey results described previously. Arguments for sustainable use will need to address the viability of any use and development option, both genetic and economic, and this will demand consideration of agricultural, environmental and cultural contributions of the livestock breeds. The equitable sharing of the benefits from conservation demands consideration of breed improvement, management improvement or integration of the breed into a breeding scheme (e.g. *sustainable crossbreeding*).

3.2.1   Livestock and biodiversity.

What needs to be established is that the indigenous breed has actual or potential use for humanity and in so doing contributes to biodiversity. The first step is to develop the network   of   interacting   concerns   (agricultural, environmental, cultural, social and, where draught animals

are involved, energy and transport) that may place a livestock breed as a priority for national and international conservation action.

A particular livestock breed can influence agricultural output through: specific products that are novel or of high quality; particular combinations of products or product qualities; production of products more efficiently through lower input of feedstuffs, or lower quality inputs (waste products from other enterprises, foraging or scavenging); easy care, possibly through an adaptation to the environment, resulting in longevity, reliable breeding of replacements and production of products, or good disease resistance; or its characteristics for management in special land areas such as mountain pastures or swamps. Documentation for these qualities can be produced from utilizing databases and the good design of the surveys, i.e. asking the right questions (see Chapter 2).

A livestock breed will be an integral part of its environment if (for example): it can be argued that it has a unique ability to survive in environments that are generally considered harsh, perhaps because of the local feedstuffs, prevalent diseases or climatic conditions; the grazing habits of a breed or species may favour particular flora, and in doing so maintain unique plant combinations. In such cases the livestock breed may support an ecosystem and a rural economy. The justification for conservation of biodiversity in livestock is then further underpinned by the arguments for biodiversity in the other taxa and the arguments for the rights of the indigenous community. Under the Convention, governments undertake to designate and protect environmentally sensitive areas, and the association of a breed with such an area would be an important argument in favour of conservation measures.

There are cultural values that are relevant: a major use of livestock is as draught animals, which may be an integral part

of sustainability; various societies may place specific social values on stock of particular breeds.

Examples

Namchi and Kapsiki cattle are used as dowry in Cameroon, and therefore play an important cultural role (AGRI 13:25)

The N=dama breed of cattle can survive in areas where the Tsetse fly spreads diseases caused by trypanosomes, its adaptation to this environment allowing it to tolerate the trypanosomes. Not only does this give the breed a role in the ecosystem which cannot be played by other breeds, but also it provides a foundation for an indigenous human culture.

3.2.2   Sustainability

In order to develop a project, funding bodies and other participants must view the project as having a high chance of being sustainable. Therefore the selection pressures against the breed must be identified. If the selection pressures are not addressed, the survival of the local breed can only be maintained as a live population by repeated long-term grants. In the absence of any plan to diffuse the selection pressures, the rational response by funding agencies might be that the conservation objectives can be achieved most easily and most economically by cryoconservation.

Where a breed is neglected due to a commonly held viewpoint that it is not profitable or fashionable to own then specific initiatives need to be taken and these are detailed in 3.3.

If the decline has been due to natural disasters such as drought, diseases, wars or other forms of social unrest and strife then the decline is transient and the long-term profitability of the breed is predictable without further

measures, and the sustainability of a population of live animals is assured once numbers have recovered.

If the decline in census or survey numbers has an indirect cause, through the degrading of the environment of the local breed, then addressing this decline should be bound up with protecting its environment. Indeed the decline may act as an early warning of a much wider environmental problem. Schemes to protect and conserve whole environments will need to deliver benefits to the local communities and the livestock will be one component of a much wider whole. The arguments for sustainability of the local breed are then subsumed into the wider arguments for the sustainability of, and/or the means of sustaining, the ecosystem.

3.2.3   Equitable sharing of the benefits arising from Genetic Resources.

The requirement for equitable sharing is particularly relevant where the decline of a local breed is directly due to a commonly held viewpoint that the breed has a poor economic performance. It is important that either this view is rebutted or the profitability from keeping the breed must be increased (see 3.3). Ensuring that a conserved local breed will not leave farmers assisting in the conservation process economically disadvantaged is a positive response to the Convention's benefit sharing objective: an uneconomic breed may be considered an asset by observers from far away, but it is essential that it is seen as a benefit by those farmers still supporting the breeds current conservation. If this cannot be achieved then the most likely form of conservation (if at all) will be by cryoconservation alone.

▶ ·   ### 3.3   Opportunities for improving economic performance.

How might the profitability of local breeds be secured?

Economic performance is a holistic assessment of the profitability of keeping a breed and includes: replacement rates accounting for mortality rates, re-breeding intervals and litter sizes; costs of rearing replacements accounting for quality of feed and needs for housing and other special management; production costs accounting for quality of feed, capital requirements such as housing, and labour requirements; veterinary costs; and returns from product sales. The economic performance is closely related to lifetime profitability, and is certainly *much* more than a simple calculation based on value of primary product per head at one point in time.

There are at least three possibilities as to why a breed may be commonly viewed as having a poor economic performance: the products are desired but the breed is commonly held to have a poor performance relative to an introduced breed; the products are desired but are being competitively produced elsewhere; the products are themselves in decline. It is the first of these that will prove to be the most common cause in a global context, where crossbreds between the local and exotic breed are produced and are then backcrossed to the exotic.

The strategies to confront declining support for a breed will be considered in the context of a commonly held viewpoint that the local breed has a poor performance relative to an imported exotic or crossbred, but all except 3.3.1 and 3.3.2 are relevant to other causes of poor economic performance. Table 3.1 gives a perspective on the potential of the different approaches in relation to the cause of the decline. These approaches are not mutually exclusive.

3.3.1   Determine the facts about economic performance.

The comparisons between crossbreds and indigenous breeds with regard to productivity have often been made with poor experimental design leading to very misleading results. Crossbreds generally benefited from preferential treatment and factors such as reproductive fitness, longevity, and the necessary level of input, which are important economic factors, were not appropriately taken into account. Detailed scrutiny of the holistic performance of the exotic and/or its cross with the indigenous breed, including production risks over time, may demonstrate that the indigenous breed is very competitive. Comparisons will also need to examine subsidies paid to the farmers which may favour the introduction of exotic breeds. It is notable that the introduction of exotic breeds to the environments of developing countries resulted in many failures.

This option requires scrutiny of the documented evidence (research and development studies, project reports) available and an expert assessment of whether the existing evidence is in any way convincing. A well designed comparison should be conducted and analysed to rebut or confirm the common viewpoint. This will require genetic, statistical and economic expertise to be involved from the start of planning onwards and will need to account for both input and output differences over time, and the risks associated with each alternative.

● *The key question is: "has the economic value of the indigenous breed been underestimated?"´*

3.3.2   Incorporate the indigenous breed into a crossbreeding scheme.

The holistic assessment of the economic performance of the indigenous breed may indicate that crossbreds are superior. Nevertheless the indigenous breed still has an important

economic value providing the crossbreds are superior to the exotic; it may be very clear that this is so, but a positive answer provides further opportunities for conserving the indigenous breed.

The indigenous breed may be amenable to profitable incorporation into breeding schemes involving crossbreeding. Such an option has the objective of exploiting any hybrid vigour in the crossbred to provide a genotype that can combine an ability to overcome the difficulties of the environment with the ability to produce more primary product. The greater the distinction between the environment of the local breed and the environment of the exotic breed the more opportunities this strategy may have to succeed (although this cannot be guaranteed). However involvement in crossbreeding does not necessarily guarantee the survival of the indigenous breed as a distinct entity.

Two routes may be considered: (i) sustained crossbreeding where both pure breeds are maintained as viable populations; (ii) development of new breed from the crossbred and where the genes of at least one of the breeds is maintained only in the new breed. Both these options can be combined with selection, in the first case within the purebred with the objective of improving the crossbred, and in the second case within the new breed itself.

►   Sustained crossbreeding.

With high reproductive rates (e.g. such as in pigs and poultry) this system may be carried out using only parents of the local breed and the exotic: the purebred females will have sufficiently high litter sizes to produce both purebred replacements and to breed the crossbreds, whose products are commercially more valuable. This option is in operation to aid conservation of the Tapaong pig in Togo, where females are crossed with the Large White to produce more

profitable offspring; and of the Thimadit sheep in Morocco which are selected for reproductive performance in their own mountain environment, and their crosses with a meat-type breed are raised on the plains.

With low reproductive rates, the crossbred females themselves may need to breed replacements and systems of rotational mating need to be employed where the crossbred female is mated to either the local breed if her sire was an exotic, or *vice versa*. The practical difficulty of such an option arises in maintaining a rotational mating structure, with the danger that progressively more matings are to the exotic but this can be overcome by good planning and management.

▶   Development of new breeds.

In this option the crossbreds are bred amongst themselves as a composite. In this case there is a risk that the local breed will disappear as an entity. However its genes are contributing to the crossbred. Examples of this in practice are the Jamaica Hope and the Australian Friesian Sahiwal. This possibility is an equivalent outcome to one of the main objectives set for cryoconservation (see 5.1.2).

● The key question is: "does the crossbred have better economic value than the imported exotic?"

3.3.3   Selection within the indigenous breed.

Improvement may be obtained from within the breed itself through selection, which has the benefit of maintaining the breed as a distinct entity. Selection lends itself to incorporation into nucleus operations (see 4.4) which are an effective means of managing small populations at risk. The benefits of selection are enhanced through publicity on the improvement scheme which promotes the advantages of the

breed and breeding goals of the scheme. Such publicity raises the profile of the breed as being dynamic and developing. Such messages in themselves will underpin and expand ownership. A successful breed also benefits from a well-considered improvement scheme.

This option is most practicable with breeds that are either not yet at critical levels or have recovered in numbers. A sustainable conservation programme with effective population sizes less than 50 should be concentrating on multiplication and spreading the genetic base before embarking upon selection, and this will limit the opportunity for selection. If the local breed is very uneconomic then selection alone will not remove the problem except in the long term, since rates of progress from well-run selection schemes may only achieve 1-2% improvement per annum. However the genetic gain is permanent and cumulative (so 5-10% improvement after 5 years may be possible) and for a breed which is suffering a gradual erosion in profitability and numbers, a selection scheme is a very effective option.

Where the product demand is in decline, small changes in the product might halt or reverse this trend. This may easily be achievable through selection.

The selection may have consequences for other traits that are considered valuable in the breed and may compromise the adaptation of the breed which may be a motivation for its conservation! This opportunity therefore needs careful consideration on selection objectives to avoid deterioration of important secondary traits associated with fitness for the environment; however faced with the eventual extinction of the local breed (or its conservation *ex-situ*), two breeds that are economically viable in the locality are a gain in biodiversity compared to one (i.e. the exotic).

The information on possible responses to selection could be obtained from research and development studies, both on the responses selected for and the consequences for other traits. This information is unlikely to be on the local breed but information from other breeds matched as closely as possible for environment and management will be a good first estimate. This can be refined as further information becomes available on the local breed.

● The key question is "will development by selection restore profitability to the local breed?"

3.3.4   Examine the potential of niche markets for high quality products.

The product from the local breed may be capable of being marketed at a higher price than the comparable product from the exotic breed. For endangered breeds, the products already have a rarity value, but this needs to be capitalized upon. If this is possible, income may increase from local and export markets.

This added value can arise simply *because* the local breed is traditionally reared with the products traditionally processed. This can be sufficient to identify the product as being of a higher quality and therefore demanding a higher price in the market place. This may require the need to more closely identify the breed with the region, to emphasise the management of the breed and to market the products accordingly.

There are other opportunities for adding value to the product: highlighting additional qualities in the product from the local breed compared to the exotic (e.g. larger proportions of fat as polyunsaturated fatty acids rather than saturated fatty acids).

● The key question is: "can the product be differentiated to make it command a higher market price?"

3.3.5   Developing novel products.

When in competition from another breed this option is not easy since what can be made with one breed is likely to be made with another. This therefore depends on the imagination. One route to explore is the identification of a traditional product that is no longer produced: if this is identified, competition from other breeds may be overcome by the kind of marketing associated with niche products

One kind of novel product should be mentioned, namely, the breed itself. It may be possible to generate tourist income from maintaining a breed by incorporating it into a farm park or similar venture, perhaps in combination with wildlife indigenous to the region. This option has its problems. It may only work in regions where: (i) other tourist attractions make it feasible to generate sufficient income; or (ii) a sufficient number of farmers can keep livestock as a hobby, associated with other commercial enterprises. If there are only a few participants, each with small numbers it may be difficult to maintain a viable population. Although the sustainability of this option is in general less clear, it may in the short term conserve the breed and generate the momentum for developing the other options.

● The key question is "can the breed be marketed to generate income from non-traditional products?"

3.3.6   Incentive payments.

These have the objective to compensate the farmer or the one who keeps the animals, for the difference in production potential between the breed which is to be conserved and the replacement breed in the same management system and environment. This will ensure that good farmers will still be attracted to the scheme (Henson, 1992). Incentives are subject to political decisions and so are unlikely to be sustainable in the long term, but in the short term it may be

feasible to make use of incentives while other options are being developed.

Although the payments are another form of subsidy, it might be hoped that such subsidies, paid in the context of Convention on Biological Diversity, would have a special position within the World Trade Organization framework.

Incentive payments can be made in different ways: (i) per purebred male or female kept for straight breeding; (ii) purebred offspring raised to weaning age; (iii) per unit of products from the purebred; (iv) by providing government-owned areas for grazing; (v) by subsidizing matings (e.g. AI from purebred males made free of charge); (vi) by subsidizing commercialization of products. Of these, item (i) is unlikely to be tractable since the breeding use of purebred individuals would be difficult to verify, and (ii) and (iii) may be difficult to implement because purebred individuals (or products) need to be distinguishable from crossbred individuals (or products). It may also be difficult to obtain accurate and reliable numbers.

The question of incentives may be of particular interest in establishing and maintaining a sustainable crossbreeding scheme (see 3.3.2 above), since subsidy of purebred inseminations to both crossbred and purebred females may provide added stability to the crossbreeding scheme.

If incentive payments are being made, ownership of the products and priorities when selling or distributing these products have to be clearly identified.

● The key question is: "can a fair incentive scheme be funded?"

3.3.7   Improving the management.

Improving the management, depending on the circumstances, can make significant improvements in

profitability in a short period. This may often take the form of additional care in the bad season. However this improvement will almost certainly require financial investment by the local farmers (costs of extra, or higher quality, feedstuffs; housing) and this needs to be explicitly included in the cost benefit of management improvements.

As with selection, changes in management may compromise the traits in the local breed that are of value. It may therefore be advisable to decide to what limits management may be altered, and how this limit can be enforced, if at all. Nevertheless this should be considered if this provides a means of providing conservation within the same region and avoids conservation outside the region or by cryogenic means. Two economically viable breeds may be argued to represent a gain in diversity over one.

TABLE 3.1.   A cross-classification of the options to sustainably reverse the breed decline and the reasons for decline. The number of asterisks indicate a subjective chance of success, which may help decide on the priorities for evaluating the options. N/A means not applicable.

Option	Local breed is commonly viewed as uneconomic	Local products are produced more economically elsewhere	Product demand is declining
Demonstrating value	***	N/A	N/A
Crossbreeding	**	N/A	N/A
Selection	**	**	**
Niche products	**	***	***
Novel products	*	*	***
Incentives	**	*	*
Management	*	*	*

Because of the modification of the environment implied by this option, it has a lower priority compared to other options that would allow the maintenance of a live population within the region.

● The key question is: "where other options are limited, can cost-effective improvements in management be made?"

▶· 3.4   Choosing the conservation strategy

What methods of conservation should be used?

3.4.1   Categorization of conservation strategies.

Consideration of the above will have refined which conservation projects are needed and what the options are feasible to accomplish *in vivo* conservation for a breed. What remains is to define the objectives for the programme. To do this there is a need to prioritise and select the appropriate conservation strategy.

To recap a very important point of Chapter 1, the primary categorisation is between *in-situ* and *ex-situ* conservation.

▶   *In-situ* conservation.

In the context of domestic animal diversity this is primarily the active breeding of animal populations for food and agricultural production such that diversity is best utilized in the short term and maintained for the longer term. Operations pertaining to in-situ conservation include performance   recording   schemes   and   development   (breeding) programmes. In-situ conservation also includes ecosystem management and use for the sustainable production of food and agriculture. It is

accepted that such improvement may alter the allele frequencies in the gene pool.

▶ *Ex-situ* conservation.

In the context of domestic animal diversity this means conservation away from the habitat and production systems that developed the resource. This will include both storage as live animals away from the habitat and cryoconservation.

> The second categorization is an important qualitative and technical sub-division of *ex-situ* conservation.

▶ *Ex-situ* live conservation.

This is simply *ex-situ* conservation storage as live animals. As for *in-situ* conservation it is accepted that improvement and natural selection in the new environment may alter gene frequencies in the gene pool.

▶ Cryoconservation.

This is the collection and deep-freezing of semen, ova, embryos or tissues which may be used to regenerate animals.

> A final category must be considered: to take no active conservation step and simply allow the pressures surrounding the breed, economic or unrest or other, to resolve themselves and to accept the risk of extinction for a breed with no recourse to stored material

3.4.2   Priorities among strategies of active conservation.

The Convention on Biological Diversity lays clear guidelines for the priorities among the strategies. Article 8 advocates the development of *in-situ* conservation activities where appropriate, whilst Article 9 calls for the development of *ex-situ* conservation schemes but primarily as a complement to

in-situ efforts. Therefore the Convention identifies that *in-situ* conservation as the method of choice wherever possible.

Integral to the definition of *in-situ* is the concept that the breed should continue to occupy the environment in which it was developed. In the context of what has gone before, of the seven options in 3.3, the management changes run the risk of turning the project into an *ex-situ* project. However such a change would conserve the breed within its own locality, and so it would continue to have a relationship with its indigenous keepers, and this must be considered of greater value than complete removal of the breed from the locality.

Conservation schemes can be envisaged that maintain live animals both *in-situ* and *ex-situ* and involve the regular interbreeding of the subgroups. Strictly speaking such schemes should be considered as *ex-situ* live animal conservation, but common sense must tell us that these have some higher quality than other *ex-situ* options.

Cryoconservation must be considered as the strategy with the lowest priority. Therefore its sole use is only justified when *in situ* conservation is not feasible, and *ex situ* live conservation cannot be carried out to the standards described in Chapter 4.

3.4.3   Aspects particular to cryoconservation.

Although cryoconservation has the least priority among active strategies it can involve a high cost of obtaining the required samples and must again be justified. Much of the case for justification has already been done to get to this point, even though the principal focus has been on *in-situ* schemes. However priorities for cryoconservation should be breeds which have no viable *in vivo* conservation programme and, in order of priority: (i) have phenotypic adaptations to their environment or have novel products; (ii) have social or cultural importance; (iii) after considering these other

priorities, breeds which appear to be genetically distant from other conserved breeds based upon DNA methods and finally, other breeds.

Cryoconservation may store semen or embryos or, in the near future, cells. In general the cost of obtaining embryos is greater than the cost of obtaining semen, whilst the storage costs for both are the same. The precise objectives for cryoconservation are discussed in more detail in Chapter 5, but semen is as effective or more effective in achieving these objectives except where the objective is to re-establish a breed that has become extinct. Therefore the priority material for conservation should be semen. Where it is considered that there is a high probability of access to the stored material for re-establishment the storage of embryos should also be considered as an addition (although this situation is not considered to be very likely).

Even once the samples of genetic material are obtained a cryoconservation programme is not without risk. The samples need to be continuously maintained in liquid nitrogen and this requires appropriate storage facilities and regular maintenance (described in Chapter 5) and failure to carry out the procedures will result in the loss of the material.

One of the most useful aspects of cryoconservation is as a support to *in vivo* conservation (see 4.7, 5.2.3). In this case it is an adjunct of a wider conservation programme, especially an *in-situ* project, in which cryoconservation of genetic material is carried out as a reserve in case of catastrophe, or a genetic problem arising from the accumulation of deleterious recessives in the live population. Where the breed will be used in a new breeding programme using selection or crossbreeding (especially), where management changes are being made, or where some element in its natural environment is changing, cryoconservation at an early stage

may also be advantageous. It must be stressed however that cryoconservation is not a necessity for successful *in-situ* conservation, and is simply a means of risk reduction where funds and facilities allow it.

3.4.4   Making the decision.

The final decision on the conservation strategy in a particular case will depend on: (i) the risk status of the breed; and for each form, (ii) the priority accorded by the CBD as described in 3.4.2 and including an assessment of sustainability and benefit sharing, (iii) short-term and long-term costs, (iv) the technical difficulty, and (v) the risks of failure.

The total costs of the various strategies are not only qualitatively different in magnitude, but differ also in the timescale over which they are incurred. The costs will also vary widely within conservation strategies; for example, the costs of *in-situ* conservation depend heavily on the competitiveness of the breed. Costs may also vary according to the core activities of stakeholders; for example, maintenance of animals *in vivo* may be combined with training animal husbandry in a teaching organization.

The importance of technical difficulty, with its associated demands on human resources, and the assessment of risk of failure will be dependant on the country concerned: for example, cryoconservation may be perceived to have no insurmountable technical difficulty and very low risk by a developed country, whilst the opposite may be true for a developing country.

Table 3.2 gives a summary of the comparative cost and benefits of the different forms. The following perspectives may apply:

Table 3.2   Comparative advantages between the different forms of conservation
(the more +•s and fewer -•s the better). This comparison assumes that the
strategies are fully implemented as described in these Guidelines.

Form of Conservation	Contribution to Biodiversity	Maintenance of adaptive fitness	Sustainability	Cost of establish-ment	Cost of maintenance
Cryoconservation	+	++	+	---	--
Ex-situ live	++	+	+	-	----
In-situ	++++	+++	+++	-	-

> ▶   *In-situ* conservation.

A well-constructed *in situ* conservation project will move towards self-financing and will draw upon a wide and robust community involvement. This will enhance sustainability and minimize costs, both in the short and the long term. The technical requirements are low compared to cryoconservation. The development of this Chapter has been directed to promoting this primary route.

> ▶   *Ex-situ* live conservation.

The key question for this strategy is whether or not long-term finance and commitment is available to maintain generations of animals to the standards required for successful conservation (see Chapter 4). The maintained livestock will be producing outside their own environment of origin, and consequently the production may be uneconomic and management may be demanding. The technical requirements in *ex-situ* live conservation are low compared to cryoconservation.

> ▶   Cryoconservation.

The key question for cryoconservation is whether, in the short term, the facilities and expertise required for the collection of the samples can be financed and put in place. The logistics of providing and maintaining storage facilities will need to be addressed before the cryoconservation is

carried out, but the long-term costs associated with storage are low compared to *ex-situ* live.

The decision aids in this Chapter have developed the simple conclusion from census and survey that a conservation of domestic livestock is required into an outline plan for a breed. With the conservation projects integrated into wider national action plans, and an analysis of opportunities and options, the form of the conservation project will stem from application of the priorities expressed in the Convention on Biological Diversity. Where these involve an *in vivo* animal project (both *in-situ* and *ex-situ)* the design considerations are dealt with in Chapter 4, and where cryoconservation is used, either as a last resort or as a support, the design considerations are given in Chapter 5. It may be that the considerations in Chapter 2 and in this Chapter lead to the conclusion that certain breeds have a low priority for conservation and no further organized conservation will be undertaken.

References

Henson, E.L. (1992). In-situ conservation of livestock and poultry. pp. 77-78. FAO Animal Production and Health Paper 99.

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▶ 4. The Design of *In vivo* Conservation Programmes

- *An introduction to some relevant genetic concepts*
- *Coping with the history of the population*
- *The genetic structure of the population*
- *The physical structure of the population*
- *Recording, monitoring and research*
- *Coping without pedigree recording*
- *A note on the use of cryoconserved material*
- *Expertise required*

This chapter will describe the technical details of how to set up and run an *in vivo* conservation programme. The design options are displayed in Fig. 4.1. It is assumed that it has been decided that the endangered breed is to be conserved as live animals. The technical design is independent of whether the scheme is *in-situ* or *ex-situ* live conservation and is largely independent of the species. The aim of the conservation programme is to maintain the remaining genetic variation in the breed. For more detailed consideration of the genetic concepts see Falconer (1989) and Wiener (1990).

▶· 4.1 An introduction to some relevant genetic concepts

What are the important genetic principles?

4.1.1 Genetic variation and rates of inbreeding.

In any population it is inevitable that some genetic variation will be lost over time.

Figure 4.1: Designing an *in vivo* conservation scheme
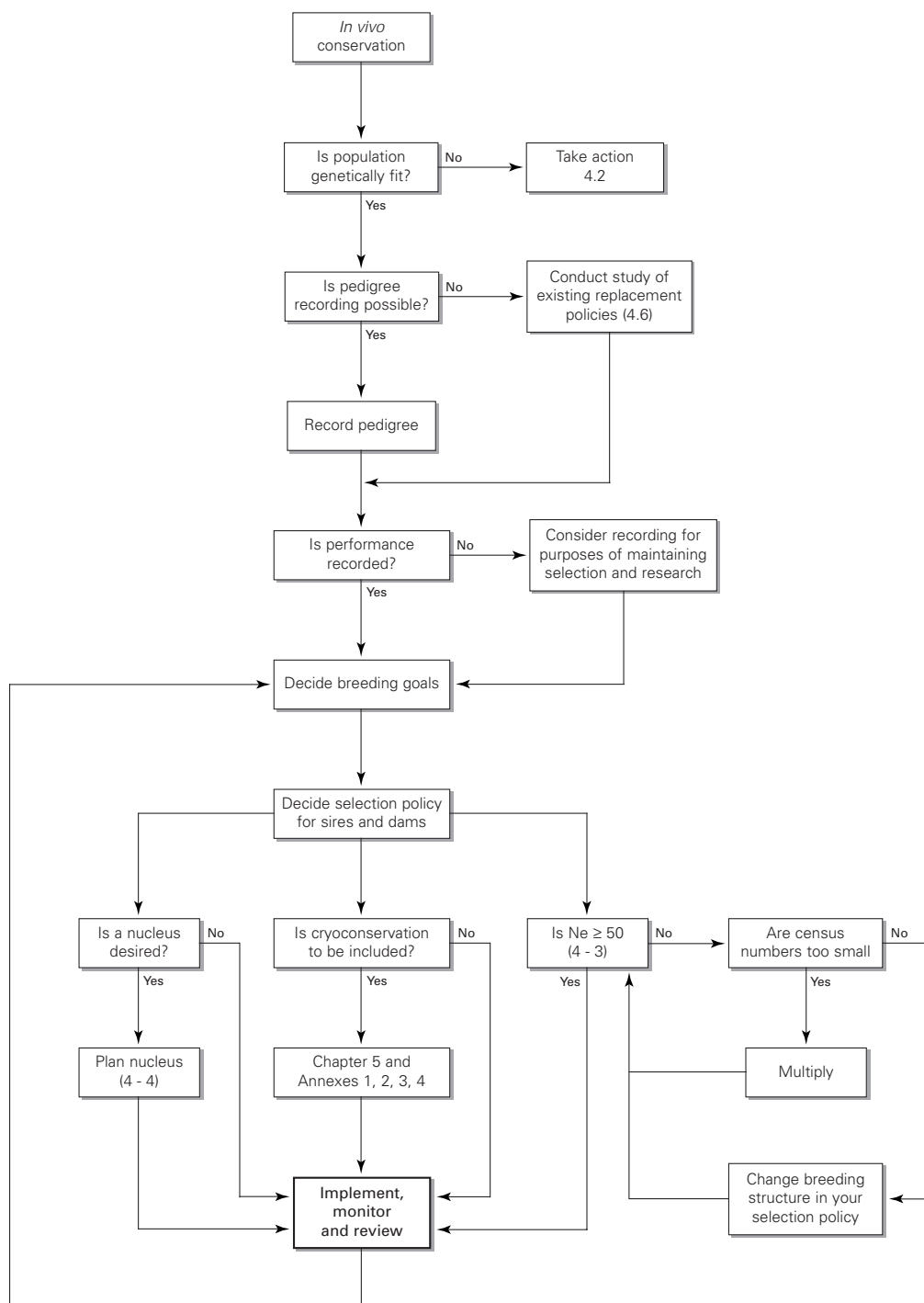
Although, we cannot measure genetic variation in all traits, we can measure its rate of loss and, through controlling this rate, keep it at low levels. Minimisation of the loss of genetic variation is equivalent to minimisation of the rate of inbreeding in a population (see Box). *Thus, the rate of increase of inbreeding (ΔF) is the most important parameter in programmes that maintain genetic diversity.*

Inbreeding occurs when related animals are mated. The offspring of these matings are more likely to inherit identical genes from each of its parents, and, as a result, they obtain less benefit from hybrid vigour which arises from inheriting different genes. The closer the relationship between the mates the more inbreeding will occur. Inbreeding can not be completely avoided in small populations, because if we trace the pedigree back far enough all animals are related. The degree of inbreeding varies, and is measured by the inbreeding coefficient, F, which lies between 0 (not inbred) and 1 (completely inbred, with no genetic variation); most animals in a population will be partially inbred with a coefficient >0 but <1.

The relationship between loss of genetic variability (••g2) and the rate of inbreeding (•F):
The rate of inbreeding per unit time is the change in inbreeding in the time unit, expressed as a ratio to the inbreeding yet to go i.e. 1-F. The rate of inbreeding has predictable forms, and has a very important relationship with loss of variation: if σ_g^2 is the genetic variation then the loss in a unit of time is: $\Delta\sigma_g^2 = \Delta F\, \sigma_g^2$

The rate of inbreeding is more important than the actual level of inbreeding, because the actual level of inbreeding is relative to some base population, which is assumed unrelated and non-inbred. In practice, the base population is the population when pedigree recording started, i.e. it is the population for which the sires and dams are unknown in a pedigree. Consequently, populations with many generations of recorded pedigrees will tend to have high coefficients of inbreeding, and populations in which pedigree recording started recently will tend to have low coefficients of inbreeding, irrespective of the *rates* of inbreeding in the

populations. The inbreeding coefficient (F) is therefore not a suitable parameter to describe a small population.

The genetic size of a population is most easily described by the effective population size (N_e) which is a number that is generally smaller than the census size of the population (see Box). The *effective size is determined by the rate of inbreeding* (and vice versa): $\Delta F = 1/(2N_e)$.

Thus, the proportional rate of loss of genetic variation, the rate of inbreeding, and the effective population size are parameters describing identical concepts.

Some technicalities with respect to the rate of inbreeding ΔF

The rate of inbreeding is often predicted by the formula: $\Delta F = 1/(8N_s) + 1/(8N_d)$ where N_s is the number of sires, N_d is the number of dams per generation. This formula *only* holds when selection is at random, i.e. no selection. With selection, ΔF is substantially higher than predicted by the above formula (Wray and Thompson, 1990). This publication clearly shows that the parameter ΔF comprises more than merely the number of sires and the number of dams. Therefore the formula given above must be used with care. If 5 sires and 25 dams are used in a generation and *if selection is random*, $\Delta F = 0.025 + 0.005 = 0.03$.

Parameters have been described as alternatives to the rate of inbreeding that might take better account of founder animals. For example, Alderson (1990) used the Genetic Conservation Index (GCI) to calculate an effective number of founders for an animal: $GCI = 1/\Sigma P_i^2$, where P_i is the proportion of the genes passed to a current animal that are from founder animal i, and Σ denotes the sum over all founders. The GCI will depend on the depth of pedigree available, but if one traces the descendants a large number of generations back to the founders, the GCI (and all the component P_i) becomes the same for all descendants and equals half the effective population size N_e as defined in 4.1. Hence, as shown by Wray and Thompson (1990), if the founders are traced back many generations with full pedigree recordings: $\Delta F = 1/(4\ GCI)$. This equivalence in concept between GCI and ΔF motivates us to stick to ΔF as the single parameter to describe small population size.

Genetic variation can also be generated in a population through new combinations of genes and mutations. This is a

natural process maintaining variation in all populations. The rate at which this occurs is slow, but it is, in the long term, sufficient to maintain some genetic variation in the population. The amount of genetic variation that is maintained in the population in the long term is proportional to the effective population size. Hence also in the long term, the effective population size or, equivalent, the rate of inbreeding determine the amount of genetic variation that is maintained.

It is strongly recommended that the pedigree of the animals is recorded in a conservation programme, so that the rate of inbreeding can be calculated. This requires only the recording of the sire and dam of every animal. Where pedigree is recorded animals with unknown sires and dams are assumed to constitute the base population. From the pedigree, the relationships between all animals can also be calculated (Falconer, 1989), which is another useful tool. If it is not possible to record pedigrees, see 4.7.

In the Guidelines the target is to have an effective population size is 50 animals per generation or more. The effective size of 50 corresponds to a rate of inbreeding of 1% per generation. *All conserved populations should have achieving this minimum effective size as their first objective (see 4.3.1).*

4.1.2   Generation intervals.

The *generation interval* is the genetic unit of time for populations. It is a measure of how long it takes to replenish a set of parents. Since males and females each contribute half the genes to the population the generation interval is the average of the generation interval for the breeding males and for the breeding females. These periods may differ between the sexes. The generation interval for males (or females) is the average age of the male (or female) parents at the birth of their straight-bred replacement. In many cases the generation interval for a sex will be approximated by the average age of

the parents of the sex at the birth of all the offspring, but this need not be so if crossbreeding is part of the production system, see 2.7.

Definition of generation interval:
The generation interval for males (L_m) is defined as the average age of the male parents at the birth of their replacement. The definition for females (L_f) is similarly defined. The generation interval for the population is then $2(L_m+L_f)$.

Example:
If the offspring are born when sires are one year old, and 60% and 40 % of the offspring are born when the dams are one and two years old respectively, then L_m=1, L_f= 0.6*1 + 0.4*2 =1.4 years. The population has a generation interval, L=2 * (1+1.4) = 1.2 years.

The Guidelines use the genetic timescale of the population and therefore consider target inbreeding rates per generation. Generally the factors that counteract inbreeding, such as, natural selection, recombination between closely linked genes, and mutation, are effective per generation. Consequently, the inbreeding rate per generation is more relevant than the inbreeding rate per year for the genetic constitution of the population, and so will be considered here. In some circumstances longer generation intervals are desirable since then the rate of loss of genetic variation is slower in relation to human decision processes which are measured in terms of years.

Most practical breeding schemes involve *overlapping generations* where there are parents of different ages being used. Sometimes breeding schemes can operate in what is termed *discrete generations*, where all parents breed only at one age and the same age for both the sexes.

4.1.3   The average relationship of a population.

Complex relationships between two animals can be described by a coefficient which is a function of their pedigree. It can be calculated when the pedigree of the population is known, i.e. when the sire and the dam of every animal is known for 1 or more generations. The usefulness of relationships as a tool increases with the number of generations of known pedigree. Falconer (1989) shows a tabular method to calculate the relationship coefficients and simultaneously the inbreeding coefficients. If necessary, there are much faster computer algorithms available (e.g. Meuwissen and Luo, 1992).

The average relationship in a group of individuals equals one quarter the average relationship among the males (calculated from the relationship coefficient for every pair of males in the group) plus one quarter the average relationship among the females (calculated from every pair of females in the group) plus one half the average relationship between the males and the females (calculated from every sire and dam combination).

The inbreeding coefficient of the offspring equals half the relationship between the sire and the dam. Thus, if we calculate the relationship between every possible sire and dam, we can choose the sire and dam pairs that yield the offspring with the lowest inbreeding coefficients. This decreases the immediate inbreeding in the offspring. If we want to decrease inbreeding in the longer term, we should choose a group of sires and dams that have a low average relationship amongst themselves.

►· 4.2 Coping with the history of the population

What kind of problems may be present at the start?

4.2.1 Bottle necks.

The endangered population may have recently gone through one or more genetic bottle necks, i.e. the population size has been very small during some period(s). In itself this is not a problem if the population has emerged from it with no genetic defects and without depressed performance (the population is said to be *fit*), but it is likely that some deleterious genes have drifted to high frequencies. If this is the case see 4.2.2. A very recent bottle neck may be ameliorated by selecting animals for the conservation plan that are as little related as possible (see 4.4.1).

Regardless of bottle necks, the conservation programme will try to conserve the remaining genetic diversity as much as possible. If the population size is currently very small, every animal is extremely important for the population, because the loss of an animal has a large impact on the total population size.

4.2.2 Genetic defects.

The population may show genetic defects in high frequencies, for example more than 10% of the animals show a particular genetic disease. Often genetic defects are only expressed if the deleterious gene is present in homozygote form (i.e. the affected individual carries two copies of the defective gene, and heterozygote carriers which have only one copy are fit). The following animals should then be excluded (as far as possible given the desired number of

parents) from being parents of any generation of the conserved population (in order of priority):

1.   animals showing the genetic disease (homozygotes);

2.   offspring of animals that showed the genetic disease;

The important point is to put in place the control measures described above to control the spread of the disease and to ensure that breeding individuals are used in a balanced fashion, so that the inadvertent use of a heterozygote carrier has only a limited impact on the population. Once under control, the rate of removal of the defect can be as rapid as numbers allow: beware creating new bottlenecks and new problems from using too few parents. Over time from following the policy described above the defective gene frequency will decrease and the disease incidence will become sporadic; however copies of the defective gene may still be in the population as heterozygotes.

When the defective gene is at low frequency, a test for heterozygotes is particularly valuable since it facilitates complete elimination of the defect by avoiding heterozygote sires and dams *providing* numbers allow. This is most easily achieved if a DNA test for the gene is available. In the case of a fast reproducing species and early diagnosis of the disease, a progeny test can sometimes be conducted if a DNA test is unavailable: the candidates are mated to diseased animals (if the disease is not fatal) and any diseased offspring indicates that the candidate is a carrier. All offspring from these test matings are discarded from the conservation programme where numbers allow. Where carriers must be used as parents, then one carrier should not be mated to another unless this is unavoidable; this will minimize the number of diseased offspring produced.

For pedigreed populations there are good computational techniques available to identify the probability that a particular animal is a carrier, and this information can be used to help selection and mating; this may be particularly useful in removing all carriers when no other test for heterozygotes is available, so seek advice (from research institutes, FAO).

There is considerable interest in identifying single gene mutations causing disease since they provide immense scientific opportunity to advance knowledge in physiology and in medical and veterinary science. If the population has a genetic disease that is well defined, then funding for a collaborative project with a scientific institute may be available with the objective of identifying the defective gene. Such funds may help support the population during the period needed to bring the incidence of the disease to an acceptable frequency and, depending upon the outcome of the project, may provide longer term funding for the breed.

4.2.3   Populations in genetic melt-down.

A population that is in a genetic melt-down is one which is not fit enough to reproduce itself and thus the number of animals are irrevocably decreasing every generation, i.e. the population is heading for extinction. In a genetic melt-down, the rate of inbreeding has probably been too high in the past. It can be conserved *in vivo* by:

1.   limited crossing with another breed that is fitted to a similar environment. A small number of genes from the crossing breed can have a big effect (see Box). Ideally, the introduced breed should be phenotypically similar to the breed that is to be conserved.

2.   changing the environment of the animals, so that their fitness increases and natural selection can further increase its fitness. When the fitness has increased, the

population may be returned to its original environment and measures in 4.2.2 can then be applied to any further problems.

3. cryoconservation. However, cryoconservation will only postpone the melt-down, and the only viable option post thawing is either using semen to cross to another breed, which is similar to (i), or (ii).

Introducing foreign genes:

If a proportion p of foreign genes is introduced the proportional reduction in inbreeding is $1-(1-p)^2$. Therefore with 10% of foreign genes, $p=0.1$, the inbreeding coefficient is 0.81 of what it would have been without the foreign genes e.g. instead of F=0.30, F would be 0.24.

► · **4.3 The genetic structure of the conserved population**

How should conserved populations be maintained and improved?

4.3.1 The desired effective population size.

The desired effective population size is about 50 animals per generation. This leads to a rate of inbreeding of 1% per generation. When selection is within families, i.e. a son replaces the sire and a daughter replaces a dam, the rate of inbreeding is as low as possible for a given number of sires and dams. Table 4.1 provides some numbers of sires and dams that lead to an effective size of 50 animals per generation. The numbers for within-family selection hold only when this selection scheme is strictly followed. If strict within-family selection cannot be guaranteed, the numbers of sires and dams for random selection should be used.

Table 4.1   Number of sires and dams needed per generation to achieve an effective population size of 50.

Random selection		Mass selection[1]		Within family selection[2]	
Sires	Dams	Sires	Dams	Sires	Dams
25	25	35	35	13	13
20	34	30	45	12	14
16	56	25	65	10	50
14	116	20	300	9	1000
Smaller number of sires: not possible					

[1] These numbers are representative but are derived assuming $h^2=0.4$ and 6 offspring per female reproductive lifetime.

[2] These numbers are only valid when strict within family selection is applied, otherwise use numbers of sires and dams for random selection.

Note that the numbers of sires and dams in Table 4.1 are per generation. With a generation interval of 4 years and each year 2 young sires enter the breeding cycle, the number of sires per generation is 8. It follows that an extension of the generation interval leads to the use of more sires, if the same number of young sires enter the breeding cycle each year. In this way, a prolonged generation interval can increase the effective population size.

When the desired effective population size is achieved, it should not be allowed to decrease, because the effective population size over a long period of time is mainly determined by the smallest effective sizes within that period.

Prediction of the rate of inbreeding:

Within family selection, i.e. a sire is replaced by one of his sons, and a dam is replaced by one of her daughters, leads to the smallest possible inbreeding rate at a given number of sires and dams, namely: (Gowe, Robertson and Latter, 1959):

$$\Delta F = 3/(32\ N_s) + 1/(32\ N_d)$$

where N_s and N_d are the numbers of sires and dams per generation. This low rate of inbreeding is also achieved when the selection is based on phenotypes or a selection index, as long as the selection is entirely within families. Hence within family selection is a very safe method to achieve genetic improvement in small populations.

The above rate of inbreeding is approximately half the inbreeding that results from a random choice of sires and dams across the families which is predicted by the formula of Wright (1931):

$$\Delta F = 1/(8N_d) + 1/(8N_s).$$

This is often misused, since if the sires and dams are selected for phenotype or selection indices across families, the rate of inbreeding increases even further and advice is available for helping design in these instances.

4.3.2   Cryoconservation as a means of reducing inbreeding.

As shown in 4.3.1 the longer the generation interval, the lower the rate of inbreeding. In live animals the generation interval is limited by the maximum age of the animals, but if we can freeze the embryos, the generation interval can be much prolonged. This is one justification for cryoconservation, where there is no inbreeding until the embryos are used again. However, the aim here is to design an *in vivo* conservation programme. If we can cryoconserve semen, we could increase the generation interval of the sires alone and inseminate the dams with semen from old sires that died a long time ago. This is an efficient way of increasing the number of sires used per generation.

Uses of cryoconservation for *in vivo* conservation are summarized in 4.7.

An interesting situation occurs if we could cryoconserve enough semen from a current batch of bulls such that we could use it for a virtually unlimited period of time to fertilise the females. The inbreeding coefficient asymptotes to a value of $1/(2Ns)$ (relative to the current inbreeding level of the sires, i.e. setting their current level of inbreeding to zero, with Ns being the number of cryo-conserved sires. The genes of the frozen semen will replace all the other genes in the population. The latter implies that:

(i)   selection in females will lead to very little selection response;

(ii)   genetic adaptation is limited;

These 2 points make an indefinite generation interval on the male side a poor *in vivo* conservation option, but some increase of the male generation interval by the use of frozen semen may be worthwhile in very small populations.

4.3.3   Selection of sires and dams.

The recommended rule for the initial stages of a conservation programme is that selection should be within families, i.e. a sire is replaced by one of his sons, and a dam is replaced by one of her daughters. This minimises the rate of inbreeding. If selection is not within families and selection is not at random, the rate of inbreeding can be substantially higher than expected.

The rule that a sire is replaced by one of his sons and a dam by one of her daughters, leaves some room for selection. Although the existing genetic constitution of the population is best conserved when this selection is at random, a really successful conservation programme eventually leads to a breed that is commercially acceptable, and thus the Convention on Biological Diversity recognizes the need for improvement of the genetic constitution of the population.

Demonstration of within-family selection:

Consider the following simple scheme that mates 2 sires (A and M) with 4 dams (B, C, N and O):

$A_♂ * B_♀ =>$ $D_♂, F_♂, G_♀, H_♀$
$A_♂ * C_♀ =>$ $I_♂, J_♂, K_♀, L_♀$
$M_♂ * N_♀ =>$ $P_♂, Q_♂, R_♀, S_♀$
$M_♂ * O_♀ =>$ $T_♂, U_♂, V_♀, W_♀$

For within family selection, one of the male offspring of A namely D, F, I, or J is selected to serve as a sire in the next generation, and one of the males P, Q, T, or U is selected to serve as a sire. Similarly, G or H is selected as a dam, K or L is selected as a dam, R or S is selected as a dam, and V or W is selected as a dam. This selection yields again 2 sires and 4 dams. The matings between the 2 selected sires and 4 dams should be such that the sire * dam combinations have the lowest possible relationship.

Thus we might select for phenotypic records within the families. If we want to improve several traits simultaneously, one can either estimate the genetic parameters and set up an index for those traits (see Falconer, 1989), or try to combine the traits into a super-trait. For example, if we want to increase growth rate and decrease fat content of pigs, we can select for lean growth rate as a super-trait. In the case of many traits with their economic values, a simple approach is to calculate the profit for every animal and treat that as a super-trait.

Often endangered breeds have very specific characteristics which we wish to preserve as a priority. Even at the reasonably large population sizes recommended here, these characteristics might undergo genetic drift in an undesired direction. If this occurs one can always select against it, but it is also possible to use BLUP-EBV (Best Linear Unbiased Prediction - Estimated Breeding Values; see Mrode, 1996) to select sires and dams that have a near average EBV equal to the population mean. This would minimize the drift for such a characteristic.

The selection against genetic defects is described in 4.2.3.

When mating the selected sires and dams, the mating of sires and dams that are full-sibs should be avoided, and where possible half-sibs. Complete avoidance of other relatives is impossible after the initial generations. Although this avoidance of the mating of relatives will have little effect on the long term rate of inbreeding, it reduces the actual level of inbreeding in the offspring (offspring of full- and half-sibs have $F \exists 0.25$ and $\exists 0125$ respectively). This is important because it avoids inbreeding depression in these families which may reduce the fertility and general fitness of the animals.

Where females produce more than one litter, then factorial mating (Woolliams,1989) should be used. The production of large full-sib families carries a penalty for inbreeding, if any selection, natural or artificial, is occurring. Factorial mating ensures different litters from the same dam are produced by different sires. For example, if a sire is carrying a deleterious gene and a dam is always mated to the same sire then all the dam=s offspring are at risk (and consequently her future contribution to the population); if mating is factorial then other litters from the dam will escape this risk.

As the breed becomes more profitable, the census size of the population will increase and there will be many more candidates for selection as breeding individuals. At this point selection between families becomes possible. More sires and dams need to be selected each generation, otherwise rates of inbreeding will go above the recommended target. The simplest form of selection that involves some selection between families is mass selection and Table 4.1 gives a recommended number of sires and dams in this case.

It is possible to improve on mass selection to obtain faster rates of progress by using indices incorporating information from relatives or evaluation using BLUP even when inbreeding is restricted. In these circumstances the number of male and female parents needed to maintain the target inbreeding rates needs further evaluation; *if the improvement scheme is able to use these more advanced indices it is also able to use the companion computer-aided decision tools to control inbreeding* (e.g. Meuwissen, 1997; Grundy *et al.*, 1998), so seek advice. With these sophisticated selection methods, the organization will need to be effective for the purpose (pedigree recording, gathering together performance records, and entering data on computer) and the costs of the conservation programme will increase, but this may be justified when considering the future income from the market niche.

4.3.4 Commercial use.

A conservation programme is most likely to maintain its funding when there is some commercial use of the animals. The animals that are not selected as sires and dams can be sold and therefore help the funding of the project. Selection towards some specific market niche can make the animals more valuable. Examples are the production and marketing of special cheeses, or better adaptation to local environments. Also the development of special crossbreds that may perform well in an environmental niche, may demand that the purebred is maintained and so increase the value of the purebred (see 3.3).

The possible niches will vary from case to case and a lot of investment and determination may be needed to achieve them. When a niche has been identified, a breeding scheme has to be set up to achieve the niche:

1   The breeding goal should be derived. This is achieved by determining the increase in profit when one trait is

improved by one unit. The latter yields the relative value of each of the traits, which can be summed to form the breeding goal (see Weller, 1994 for a general review of economics in animal breeding).

2. A selection index has to be set up from traits that are measurable and which correlates as highly as possible to the breeding goal (Falconer, 1989).

3. The selection should be performed as described in 4.3.3. This selection is reasonably efficient. If possible long generation intervals should be reduced to increase the rate of genetic gain (Falconer, 1989).

If the endangered breed is particularly poor for some trait, the limited introduction of some genes from a foreign breed, i.e. crossing some of the endangered animals with the foreign breed, may increase the scope of selection for that trait. This may lead to the development of a new breed. The fraction of genes coming from the foreign breed must be minimised in order to conserve as much as possible the endangered genes.

4.3.5   The introduction of new unrelated animals.

Sometimes serendipity occurs and are found in some remote herds that are of the same breed but are apparently unrelated to the animals in the conservation programme. Generally this will have a very positive effect on the rate of inbreeding but there are pitfalls:

1 firstly, what is meant by unrelated? For how many generations back can separation from the remainder of the population be firmly established? If the known ancestry is very limited then considerable care should be taken, and whilst the >new= stock are valuable it may be worthwhile seeking advice.

2   if matings are devised based solely upon the minimum coancestry or minimum relationship principle, the new animals may obtain a too large proportion of the matings because they are unrelated to the old animals. This may result in a new bottleneck, especially if the >new= stock are limited in number: the later generations will all be related through the small number of newly introduced animals!

3   the 'new' animals are unrelated to the 'old' animals but may be highly related among themselves, which limits their use. The 'new' animals should be chosen as unrelated among themselves as possible.

Keeping in mind these pitfalls, if the >old= population had too high rates of inbreeding then the >new= population can simply be added to the >old= population and the total number of parents can be increased.

If the >old= population was of considerable size, i.e. Ne ∃50, then the >new= population can still be used to decrease the inbreeding. Sensible use of the new unrelated animals is made by minimizing the average relationship of the selected sires and dams across the >new= and the >old= animals, without reducing the total number of parents used per generation. It will be assumed here that the >new= animals are not more highly related among themselves than the >old= animals. In this case, both groups of animals should contribute approximately 50% of the genes of the new population that will be established from the >old= and the >new= animals. For example, the 50% contribution from the >new= animals can be achieved by letting all sires be >new= animals, and all dams be >old= animals; the number of sires selected from the 'new' population will equal the number of sires that normally would have been selected from the >old= population. In this example, if there are not enough >new= sires to replace all >old= sires, some >old= sires should still be used chosen.

4.4   The physical structure of the conserved population

What role do nucleus populations play in conservation programmes?

It is often desirable to identify a subset of the population upon which to concentrate limited resources. This will safeguard a core, conserved population from which to expand. This will be called the nucleus population. It is possible to focus the organization of the programme within the nucleus, at least initially. After the conservation programme is firmly established it is a good base for selection because of this organization. It is not necessary to define a nucleus but it is recommended. There are issues to address: size, establishment, location and management.

4.4.1   Selecting the sires and dams to enter a nucleus population.

The numbers required will be determined by financial and other resources available. It is recommended that, where possible, the nucleus should have an effective population size of 50 or more (see 4.3.1). If resources are limited the nucleus should be as large as possible. The actual numbers of sires and dams needed for establishment has already been described in 4.3.1, and this will equal the number of parents required in later generations. In slow reproducing species, it may take some generations or years before these numbers are achieved. This is not a problem if the principles to minimize loss of variation, described earlier, are followed as closely as possible.

If there are sufficient animals to allow selection of a nucleus population then the average relationships among the sires and dams chosen to produce the first managed generation of the nucleus should be minimised. The unrelated animals will be more representative of the wider population. This form of selection is only possible when: (i) there are more selection

candidates than the number of sires and dams needed; (ii) the relationships between the animals are known, i.e. when the pedigree of the animals is known. If the pedigree of the animals is unknown, sires and dams will have to be chosen at random or, maybe, DNA markers can be used to determine the genetic relationships among the animals.

4.4.2   Location of the nucleus.

The conserved population could be housed in a central nucleus herd or in several dispersed herds. It is recommended to choose the dispersed herds option, because a central nucleus herd could be wiped out by diseases, fires, and other natural disasters. Also, the contact of the farmers with the animals is increased when they are kept at dispersed herds. However some agreed uniformity of management is desirable.

With a central nucleus, there is a risk to *in-situ* schemes from genotype by environment interactions (in which genotypes perform differently in different environments) since the population may adapt over time to the local environment of the nucleus. With a dispersed nucleus, a risk to *in-situ* schemes is that the dispersal extends beyond the environment of origin. A further consideration on dispersal is the very practical question of the location of those farmers interested in keeping the breed.

Generally, genotype by environment interactions favour the keeping of the animals in an environment that resembles their natural environment as closely as possible. *Ex-situ* live conservation programmes will not be successful when genotype by environment interactions are strong, and *ex-situ* cryoconservation programmes may also fail if the interactions are strong and important aspects of the environment change since cryoconserved animals cannot adapt.

Therefore whilst a dispersed nucleus is recommended, the extent of dispersal will depend on the situation and common sense applied to the specific problem must be used.

4.4.3   Mating systems for a dispersed nucleus.

With the animals being kept at dispersed herds, we must avoid the occurrence of small distinct populations in each of the herds with no connections between the herds. This is most efficiently achieved by setting up a rotational breeding scheme. For example, in a 3-herd scheme, the selected sires from herd A are mated to dams at herd B, the sires from herd B to dams of herd C, and sires of C to the dams of A.

This may be an important role for cryoconservation, since it will allow the use of AI. This greatly facilitates such mating systems, when anything other than walking distance is involved.

4.4.4   Recording.

One of the drawbacks of conservation programmes with a dispersed nucleus is that the quality of the recording of the pedigree and traits may be poor in some or all of these herds. In a central nucleus programme the possibilities for accurate recording may be much higher. Accurate recording is the basis for success of any breeding scheme, and thus some balance between many dispersed herds, spreading the risks widely, and few nucleus herds, with accurate recording, has to be found.

► ·   4.5   Monitoring, recording and research

What records need to be kept?

4.5.1   Recording and the database.

It is strongly recommended to record at least the sire and dam of each animal such that the pedigree can be constructed. This requires identification of the animals, e.g. by ear tags in larger species. Traits that are worthwhile monitoring include the traits that characterise the breed, the economically interesting traits, traits for which selection is desired, (genetic) diseases, and others.

The recordings should enter a databank of which a back-up copy should be stored at another place. It is preferred to build a relational databank that links animals to sires and dams and to its records. The databank should also point to information stored in other databanks. This will serve as a basis for answering survey questionnaires (see 2.7).

4.5.2   Monitoring.

The recorded pedigree makes it possible to monitor numbers of stock, the increase of the average inbreeding coefficient of the animals, and hence to check whether the target for the inbreeding rate is being met. More recent selection decisions are better monitored by calculating the increase of the average relationships among the animals born in each cohort. For a scheme in equilibrium, the average relationship increases at twice the rate of inbreeding, i.e. at 2% per generation when the inbreeding rate is 1% per generation. The average relationship reflects what the inbreeding will be in the future.

The genetic level of the interesting traits can be monitored by evaluations of varying sophistication. It is likely that climatic variability and the other sources of environmental errors will obscure the genetic trends in anything other than a long-term study. Nevertheless routine monitoring is important so as to be up-to-date. It may also be possible to spot the introduction of crossbreds. The most sophisticated analyses will make use of BLUP evaluations (Mrode, 1996). These yield estimates of breeding values for all individuals in the population (EBVs) which are corrected for possible environmental changes, e.g. a poor year.

Seek help for monitoring if the required expertise (statistical and genetic) is not available.

4.5.3   Incorporating some research.

At least in the early years of establishing a conservation programme it may be useful to associate some research with it to learn more about the breed i.e. upgrade the phenotypic and genetic characterization. The findings may well suggest simpler avenues for conservation e.g. if unforeseen qualities are identified.

A well considered proposal for characterization may provide a means of obtaining national or international funds to support the initial phases of the conservation programme.

▶·   ## 4.6   Coping without pedigree recording

What needs to be done if pedigree recording is not possible?

Many parts of the conservation plans described above assume an identification system for the animals, e.g. tags. Although the use of such a system is strongly recommended, in some situations and species this may not be possible.

The general principles of conducting the scheme still apply, but it will be harder to estimate the rate of inbreeding in the population. In these situations we should try to estimate the number of sires and dams that are used per generation. This estimation can be part of a census or survey as described in 2.7, but may be the subject of a special study. The census or survey should ask questions such as:

1.   how many sires does the farmer use per season;

2.   from where does the farmer obtain sires (there may be a small breeding nuclei somewhere in the population, so many sires might be related);

3.   how many dams does the farmer use per sire (the number of dams per sire times the number of sires yields the total number of dams);

4.   the litter size of the breed;

5.   selection of replacement dams.

Using this information it may be possible to establish the links between groups of keepers, and whether or not a hierarchical population structure exists within the breed. An early example of this within UK sheep breeds was presented by Wiener (1953).

Once this information is collected then, together with survey information on breeding ages, allows estimation of the inbreeding rate per generation (help may be obtained from research institutes, FAO). If the estimated rate of inbreeding is more than 1%, the conservation plan should try to get the farmers to use more sires. Possibilities for this may be education, financial incentives, establishing alternative sources of sires etc.

Until this information becomes available, then the breeding scheme should ensure that wherever possible a minimum of 6 villages take part in the conservation scheme and that a system for rotating the breeding males among these villages is adopted.

When it is required to choose a group of animals with maximum variation, say for the establishment of a nucleus (4.4.1), then the procedure described in section 5.3 can be used to overcome the lack of pedigree.

▶· 4.7 A Note on the Use of Cryoconserved Material

How can frozen semen and embryos help a live animal conservation programme?

The first point that must be made is that it is not necessary to use cryoconservation to conduct a live animal conservation programme. However its use can help to secure the programme and to make its day to day operation easier. Chapter 5 describes how to obtain the samples.

4.7.1 Controlling genetic defects.

Genetic defects can spread quickly in a small population, particularly if the rate of inbreeding has not been properly maintained. Many are inherited as complete recessives and by the time these are observed there will generally be many carrier animals. Section 4.2.2 described how genetic defects may be controlled in a population. An additional tool that can be used is the use of cryoconserved semen or embryos from previous generations of the same breed as parents rather than the use of carriers in the present generation. Two caveats must be mentioned: the donors of cryoconserved material are likely to be ancestors; and, the donors may be carriers too, with samples taken when the defective gene was still at a low frequency. For both these problems it is useful to have pedigrees available. The first problem is part of the normal maintenance, avoiding the mating of close relatives (parent, grandparent in this case) and avoiding if possible excessive contributions from particular ancestors. To overcome the problem that the donors may have been

carriers, the pedigree should be analysed to assess the probability for each donor. (Computer aided techniques for analysis of complex pedigrees are available; seek advice from research institutes, FAO).

Cryoconserved semen and embryos may help the population through a severe bottle neck, providing the stored material comes from an adequate sample of the breed and pre-dates the origin of the bottle-neck. When the population is in a genetic melt-down, some old cryoconserved semen or embryos may be used to save it, very much like the use of animals from a different breed that was suggested in 4.2.4, providing the stored material pre-dates the origin of the melt-down and was from genetically fit animals.

4.7.2   Dispersed nucleus.

With dispersed nucleus schemes it is necessary to pass breeding material from unit to unit, since each unit may in itself only have a small number of animals. Where the distance between units is only walking distance, live animals may be moved. Over larger distances it may be possible to use fresh semen and AI. However greater flexibility in time and organization is obtained when using cryoconserved semen. This requires access to more sophisticated resources and requires further training, but it does allow semen to be obtained when convenient, and moved where it is necessary when it is necessary.

▶·   4.8   Expertise Required

The designing, implementation, maintenance, and adaptation of a specific conservation programmes to changing circumstances requires expertise of somebody with post graduate training in quantitative genetics. Such a person would also have much of the statistical expertise required. Depending on the species, further expertise may be required for the reproductive technologies that are possibly used in the conservation scheme. In the case of very small populations, the value of every animal is very high. Hence, the expertise of achieving a very good management and health care of the population is needed and a veterinarian may well combine this expertise with the expertise on reproductive technology.

The general principles of the day to day management of the animals and the recording procedures should be written down in a manual. Attention to detail, arising from good training is an important factor in the success of the project.

Further details of training and expertise are described in detail in Chapter 6.

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▶ 5. Cryoconservation

- *The basics*
- *Achievable objectives*
- *Choosing donor animals*
- *Overview of standards and procedures for collection*
- *Storage of samples*
- *Grading for health*
- *Numbers of samples*
- *Access and use*

Ex-situ conservation can be achieved by cryoconservation (of embryos, ova, semen, cells or DNA), alone or together with maintenance of animals *in vivo*. DNA is the simplest and most inexpensive of these to store and can be used as a source of single genes for animal improvement. However, it is insufficient alone as it cannot be used to obtain live individuals with more than just a few specific genes derived from the DNA sample (nor is it clear yet that this will ever be a practicable route). DNA is particularly valuable as an inexpensive research and training resource. Since research is essential to identify potentially useful breeds and genes, as a prelude to their exploitation, DNA should be preserved for this purpose.

For the near future, at least, cryoconservation will largely consist of cryoconservation of semen and embryos. Ideal conditions for the collection of good quality sperm or embryos occur when healthy donors, tested pathogen free, are kept in a disease free environment by technically competent people with the appropriate equipment. It is in the nature of conservation programs that for some breeds which are threatened by extinction, the collections will have to be done under less than ideal conditions in order to save the breed. This might be particularly true for developing countries. In such a conflict of interest between saving a gene pool and having pathogen free germplasm,

priority should be given to the conservation of such a gene pool. Therefore a graded approach to health requirements is needed.

Recent results (Wilmut *et al.*, 1997) have suggested that in the future it may be possible to produce a live animal from a stored somatic cell. This possibility is important since the protocols for collecting samples of somatic cells are less demanding than for collecting semen and embryos, yet it remains unclear if these samples will be effective in producing live animals. Consequently, the use of somatic cells for conservation now (i.e. in 1998) would be in anticipation of the scientific progress required to achieve the full objectives of cryoconservation. However the approach has enormous potential and the scientific progress will be closely monitored by FAO. Therefore, whilst the Guidelines recognize and discuss the possible use of somatic cells, the Guidelines **only** recommend their use subject to FAO approval of the project (see New Developments in Biotechnology and their Implications for the Conservation of Farm Animal Genetic Resources – Reversible DNA Quiescence and Somatic Cloning, Report of Workshop FAO 1989)

Therefore what is described in this Chapter are: an overview of cryoconservation; achievable objectives in conservation; obtaining stock for collection of material; an outline of facilities and techniques; an outline of maintenance and care of livestock during collection; grading of samples on health criteria; the numbers of samples required for achieving objectives; managing a genome-bank, including access and replenishment. The technical requirements for facilities, techniques, maintenance and care are demanding and detailed and are addressed in Annexes, nevertheless they are an *essential* component of this Chapter. Fig. 5.1 shows an outline of the decision pathways to obtain and store samples, whilst Fig 5.2 outlines the decision pathways for access and use of a genome-bank.

Figure 5.1: Conducting cryopreservation
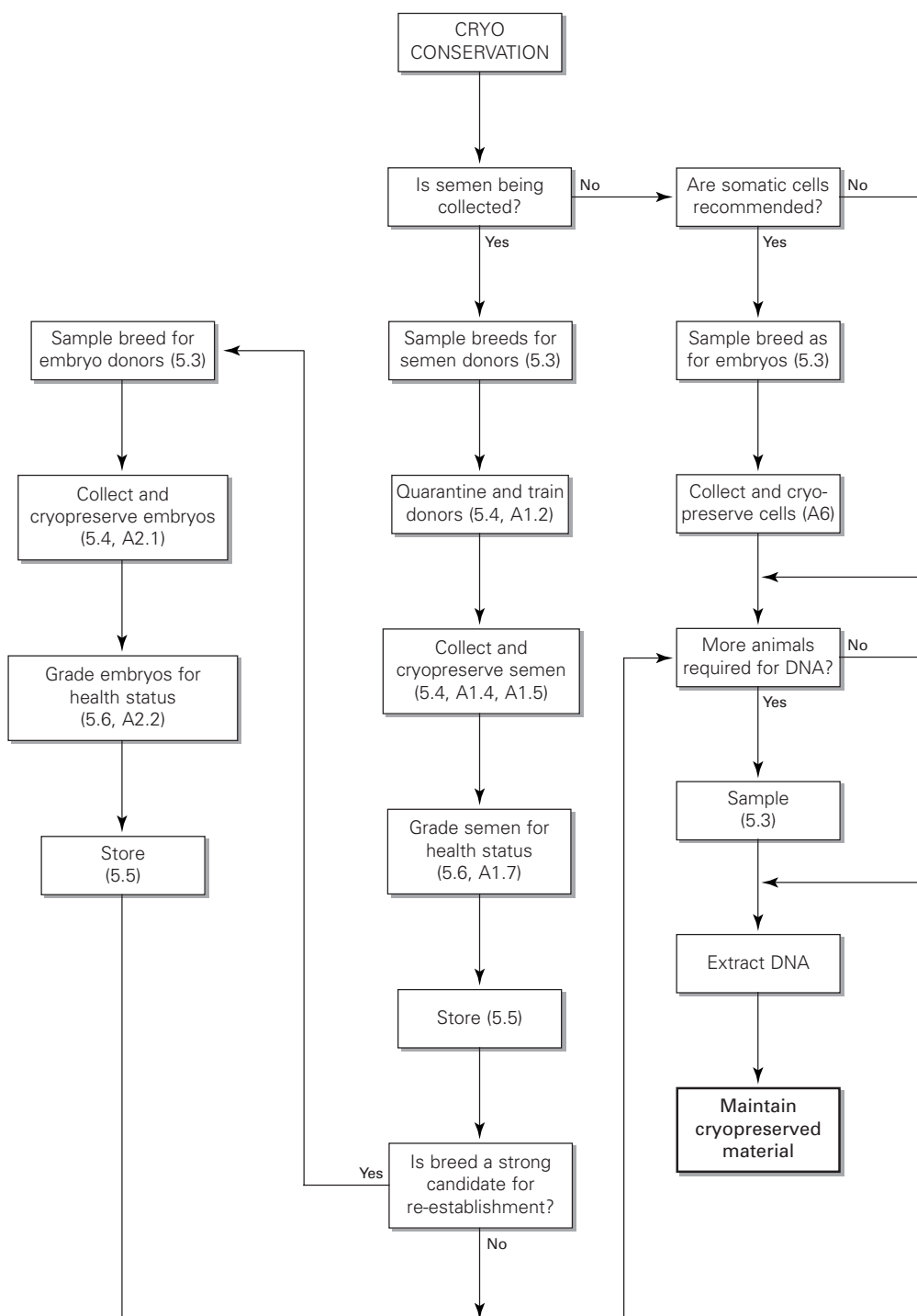
Figure 5.2: Suggested framework for access to genome bank
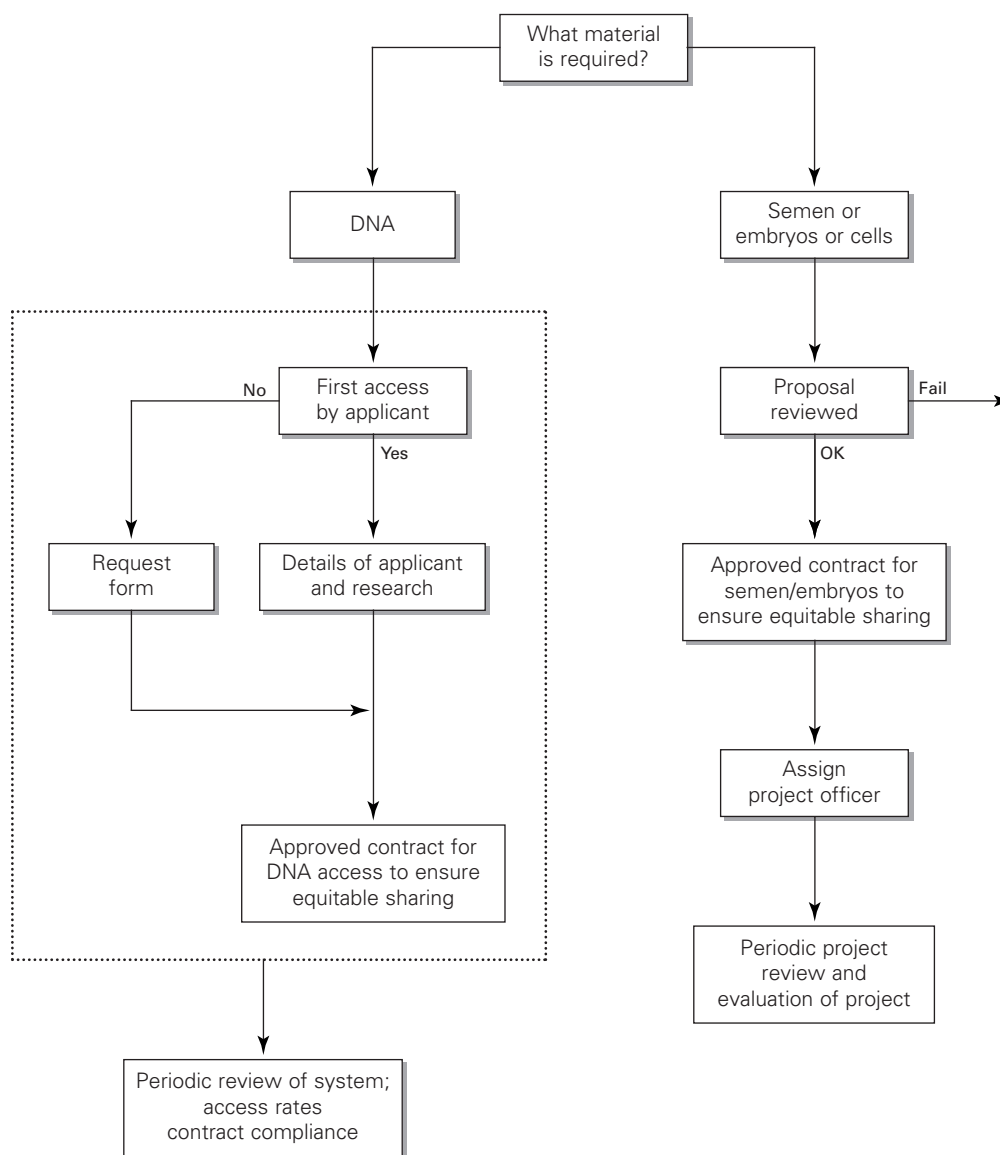
▶· 5.1 The Basics of Cryoconservation.

The long term storage of living cells is possible at very low temperatures, such as the boiling point of liquid nitrogen at -196^0C. Such low temperatures prevent enzymatic and chemical reactions to take place and a sample can be conserved almost indefinitely! It has been shown that semen frozen and stored 30 years ago can be used successfully in the preservation of endangered breeds.

Therefore the first requirement for cryoconservation is access to facilities for storage of samples in liquid nitrogen (LN_2) and the ability to replenish the storage containers regularly with LN_2. Liquid nitrogen is industrially produced in many countries, although sometimes at prohibitive prices.

With the present state of the art, only two types of biological material allow the reliable recreation of a living individual, sperm and embryos. Embryos are only feasible for mammalian species and can be obtained either by recovery *in vivo* or from an oocyte matured and fertilized *in vitro*. To use sperm and embryos after thawing will always necessitate a female of the same species, but not necessarily of the same breed, to be inseminated or to act as a recipient of an embryo. The techniques are not equally advanced in the different species, and Table 5.1 summarizes these differences.

5.1.1 Semen.

Semen can be obtained through sperm collection of sires by well-designed technical procedures widely used in the Artificial Insemination (AI) industry: with the help of an artificial vagina (AV) which is the preferred technique; or via electro-ejaculation (to be restricted to exceptional cases because of the poor quality of the ejaculates obtained and the welfare of the animals).

Table 5.1 State of the art in the freezing of gametes and embryos.
(+, routine technique available; 0, positive research results; -, not feasible in the present state of the art; ?, unknown; *, some research hypotheses).

Species	Semen	Oocytes	Embryos
Cattle	+	0	+
Buffalo	+	?	0/+
Goat	+	0	+
Sheep	+	0	+
Pigs	+	-	0
Horse	+	?	0
Lamoids/Camelids	0	?	?
Rabbit	+	0	+
Poultry	0	*	-

Each good ejaculate contains more spermatozoa than is necessary to achieve fertilization, allowing its dilution with physiological extenders (for the composition of these see Annexe 3), and its fractionation into several doses before preservation.

There is a large variability in sperm quality within each species and within individuals. Sperm quality is defined mainly by the following parameters: volume of the ejaculate, sperm density, motility, the occurrence of pathological forms and freezability (generally expressed by post-thaw motility). Sperm quality and output also depend considerably on the sexual behaviour of the donor animal at collection. In order to obtain maximum output a training period should precede routine collection (see Annexe 1). Indications of the average sperm quality, minimum requirements for the use in AI, as well as average number of doses per ejaculate and the frequency of collection for different species are given in Table 5.2.

5.1.2   Embryos recovered *in vivo.*

Embryos are generally obtained *in vivo* after flushing the genital tract of a donor animal using a physiological solution (generally phosphate buffered saline; PBS). This delicate procedure can be done non-surgically in some species such as cattle, buffalo and horses on standing animals, but requires a surgical approach in other species such as sheep, goats and pigs. It *always* requires a team of specialists.

In order to increase the number of embryos per recovery, females are stimulated with hormone preparations (superovulated) to produce more oocytes. Detailed explanations of the techniques available are presented in the FAO documents: No.77 for cattle, No.84 for buffalo, and No.115 for sheep and goats. Table 5.3 provides indications of the number of freezable embryos that can be obtained after one superovulation and recovery, as well as on the number and the frequencies of recoveries achievable for one donor female in one year. The success rate of obtaining an adult from the transfer of a frozen embryo is also given in Table 5.3.

5.1.3   Embryos produced from *in vitro* maturation and fertilization.

Oocytes can be matured *in vitro* (IVM) and fertilized *in vitro* (IVF). The resulting embryos can be used for rescuing endangered breeds. However, for the present, it seems too early to consider routine application of this technology in cryoconservation, but since decisive improvements in the technology can be expected in the near future a brief summary of the technique is given in the following. The basis of this technique is to produce embryos in the laboratory (*in vitro*) from immature oocytes that have been consecutively matured, fertilized and cultured, to a stage where they can either be transferred fresh or cryoconserved. There are two possible sources for immature oocytes:

Table 5.2   Efficiency of Sperm Cryopreservation

Species	Frequency of collection			Straws of frozen semen / collection		Straws / female	Fertility rate (a)	Straws/ pregnancy [or hatched egg] (g)	Pregnancies [or hatches eggs] / collection
				Volume (ml)	Number				
Cattle	1	3 days		0.25	150	1 / oestrus	0.50	2	75
Buffalo	3	week		0.25	60	2 / oestrus	0.40	5	12
Sheep	1	day	in season	0.25	40	1 / oestrus (c)	0.60 (c)	1.7	24
Goat	3	day	in season	0.25	30	1 / oestrus	0.60	1.7	18
Pig	1	5 days		0.25	50	12 / oestrus (d)	0.50	24	2.1
Rabbit	1 / day			0.50	10	1 /oestrus	0.50	2	5
Horse	3 / week			0.50	160	20 /oestrus (e)	0.40	50	3.2
Chicken	3 / week			0.25	5	4 /week (f)	0.42 (g	2.3 (h)	2.1
Turkey	2 / week			0.25	4	6 /week (f)	0.42 (g)	3.5.(h)	1.1
Duck	3 / week			0.25	4	4 /week (f)	0.42 (g)	2.3 (h)	1.7

(a)   Number of pregnancies / number of oestrus for mammals; low estimate

(b)   Full term pregnancy (mammals) or hatched eggs (birds)

(c)   Uterine insemination

(d)   2 inseminations per oestrus, 24 hours apart, with respectively 7 straws and 5 straws

(e)   1 to 3 (mean 2.5) insemination per oestrus, with 8 straws per insemination (see A3)

(f)   2 straws per insemination with 2 inseminations per week; 3 straws per insemination for turkeys (see A3)

(g)   3 hatched eggs from 7 laid eggs

(h)   4 laid eggs per week /female resulting in 1.71 hatched eggs per female per week

Table 5.3   Efficiency of Embryo Cryopreservation

Species	Freezable embryos / recovery		Embryos stored / dose (b)	Survival rates			Fertile offspring	
	Mean	Variance (a)		Pregnancy rates	Mean litter size	Fertile adults / neonate (c)	per embryo (d)	per dose
Cattle	4	1.2	1	0.40	1	0.85	0.34	0.34
Buffalo	2	1.2	1	0.30	1	0.75	0.22	0.22
Sheep	5	1.2	2	0.30	1.2	0.80	0.14	0.29
Goat	6	0.9	2	0.30	1.6	0.80	0.19	0.38
Rabbit	15	1.2	8	0.50	6	0.80	0.30	2.40

(a)   Expressed as the squared coefficient of variation for the number of freezable embryos recovered
(b)   One dose (straw) thawed at each transfer without further screening of the embryos
(c)   Includes perinatal and pre-puberal mortality
(d)   Product of the three previous columns divided by the number of embryos per dose: corresponds to the probability that a frozen embryo when thawed and transferred will produce an offspring that survives to breeding age.

► Slaughterhouses.

Ovaries from cows or heifers are recovered at slaughter and brought to the laboratory. The oocytes are aspirated from the follicles, subsequently matured, fertilized, cultured and transferred either fresh to a recipient, or frozen. This technique allows offspring to be obtained from casualties or old, or sterile cows. In the context of a conservation programme one might systematically recover all ovaries from females of an endangered breed which go for slaughter. The reason for slaughter is of little importance, even in the case of compulsory slaughter because of an epizooty, as it is still possible to produce disease free embryos, provided certain precautions are taken!

► Repeated follicular aspiration (ovum pick-up, OPU).

Repeated follicular aspiration is carried out *in vivo*, and two principal techniques have been developed: ultrasound-guided (cattle, buffalo, horses) and endoscopic (cattle, sheep, goats, pigs) follicular aspiration. Both these procedures have variations depending on the group using the technique. OPU does not require any hormonal treatment of the donor. The frequency of recovery can be much higher than for embryo collection after superovulation (up to 80 recoveries during one year in cattle compared to no more than 6 when embryos are collected). Oocyte collection has even been performed successfully in pregnant cows.

The efficiency of *in vitro* development after fertilization is currently low, as only about 30% of the oocytes develop to the morula or blastocyst stage where they are transferable or freezable and about 40% of the transferred embryos result in a live offspring. Even more critically for cryoconservation, the survival rate after freezing and thawing of cultured embryos is significantly lower than for embryos obtained

from *in vivo* recovery (20% as compared to 40%). However if the survival of a breed depends on only a few animals, the technique may aid the saving of the breed.

IVF embryos have also been produced in other species such as buffalo, sheep, goats, horses and, more recently, in pigs. More research is necessary to make the technique an efficient tool for conservation programmes. Developments may be expected.

5.1.4   DNA.

DNA is the molecule carrying the genetic information which will be transmitted to the next generation and as such is the means of heredity. This information is coded by stretches of DNA termed genes which can be identified, mapped onto segments of the chromosomes and isolated through genetic engineering.

The belief that stored DNA might one day allow the regeneration of live animals proved irresistible initially. Many were lured by the relative ease with which DNA could be both isolated and stored, not to mention the low cost involved; others by the prospect of using sophisticated technologies. However, in more recent years there has been a growing realisation that such beliefs will not be realized in the near future.

Consequently attention has shifted towards the transfer of segments of DNA from one individual to another. And although progress in the area has been considerable (various methods are routinely available for gene transfer into either the germline or somatic cells) much of the initial promise, especially for livestock species, has not been realised. Difficulties include the ability to regulate gene expression at the correct stage in development, in the correct tissues and at the correct dosage, and the lack of candidate genes for transfer. As many traits of interest are governed by numerous

genes rather than by a single gene, their cohesive regulation is complex and remains to be determined. How functionally related, yet disparate genes might be transferred into an individual and regulated in a manner compatible with other activities is still unclear.

Given that characterisation represents an integral part of conservation, one of the more immediate applications of DNA lies in its ability to determine the underlying genetic structure of populations. Various methodologies (restriction fragment length polymorphisms, minisatellites, microsatellites, direct sequencing, etc.) are routinely available to rapidly screen populations for genetic variation, providing a level of detail hitherto unimaginable. This knowledge of the partitioning of genetic variability has a role in making informed conservation decisions, and has already been used to set conservation priorities in natural species. Furthermore, such information can provide details on the levels of genetic admixture within a breed, or on the levels of introgression from other populations/breeds, thereby providing an indication of the level of genetic erosion through crossbreeding (Bradley *et al*, 1994). These uses are taken up in more detail in the accompanying FAO secondary document on the Characterization of AnGR.

5.1.5   Somatic cells.

Recent research has shown that DNA quiescence in somatic cells is reversible. The potential impact of this is clear: it *may* be possible to conserve a population simply by taking samples of somatic cells (skin or follicles) and cryoconserving them. This has been achieved experimentally from differentiated embryonic cells and cells originating from mammary tissue, but the limitations are as yet unknown. The potential impact of this is clear: it *may* be possible to conserve a population simply by taking samples of somatic cells (skin or follicles) and cryoconserving them. This has been achieved experimentally from differentiated embryonic

cells (Campbell *et al.*, 1996) and cells originating from mammary tissue (Wilmut *et al.*, 1997), but the limitations are as yet unknown. See A6.

In conclusion the principle tools for cryoconservation are cryoconserved semen and embryos, and DNA for characterization. Although other techniques may already be possible, reliable conservation is only possible when the success rates of techniques in field conditions are known and documented.

Future Prospects for Cryoconservation

Ongoing research in the biology of gametes and embryos will probably open new ways of recreating individuals from frozen material. The following are options:

Oocytes. First results in mice and rabbit indicate, that it might be possible in the future to generate offspring from deep-frozen oocytes with *in vitro* techniques. The reported efficiency rates are still very low, less than 10% of the frozen oocytes develop after fertilization and in-vitro culture to viable offspring. So far in domestic animals other than rabbits, no individual has been produced based on deep-frozen oocytes and the subsequent of IVM/IVF.

In situations where the survival of a breed depends on a few females only, freezing oocytes could be attempted from those animals which yield no good embryos. It may be possible to use these in the future, once the technology is available, in an IVM/IVF program, or for micro-injection of a sperm head (or even a non-mature sperm such as a spermatid) directly into the cytoplasm. This technique has now proven to be very successful in the mouse (Kimura and Yanagimachi, 1995).

Embryonic stem cells (ESC). These are undifferentiated cells which can be cultured *in vitro*. Today, such cell lines have been established in laboratory species and are being used intensively for generating transgenic animals carrying tailored mutations. The advantage of these cells is that they can be multiplied practically indefinitely. In the species where ESC have been identified, they are obtained relatively easily from cultured young embryos (inner cell mass of the blastocyst stage) or cultured gonads (primordial germ cells) and are routinely kept frozen before use. If the nucleus of these cells is introduced into an embryo at the beginning of its development they can influence the differentiation and the cell development in several tissues including the germ line. These cells are thus potential vectors for the transmission of genetic characters. However despite intensive research, there is today no convincing evidence of the existence of ESC in domestic species. If these cells could be isolated in domestic species, with a reasonable rate of success, then these cells would be an useful tool for the preservation of genetic diversity. A possible strategy would consist of keeping these cells in the frozen state, and after thawing use them as source of donor nuclei to recreate an individual after nuclear transfer into an enucleated recipient cytoplasm.

Spermatogonia. These cells reside within the basal layer of the seminiferous tubules of the testis and have the capacity to give rise to spermatozoa. Beginning before puberty and

continuing in the adult animal, spermatogonia undergo continuous replication, thereby maintaining their number in a process known as stem cell renewal. It has been shown recently in mice (Brinster and Zimmermann, 1994) that these stem cells, when isolated from testes of donor animals, can be processed to repopulate another testis without evidence of immuno-rejection. These cells could potentially be used to pass genetic material from one generation to the next, and when frozen it could be a means to store genes from a sire with impaired fertility because of anatomical or behavioural reasons.

►· 5.2 Achievable objectives from using cryoconserved material

What are realistic options when material has been cryoconserved?

The following sections consider what may be achieved by means of using cryoconserved stocks. Five objectives have been considered:

- re-establishing an extinct breed;

- new breed development;

- supporting populations conserved *in vivo*;

- research into identifying single genes of large effect;

- DNA studies.

Each of these objectives have differing requirements for cryoconserved stocks from the genome-bank. Therefore in order to develop a target for numbers of samples there is a need to develop a concept of how the genome-bank might be used. The approaches for determining the amount of material required given the target objective are described in Annex 5.

5.2.1   Re-establishing an extinct breed.

This may be required because of a unique set of characteristics that the breed had. However the resources of a genome-bank are limited to the re-establishment of a breed with only a moderate chance of success. This should not be surprising since: (i) cryoconservation of the breed was conducted when the breed was already in an endangered state; and (ii) increasing the numbers of individuals stored in order to increase the probability of success would increase the cost of the process of cryoconserving this one breed and, as a consequence, reduce the number of breeds, and breed diversity, conserved in total. As a result the re-established breed will need to be multiplied up before selection for desirable traits can be considered. The re-establishment may take two forms: ideally, from embryos in which a population of straight breeding males and females are immediately produced; or in a more cumbersome fashion, from semen alone in which several generations of backcrossing (i.e. grading up) are necessary before the population becomes straight-breeding (one or two backcrossed generations may be regarded as resulting in a form of creating a new breed, see 5.2.2). For reasons outlined below, re-establishment via embryos is considered to be very much more desirable than by semen.

Unless it is clear that breeding females (and males) of the breed are capable of a lifetime family size of more than 2 breeding individuals in the environment in which it will be re-established then it will be very rare that re-establishment by any means can be justified. This is a requirement because, otherwise, the re-established breed cannot be multiplied up and is ultimately doomed to re-extinction. Since fit populations, when given opportunities to expand, have a lifetime family size above this threshold, the probable cause for the lack of fitness is inbreeding depression. In these circumstances it is clear that new genes have to be

incorporated into the breed and, as a result, objectives need to be re-examined with a view to (i) a programme of backcrossing to a suitably chosen base breed and selection to restore fitness, and/or (ii) cryoconservation of semen for the use in new breeds. (See 4.1.3)

► **Achievable objectives relevant to both re-establishment via semen or embryos.**

The view has been taken that the re-establishment of an extinct breed with 12 males and 12 females will be a reasonable minimum objective. Such an objective should be achieved with 90% certainty. It is not worth embarking on an expensive task if the prospects of success were less. Twelve of each sex gives an effective population size of 24 per generation relative to the re-established base population assuming that the population were to be kept at a constant size with random selection and mating. However this need not be the case: a breed of sufficient importance to be re-established would be multiplied up and it would be reasonable to expect such a project to be required to record pedigree, which would give a more opportunity to control rates of inbreeding. With a constant number of parents and a replacement policy of son for sire and daughter for dam whenever possible, the effective population size would increase towards 48, a maximum for this number of parents. This is close to the 50 recommended in Chapter 4.

This objective has been considered as a minimum requirement but breeds with attributes that are particularly rare may be considered to merit larger reserves. However only procedures for achieving this minimum are given, but the detailed considerations presented are relevant for choosing strategies for other cases.

Further considerations for re-establishment via semen.

The re-established breed will never be totally free of the base breed used to provide initial females for the backcrossing process. Therefore an informed but otherwise arbitrary number of backcrosses has to be decided upon to arrive at some form of minimum requirement. Hill (1993) has shown how the number of generations of backcrossing will alter the mean and variance of the proportion of the genome coming from the cryoconserved breed. An achievable objective which takes account of this and other problems is to carry out 4 generations of backcrossing, since this number of backcrosses will give a 95% chance that 90% of the genome of the individuals produced will derive from the cryoconserved breed.

For a breed endangered by shortage of breeding males the prospect of re-establishment via cryoconserved semen is yet more limited. Unlike the case of re-establishment with embryos where the small number of males contribute half the genes, and the other half contributed by the females of the breed, when re-establishment is via semen the same number of males will contribute 15/16 of the total number of genes (the remaining 1/16 coming from the base breed). This inevitably increases the risks attached to the re-establishment process. Therefore the need for the breed must be very great indeed to attempt such an enterprise, and the inclusion of re-establishment via semen does not constitute a recommendation!

For a breed endangered by shortage of breeding females (F<1000) but not by the shortage of males then re-establishment by semen alone may be genetically more realistic depending upon the time scales for breeding each generation.

The base breed to be graded up should be chosen with characteristics amenable to this process, possibly phenotypically similar to the extinct breed for a range of traits. Known dominant alleles in the base breed can be removed easily by subsequent selection. Known recessive alleles in the base breed are more of a problem unless they have been tagged by a genetic marker, since carriers cannot be identified phenotypically and therefore only the homozygotes can be identified; however the frequency of occurrence will be low in the fully re-established breed. (See also 4.2.2).

A further important consideration for species with a low litter size occurs when the number of litters per female is restricted for some reason and the total number of female replacements per breeding female is less than 1, then the number of females required for the backcrossing process increases exponentially with the number of backcross generations. Where the number of female replacements bred per breeding female is larger than 1, the number of females required remains constant over generations. This means that the number of semen straws required increases either exponentially or linearly with the number of backcross generations depending on the replacement rate. This point was neglected by Lömker and Simon (1994) in their study of re-establishment costs. The replacement rate can be influenced simply by increasing the number of litters per female that are used (*all fit species must have females capable of producing their own replacement* i.e. a lifetime family size of at least 2, a male and a female). Therefore all re-establishment programmes should plan for sufficient litters to give a replacement rate ∃1; this has a penalty that the breeding programme may take marginally longer and the individuals produced may be more spread chronologically.

5.2.2   New breed development.

An important contribution of cryoconserved material is to continuing new breed development to fit new production circumstances. In this case the objective is to use the unique genes and gene combinations of a stored breed to establish a new population with desirable properties. The need for such a population may arise from: new selection objectives possibly due to changed environmental conditions or disease threats; or, to replace or improve a breed that is seen to be genetically inadequate maybe through excessive inbreeding depression, or lacking in mean performance or variation in desirable traits. The route perceived for this would be through the use of stored semen and through a number of generations of (back)crossing. Since the number of individuals that would be able to be restored from embryos of an endangered breed is limited, their use in this instance would not be warranted (unless cytoplasmic inheritance was very clearly implicated in the desired trait, and this is very rare). Semen from more than one stored breed may be used in order to provide variation in a range of characters for subsequent selection. The cryoconserved breed may provide genes to more than one new breed so that multiple use has been allowed for.

▶   Achievable objectives.

The method employed for developing a new breed, and the proportion of the genome of the new breed that is to be provided by the cryoconserved breed can vary considerably. To suggest the requirement for storage we have considered only one possibility which uses a high proportion of the cryoconserved breed. This example can be used as a model to estimate semen required when considering proposals to access the cryoconserved semen. In this hypothetical example a new breed is to be developed from a gene pool that initially has 75% of the genes derived from the cryoconserved breed,

and after the second generation of crossing, selection will take place. The requirement for selection is that there is to be 90% certainty that 100 males will be available as candidates for selection. It is anticipated that the same process would generate viable population of females for the new breed.

5.2.3   Supporting *in vivo* conservation.

The *in vivo* population may acquire lethal recessives over time. In a small population these may very quickly reach gene frequencies that are difficult to remove from the population. Therefore individuals that have been cryoconserved may aid the maintenance of the population through semen or embryos. In some cases, depending upon the extent of the inbreeding depression, semen may be sufficient to aid the removal of the recessive. Before introducing the cryoconserved semen, consideration should be given to the possibility that some of the cryoconserved individuals may also be carriers and should therefore be avoided. Available pedigree information may clarify the probabilities. (See 4.7.1)

► **Achievable objectives.**

The number of straws required for supportive breeding of conserved populations of live animals will depend on the size of the population. Assuming that the population has run into problems of lethal recessives, two generations of supportive breeding may be required, to be followed by a programme of selection against remaining carriers where identified. It will be assumed that 100 females are mated in each generation interval (including multiple litters). This is more than the minimum required to achieve an effective population size of 50 per generation (see 4.2.2) but does allow the possibility that such populations may require (or desire for reasons of selection) to sustain the population with a small number of breeding males. It will be assumed that the genome-bank

must provide sufficient semen to allow two generations of females to be mated with the cryoconserved stocks.

The regular availability of cryoconserved semen might alter decisions upon the structures of the nuclei of animals conserved alive (see 4.5, 4.7.2). Such an option will depend upon the existence of the necessary infra-structure at a national level and should be considered as a desirable but optional extra for *in vivo* conservation. It is not considered as a justification for inclusion as a component for determining numbers of doses.

5.2.4   Research into identifying single genes of large effect.

The inclusion of research into the identifying single genes of large effect as a legitimate claim on cryoconserved stocks is justified by the observation that the primary purpose of *ex-situ* conservation is to conserve a set of genes that may be valuable in the future. The initial objective of this research, often conducted in the form of genome mapping studies, is to tag such genes with genetic markers whose location within the genome is already known. This knowledge will lead to breeding programmes that can more efficiently utilize these genes, either directly through the use of the cryoconserved breed or from subsequent research in other breeds that builds upon the results of the mapping study. This activity will encourage the use of the conserved stocks.

▶   Achievable objectives.

The mapping studies considered utilize crosses between the cryoconserved breed and another breed. The hypothesis is that the cryoconserved breed is homozygous for a major gene with a large, beneficial effect that is not present (or only at a very low frequency) in other breeds. The other breed involved would be assumed not to carry this gene. The study first produces F1s and then F2s produced from within the F1 population. Alternative designs using both backcrosses would

require a heavier use of stored semen. To define the achievable objectives a 20 cM map is assumed, with 250 F2 progeny for measurement which, following the unpublished work of Haley (personal communication), gives a 90% chance for detecting a gene that is responsible for a difference of 1 phenotypic standard deviation between homozygotes. Since some important traits may be sex-limited, the requirement has been set to be 250 F2 progeny of a single sex. This can be achieved over time by 250 F1 females and sufficient semen should be allowed to achieve this number of F1 females with 90% certainty.

5.2.5 DNA studies.

A DNA bank would allow access to the nuclear DNA of the cryoconserved breeds without the need to remove or use the breeding materials. Given the small quantities of DNA required for research purposes relative to the amounts obtained in blood or semen samples (before storage) such requests should, in the absence of other considerations, be dealt with favourably on the submission of a well-considered proposal.

5.2.6 Achievable objectives for the genome-bank as a whole.

A view has to be taken on how many of these objectives should be anticipated.

The total requirement for embryos that will be catered for is the duplicate provision for the single re-establishment of a breed using embryos (assuming that the breed was deemed of sufficient importance for embryos to be recovered).

The total requirement for semen that will be catered for will allow for the duplicate provision of the following objectives given above:

- 1 x re-establishment of an extinct breed (5.2.1);

- 3 x creation of a new breed based upon a 3/4 bred (5.2.2);

- 1 x support for *in vivo* conservation (5.2.3);

- 1 x research into genes of large effect (5.2.4).

The relative weights given to item 5.2.2 reflects the foreseen usefulness of the global genome-bank and the difficulty of adequate replenishment following access for this purpose.

However the objectives 5.2.1 and 5.2.3 are not independent since if a population requires re-establishment there is no *in vivo* population requiring support and, providing adequate replenishment protocols are in place (see 5.8.4), the samples stored for supporting an *in vivo* population can be used for re-establishment in the event of a catastrophe. Anticipating the results of 5.7 (where it is shown that the demands of objective 5.2.3 are greater than those for 5.2.1), no provision will be made for re-establishing an extinct breed.

It is recommended that DNA be stored if at all possible, even if semen or embryos will not be collected.

▶· 5.3 Obtaining donor animals for cryoconservation

How many donor animals are required?

In order to determine the number of donor animals to enter a cryoconservation programme we have taken the view that *every* animal is valuable and has a utility, up to a certain limit. This limit has been taken as 25 males for semen collection and 25 males and 25 females for embryos or somatic cells. If less than this number is available then the animals are selected irrespective of the relationships between them. More than 25 is, of course, useful (although in some cases more animals does not necessarily mean more genetic variation if many of them are closely

related). However the maximum numbers considered here assume 25 of each sex. To obtain DNA then 50 individuals should be sampled (as recommended in MoDAD).

The same males can be used for both semen and embryo collection. The same individuals can be used for embryo collection, somatic cells and DNA. For DNA it is recommended that if there is less than 25 individuals of each sex available then extra individuals from the other sex should be sampled to make the total number of DNA samples stored up to 50.

It may be that there are more than 25 candidates available for use and some selection is required. Assuming that all appear fertile, selection may be carried out to reduce relationships among the donors and hence to maximize genetic variation in the samples. In many cases the depth of pedigree available will only be one generation. It is assumed that the majority of relationships arise through the high reproductive rate of males. In the absence of more than 1 generation of pedigree the known relationships are half-sibs, full-sibs and paternal. The following is a protocol for sampling males, it is intended to be robust and simple rather than strictly optimal.

1. Classify the males to be sampled into sire lines.

2. In the first round of choices, take an individual from each sire line. Where the line contains a sire and his sons then the sire should be chosen in the first instance (it is potentially less inbred). In this example this means that (4) is chosen in preference to either (8) or (9). In other lines a random choice can be made: using the example pedigree in the figure the result of the first round may be (6), (4) and (10)

3. If a further round of choices is required then select a further individual from each sire line where available. However a full-sib of a previously chosen individual should be avoided unless no other choice is available, so if (6) had been chosen in the first round, (5) would be favoured over (7). If the sire of a group of half-sibs was chosen in the

first round it should now be substituted for a pair of half-sibs (since the half-sibs are potentially more representative of the population), but not for two full-sibs. Continuing the example, the result at the end of the second round might be (5), (6), (8), (9), (10) and (12).

4.   Further rounds proceed taking individuals from each sire line if available and avoiding full-sibs where possible. (7) would be selected in a third round even though it already has a selected full-sib, since there are no other choices available within the sire line. The sire of other individuals should only be included when there are no other alternatives in that particular sire line. In the example (4) would be chosen in the third round. In the example, all available sires would have been chosen in the third round.

Where the sampling is for embryo production (or somatic cells) both donor males and females need to selected. In this case the above procedure should be followed; and most simply, sampling of male and female donors should proceed in a single process with the *less numerous sex sampled first*. The same principles regarding the avoidance of full-sibs and parent-offspring pairs should be followed. Dams should initially be selected in preference to their daughters since they may be expected to be less inbred and are less likely to have been sired by the available males; in subsequent rounds of selection the dam should not be substituted for two full-sibs (cf. item (iii) above).

The population may have pedigree with a depth of two or more generations which would include the grandsires of all the available individuals. If this is the case then it is possible to use relatively sophisticated procedures for maximizing the genetic variation in a sample. This may require expertise that is unavailable locally, if so expertise may be made available through FAO. However if the situation is an emergency the procedure outlined above may be adopted as robust and near optimum.

Example
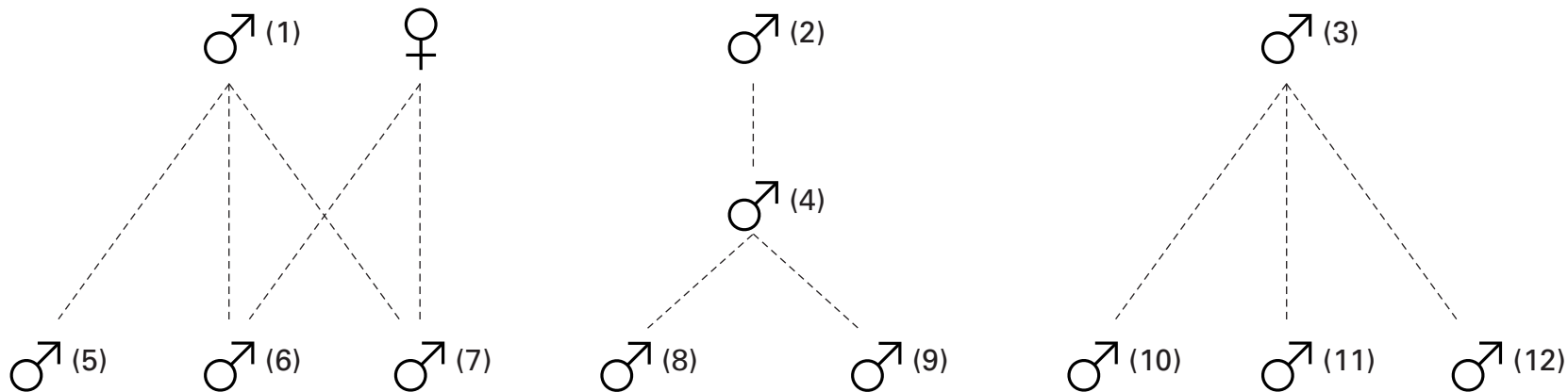
Sires ♂ (1), ♂ (2) and ♂ (3) are assumed to be unavailable and the remainder available.

► · ### 5.4   Overview of standards and procedures

What is involved in collecting samples?

This part of the process is technically and operationally demanding, both in the handling and care of the animals but also in the procedures for controlling the risks of disease associated with using the cryoconserved material, and this section only provides an overview for brevity. However it is *essential* that the details are addressed once this overview has been read. These details are given in Annexes A1 (collection of semen), A2 (collection of embryos), A3 (step-by-step techniques for cryoconserving semen, and A4 (step-by-step techniques for cryoconserving embryos). Collection of DNA is described in Annex 5.

The direct disease risk related to embryos, although being low, depends very much on the correct handling of the embryos by the embryo transfer team. This entrusts a very high responsibility to the team (i.e. the group of technicians, supervised by a team veterinarian, competent to perform the collection, processing and storage of embryos according to the conditions set out in Annexe A2). Given this high responsibility, and to ensure that the work is always done to the required high standard, it is recommended that a procedure for approving and officially recognizing these ET teams is introduced. FAO may provide such approval where no existing system of approval exists.

5.4.1   Facilities.

The health care and monitoring makes demands on the facilities used to obtain samples and it is not recommended that semen collection occurs on farms because of the lack of control of the health status, although this may be done with embryos.

Facilities that comply with international standards for semen collection are, ideally: a quarantine station, and a semen

collection centre (SCC). The quarantine station and the SCC has to be used exclusively for semen production. Adequate provisions should be made to avoid contact with other animals (e.g. fences) and pathogens being brought into the centre through other means (by provision of disinfection mats and baths etc.).

Facilities for embryo collection are either a permanent or mobile laboratory, appropriately equipped, where embryos can be examined, processed and packed. The mobile laboratory allows collection of embryos on farm.

5.4.2   Procedures for health monitoring.

In commercial embryo transfer and artificial insemination programmes utmost care has to be taken to avoid transmitting of diseases. Good quality samples are also important since these techniques are being used as a component of conservation programs and the semen or embryos are to be used after many years of storage.

► Semen.

Health procedures are carried out at both quarantine station and SCC. The quarantine station is considered the "interface" between the herd(s) or flock(s) of origin and the SCC unit. Procedures will involve the local veterinary authorities who will have to implement the proposed regulations, approve the facilities and supervise compliance with the procedures, in order to be able to certify the health status (see **5.6**) of the material. An important reference for this is the OIE Code. Particular attention has to be paid when bringing supplies, or feed to the SCC or when removing manure etc., to avoid the introduction of pathogens.

► Embryos.

The potential health risk can be high when the recommended procedures regarding collection and handling are not precisely followed. Abundant results from world wide research on the risks of disease transmission via embryos are available for the bovine. Less information is available for the ovine, caprine or porcine and is almost non existent for the other species. Any embryo collection has to be preceded by an extensive clinical examination of the donor animal, its herd or flock mates and the general environment in which the animals are kept for the presence of diseases. This clinical examination may also influence subsequent treatment for superovulation and recovery, as one can only expect good results from perfectly healthy animals. The disease risks may vary between species but this should not influence the level of attention.

5.4.3 Techniques for freezing and thawing of semen.

The technologies used today for the preservation of sperm follow the principles described by Milovanov in the early thirties:

1. prevention of cold shock to membranes during the lowering of temperature;
2. dilution of freshly collected semen in buffer solutions added with in most cases egg yolk or milk;
3. removal of accessory secretions in species such as buck, stallion and boar;
4. exposure to cryoprotective agents such as glycerol (Polge *et al.*, 1949)
5. rapid freezing in liquid nitrogen vapours
6. and thawing, rapid thawing in warm water bath.

A brief survey of the approaches used for the preservation of semen is presented in Weitze and Petzoldt (1992). As

mentioned above (5.1.1) artificial insemination is successful with a lower number of spermatozoa than required for natural mating, and semen is not only diluted but also fractionated before freezing such as in some species (cattle) several hundreds of doses can be obtained from one ejaculate. These doses can be frozen as pellets (Nagase and Niwa, 1964) but preferably in small plastic straws/paillettes (Cassou, 1965) of 0.5 ml or 0.25 ml volume. This type of packing allows for safe, hygienic and economic storage.

5.4.4   Techniques for the freezing and thawing of embryos.

Since the first reports of birth of normal offspring from cryoconserved mouse embryos in 1972 (Whittingham et al., 1972) similar successes have been reported in 15 mammalian species that include most of the domestic species with one exception the pig for which it was not yet possible to freeze embryos successfully. Embryos are generally frozen when they have 30-120 cells which corresponds to the morula or blastocyst stage which is reached about 4 to 10 days after fertilization depending on the species. They are today generally frozen in plastic straws (0.25 ml) similar to those used for semen. A recent summary of the methods and their applications is presented in Rall (1992).Because of the greater number of cells of embryos as compared to sperm cells, the freezing protocols are generally more sophisticated than those used to cryoconserve semen. Cellular properties often vary between species and between the stage of embryonic development. This requires the cryoconservation procedure to be adjusted to the species to minimize damage to the embryo and optimize survival rates. Several factors have been shown to be critical for the success of cryoconservation: the initial embryo quality estimated from morphology following examination with a stereo-microscope (magnification about 40), the time from embryo collection to the onset of freezing which should not exceed 3 to 4 hours (during which the embryos can be kept at room

temperature), and the stage of development at freezing which is critical in several species.

There are two main techniques for thawing and freezing embryos used today. The first, which is termed the >slow freezing technique= and is based upon a reversible dehydration of the cells that prevents the damaging effect of intracellular ice crystallization. The second, termed >vitrification= uses the rapid increase in the viscosity of solutions during freezing to obtain a glassy solid phase both inside and outside the cells, without formation of any ice crystals. The advantages of the vitrification technique are that it is a quick procedure which does not require special equipment, but it is technically more demanding, less robust and the fertility of the embryo is 10% less than the slow freezing technique.

Therefore the 'slow freezing technique' is recommended because of slightly better results and despite the fact that it requires a cooling machine (at a price of about 3000 to 7000 US$). These machines are available from a number of suppliers and can easily be adapted to work under field conditions. In the absence of a freezing machine or the funds to purchase one the vitrification technique is a valid technique particularly if the alternative is that the breed would become extinct otherwise.

5.4.5   Mating design during embryo recovery.

An important aspect of controlling the genetic quality of the sample of embryos is controlled during the period of collection. To obtain the required number of samples to achieve the objectives set out in 5.2, several recoveries may need to be carried out on each donor even with superovulation. Embryo recovery following superovulation is notoriously variable, for example in cattle the numbers of embryos ranging per recovery can easily range from 0 (the most likely number) to 40 with a mean of 5. Furthermore

females that do not respond well to superovulation in one recovery have a tendency not to respond well in the next.

Therefore it is important that the males used to produce the matings are not associated with the same female throughout the collection period, otherwise one sire will dominate others in the re-established breed. Furthermore, even though no overt selection is taking place, natural selection may be acting and there is a penalty arising from full-sibs: deleterious genes passed on by an individual of one sex penalizes not only its own future contribution to the population but also that of its healthy mate. Avoiding full-sibs whilst still maintaining total offspring numbers is spreading the risks (Woolliams, 1989).

Therefore the following procedure is recommended:

1. females are not mated to their son, sire or full-sib if it can possibly be avoided (this simply avoids a 25% inbred embryo which is predicted to be less viable)

2   after each recovery females and males are ranked according to the total number of transferable embryos they have produced over all recoveries, and in the next round of recoveries the male with the lowest number is mated to the female with the highest number, the male with the second lowest total is mated to the female with the second highest, and so on.

This variation in superovulated embryo recovery remains a problem for controlling offspring from the donor females but this is tackled in 5.7 and A6.

5.4.6   Collection and processing of DNA samples.

Although DNA can be extracted from many tissues it is recommended that DNA for cryoconservation be obtained from semen or blood. This is because these tissues are

relatively easily obtained (in the course of cryoconservation activities described above); and (iii) good yields of DNA are obtained per unit of sample. The processing of samples to extract DNA from blood (but not semen), and to store DNA is described in Annexe 5.

Where blood is used, 14ml samples are recommended for domestic mammals. For avian species, 1ml of blood is adequate since, unlike mammals, the red cells are nucleated as well as the white cells. The required volume of blood from a donor should be sampled into two labelled tubes, since this reduces the risk of losing all the sample from a donor prior to DNA extraction. Once the samples are obtained they may be stored at $4°C$ before extraction, but it is recommended the extraction is carried out within 3 days.

It is assumed that all pathogens which could be associated with the DNA are eliminated as part of the extraction process.

► · 5.5 Storage of samples

How are samples identified and stored?

5.5.1   Identification.

The increasing international exchange of frozen semen from cattle requires, for various reasons, a reliable identifier for each semen dose. In 1992 a European group of AI Specialists at the International Congress of Animal Reproduction and Artificial Insemination in The Hague (Netherlands) agreed on a common identification procedure for semen doses. This identification can easily be adapted to other species in adding two letters which indicate the species at the beginning of the identification number. The labelling is organized using an

alphanumeric code as shown in Fig. 5.3. Note that for health reasons (see 5.6) some additional information will have to be added. Such a labelling procedure is well adapted to the straws and is designed to minimize errors of identification. Technical devices for safe printing or for manual writing (ink that remains stable at low temperature) are available from specialized commercial companies. Generally embryos will be stored in individual straws for species with a single offspring per litter (such as cattle), or in groups of up to 10 in species with multiple offspring per litter (such as the rabbit). Precise recommendations for the identification of the straws of frozen embryos have been established by the Import/Export Committee of the International Embryo Transfer Society (manual of the IETS, 1998). Because of hand printing and space limitations, the labelling system contains only essential information for identification. It consists of an initial code number, freezing date, donor name (or number) when available, and an individual container number.

The initial code number is composed of three components: the embryo transfer organization code, the breed code and the donor registration number. The identification of the male used to produce the embryos is accessible from cross referencing data using the date of freezing. When space is available it should appear after the donor registration number. The freezing date is essential in matching up the labelling with the certificate of recovery and freezing that must be obtained and accompany each straw. An example of identification for cattle embryo is presented in Fig 5.4. Detailed information on identification and procedure for exchange of embryos are available from the International Embryo Transfer Society (IETS 1998).

Figure 5.3: Identification of semen doses

Printing on one line

| uniform bull code | | | | | | | |
stud code	breed code	bull code	bull name	ISO country code	Herdbook number	Date code	AI. Center code
3 n	2 α	5 n	24-30 α	3 α or 3 n	11 n	6-8 n	6-10 α n

Printing on two lines

	bull name		ISO country code		Herdbook number
	24-30 α		3 α or 3 n		11 n
3 n	2 α	5 n		6-10 α n	6-8 n
stud code	breed code	bull code		AI. Center code	Date code
	uniform bull code				

number of digits: total with spaces, 63-75
n = numeric; α = letter

5.5.2 Division of samples into two subsets.

An important aspect of conservation programmes is the risk reduction from storage of duplicate sets of samples in two geographically distant locations. This serves to protect the cryoconserved breed from disasters such as fire or earthquake, civil unrest, war or a simple accident. Once the samples have been collected and identified they may be divided into two near identical subsets for long-term storage.

► Embryos.

The division of the embryos obtained into two sets should follow the principle of dividing any pair of full-sibs between the two sets. When this principle can no longer be applied (i.e. embryos without a full-sib to pair with) the principle should be to divide any pair of maternal half-sibs between the two sets. When this principle can no longer be applied then paternal half-sib pairs should be divided. Finally any further embryos may be divided at random, but if further pedigree information were known then it would be desirable to take this into account. Equalizing female family sizes between duplicate sets is given priority in this procedure since there are opportunities to restore any imbalance in the male family sizes after re-establishment using stored semen.

► Semen, DNA or somatic cells.

Division of semen samples is more straightforward than with embryos: divide the straws from each semen donor into two sets. Any odd straws remaining are collected together and divided as equally as possibly between the two sets. Division of somatic cell samples and DNA samples into two sets follows the same principles as those for semen.

5.5.3. Location of samples.

After freezing, semen doses (which can be straws or pellets) are kept in gobelets in the liquid nitrogen tank. Each gobelet (various sizes are available on the market) contain only doses from one sire and one ejaculate. An appropriate recording system has to be developed to allow quick and reliable identification of an ejaculate of a given bull at a given date in the container.

For embryos, straws are placed into viso tubes of different colours and then in gobelets in the liquid nitrogen tank. As very often only a small number of straws per donor and per recovery will occur, the different colours of the viso-tubes within the gobelet represent either different recoveries or different donor animals. This procedure is necessary so as not to waste space in the LN_2 tank, although it requires an extremely accurate recording and documentation system to allow tracing a given straw without delay. Computerized programs for the management of the stock of doses are available from suppliers of LN_2 container and these are already widely used in human-tissue banks.

5.5.4 Containers.

Samples of semen and embryos can be transported in special double walled containers of about 10 to 50 litres. Semen storage containers exist in sizes from 2 litres to several thousand litres. For practical reasons, conservation programs should store the material in containers between 30 and 60 litres. This is because of the relatively small numbers of embryos per donor or semen doses per individual sire and per ejaculate, and also in view of the health classification of the samples (see 5.6). These containers have very low evaporation rates (provided of course that they are not opened frequently!) and they thus guarantee a satisfactory holding time of several months without replenishing the

LN$_2$. Furthermore in case of an unexpected container breakdown only limited damage would occur using small containers as compared to using big containers.

5.5.5 Maintaining storage.

Once stored in LN$_2$, semen and embryos must not be allowed to thaw until required for use, and then the appropriate procedures need to be followed (A3 for semen, and A4 for embryos). If accidental thawing does occur the semen and embryos will not be viable.

DNA is more robust than other materials. The temperature requirement for long-term storage is only -20^{0}C or less, so LN$_2$ is not needed. If samples are accidentally thawed, then they should be rescued and re-frozen as soon as possible.

▶· ## 5.6 Grading of health status for semen and embryos

What if the highest health standards cannot be guaranteed?

The compromises between the need to conserve the genes of an endangered breed and the health status of its last representatives and/or the availability of appropriate premises in the vicinity, has led the recommendation for the introduction of a grading system for semen and embryos according to the quality of the health monitoring carried out and the health of the donors. In view of the importance of this system the text is repeated in A1 and A2.

5.6.1 Grading mammalian semen.

This system grades the health status of the males in three categories, grades A (highest), B and C (lowest). This will facilitate storage of animals with similar health status but from different origins in the same genome-bank, and

promote future access and use. The principles are outlined in Table 5.4.

Table 5.4. Grading rules for semen samples from mammalian species.

	Compulsory requirements (A1.7.1)	Individual Health Testing (A1.7.2)	Approved quarantine and SCC (A1.2, A1.3)
Grade A	X	X	X
Grade B	X	X	
Grade C	X		

▶ Grade A health status of the sample.

To be graded 'A', the donor animal has to fulfil the entire chain of monitoring, starting with the quarantine station described in A1. Before transfer to an officially approved SCC sires have to be tested with negative results for the various diseases. Once in the SCC the sires have to pass the test for semen quality and freezability and will be kept under continuous veterinary control. It should be possible to move Grade A semen freely around the world with no restrictions, as the risk of transmitting diseases with this semen is extremely limited.

Semen doses have to be clearly identified as Grade A, for example by adding >GA.= at the end of the normal labelling of the straw (see 5.5.1, Fig 5.3).

▶ Grade 'B' health status of the sample.

To be graded 'B', the donor animal must comply with the compulsory requirements (see A1.7.1) and also to the individual health testing for the major diseases as indicated on Table A1.1.

Semen doses have to be clearly identified as Grade 'B', for example by adding >GB.= at the end of the normal labelling of the straw (see 5.5.1, Fig 5.3).

►   Grade 'C' health status of the donor.

All other samples are given Grade 'C'. They should however comply with the compulsory requirements in A1.7.1. It is obvious that semen classified Grade 'C' gives rather low security with regard to the disease risk. In emergency situations, when a given animal has to be immediately collected with no other test or indication, such collected semen is to be categorized 'C'.

If possible and provided sufficient material is available specific tests could be performed at a later stage. Obviously particular precautions will have to be taken when using these semen doses again

Semen doses have to be clearly identified as Grade 'C', for example by adding >GC.= at the end of the normal labelling of the straw (see 5.5.1, Fig 5.3).

5.6.2   Grading of avian semen.

Similar to what has been proposed for mammals, it is recommended to grade the semen doses according to the health standard of the donor birds.

►   Grade A health status of the donor.

Semen from birds that originate from flocks free of OIE list A diseases, which have gone through a quarantine station and have been tested free of these diseases and which have been collected in an approved SCC may be qualified as Grade A. Grade A semen from mammals and birds can be stored in

the same room but should not be stored in the same container.

▶ **Grade B** health status of the donor.

Birds that have not passed through a quarantine station and an appropriate SCC but which do test negative to the relevant health tests may be categorized as Grade B provided that their flock of origin was free of the OIE list A diseases. Semen from mammals and birds of the same category may be stored in the same place.

▶ **Grade C** health status of the donor.

In all other collection conditions semen must be Grade C. Semen from mammals and birds from this category may be stored in the same room also.

Again such information should be clearly stated on the containers (straws) and the accompanying documents with the same code as for mammals GA, GB, GC for grades A, B and C respectively.

5.6.3   Grading embryos.

As for semen, it may be advantageous to grade the embryo according to the health status of the donors. The grading will be A and B only.

▶ **Grade A** health status of the embryo.

Embryos categorized as Grade A will have been:

- derived from donors satisfying above health requirements
- collected by an FAO approved team, which
  - has expertise and competence,

- has followed the hygienic rules as set in the IETS Manual.

▶   Grade B health status of the embryo.

Embryos will be categorized Grade B:

- in cases where they have not been collected by an FAO approved team;

- or, if they have been collected by an FAO approved team, the veterinary health and/or other hygienic rules could not strictly be followed;

Such information should be clearly stated on the containers (straws) and the accompanying documents with the same code as for semen GA, GB, respectively for grades A and B (5.5.1, Fig. 5.4).

5.6.4   Consequences of grading on storage.

Grading of semen has consequences on the storage facilities and procedures.

▶   Semen.

Semen doses, once they are packed, are closed tightly and there is very little risk of spreading of disease directly from semen, but there is still a limited risk which arises from containers and during handling. Therefore it is recommended to store differently graded semen doses in distinct and separate rooms. Each room has to have its own equipment (forceps, basins etc.) which should be clearly marked and which should not be moved from one room to the other. Containers, gobelets and viso-tubes have to be thoroughly cleaned and disinfected before being transferred into one of the rooms.

Figure 5.4: Identification of embryo doses

Printing for straws

organization code	embryo code*	breed code	donor code
E + 3 n	2 n	2 α	7 n
	8 n	2 n	
	freezing date month/day/year	container number**	

Printing for vials or test tubes

organization code	E + 3 n
embryo code*	2 n
breed code	2 α
freezing date	6 n
container number**	2 n

* embryo code

stage of development	quality of embryos
1 = unfertilized	1 = excellent or good
2 = 2-12 cell	2 = fair
3 = early morula	3 = poor
4 = morula	4 = dead or degenerating
5 = early blastocyst	
6 = Blastocyst	
7 = Expanded blastocyst	
8 = hatched blastocyst	
9 = Expanding hatched blastocyst	

** container number
to distinguish between containers within a single collection

n = numeric; α = letter

Under practical conditions, one may presume that in countries where a basic AI structure exists semen graded 'A' and 'B' may need to be stored, while in other countries only semen graded 'B' and 'C' may need to be stored. It is considered quite unlikely that the need will arise to store 'A' and 'C', or 'A', 'B' and 'C'. The design of the SCC must plan for the range of options that will be required (see A1.5).

Samples from different countries but of the same Grade can be stored in the same room but always in different containers.

The question becomes somewhat more difficult with regard to the storage of duplicates. There is no problem envisaged with Grade 'A' as this type of semen is almost freely movable. Grade 'B' semen should be transferred to a country with similar general veterinary health conditions as the one prevailing in the country of origin.

More difficulties are envisaged with grade 'C' semen, for which a country with similar (low) veterinary health conditions but good, safe, reliable storage facilities will have to be found which will accept samples of this low grade. One cannot expect that a country with generally higher veterinary health standard will accept samples from countries with lower standards. This needs to be addressed by international organizations and governments.

The grading system should allow the highest flexibility in allowing collection from any donor which has been identified because of its genetic uniqueness, whilst giving on the other hand the maximum information to establish the necessary security measures to avoid spreading diseases when the samples are to be used.

▶   Embryos.

Similar storage considerations are relevant to the different grades of embryos.

▶·   5.7   Size of the samples to be frozen

How much semen and how many embryos are required to achieve the objectives set?

The question now arises: how many doses of frozen semen or how many frozen embryos have to be stored in order to achieve the duplicate provision for the objectives summarized in 5.2.6. Assumed success rates have been given in Tables 5.1 and 5.2 for semen and embryo cryoconservation respectively.

Given these success rates the size of samples required have been given for each species in Tables 5.5 and 5.6 for semen and embryos respectively. These numbers have been derived based upon simulations whose underlying principles are given in Annexe 6.

Some comments on these numbers should be made:

- These numbers are based upon a probability of success of 0.9 in achieving the desired sub-objective (i.e. 5.2.1 to 5.2.4) for any access to the genome-bank. This means that more samples are required than simply those estimated from multiplying up the various success rates. The latter is more likely to give a probability of success close to 0.5, which is a poor return on the investment in getting the samples and the efforts that will be required to use them. It is no good re-establishing a breed only to find that it has a severe bottleneck in males or females in the very first generation!

- It is not recommended to reduce the numbers of samples required because of the belief that it is possible to achieve a much better success rate in the procedures for a particular breed. It is very unlikely that the parameters have been sufficiently well established in a breed at risk to justify deviations from the assumed values.

- The number of semen doses required assumes that females used have the necessary number of litters to produce one male and one female surviving past puberty. This avoids the problems encountered by Lömker and Simon (1994) where numbers of females and pregnancies required in backcrossing to produce a fixed number of offspring at the end of the process increased with the number of generations of backcrossing.

- The number of semen doses per male and hence the collection periods in Table 5.5 are based upon the gathering of 25 males for AI and the realization that only 20 (i.e. 80%)of these are fertile with freezable semen. The total number of doses required will remain the same irrespective of the number of males; therefore with more fertile males with freezable semen the number of doses per male and the collection period will decrease, with fewer fertile males with freezable semen the number of doses per male and the collection period will increase.

- Reviewing the number of doses of semen required for each criteria was approximately 1:5:2:2 for 5.2.1 to 5.2.4 respectively, for all species. Following the observation that there is no need to store semen both for re-establishment and supporting *in vivo* conservation, the requirements for re-establishment are not included in Table 5.5. For all species, approximately 60% of the semen stored is intended for the development of new breeds.

- The criteria for embryos are difficult because of the variation in numbers of embryos recovered and the desire to ensure that sufficient numbers of male and female parents are represented

in the cryoconserved samples. Furthermore it is very costly having an approved ET team engaged over a long period of time trying to obtain a given number of embryos per donor female. Therefore a two-step approach has been taken:

1. to carry out sufficient recoveries to obtain enough embryos to make it 90% certain that each duplicate set of embryos will produce a total of 12 fertile males and 12 fertile females;

2. a further number of recoveries are then carried out (if more are necessary) to make it 90% certain that a subset of 12 fertile males and 12 fertile females can be identified with a special property; this property is that the distribution of family sizes for this subset has a particular characteristic (which is of genetic importance) that is better than if they had been randomly produced by 12 male and 12 female parents. This means that the population is, genetically, in better shape than if random mating of just 12 males and 12 females had produced the re-established population. Clearly care needs to be taken after re-establishment to ensure that such subsets are used wisely in producing future generations.

As a result the criteria give a minimum *total* number of embryos that must be obtained irrespective of the number of recoveries, and a minimum number of recoveries per donor female.

- Table 5.6 gives the necessary number of recoveries assuming either 20, 25 or 30 of each sex had been gathered for embryo recovery. Other numbers can be used and, in general, as more parents are used then fewer recoveries are required.

Table 5.5. Number of Doses of Frozen Semen Required for Cryoconservation of an Endangered Breed.

Species	Straws/ pregnancy or hatched egg (a)	Neonates/ pregnancy or clutch	Fertile adults/ neonate (b)	Breeding female offspring / pregnancy or clutch	Straws of frozen semen required (c)	Requirements per male (d)		
						Number of doses	Number of Collections	Time (days)
Cattle	2	1	0.85	0.43	10750	538	4	11
Buffalo	5	1	0.75	0.38	30960	1548	26	60
Sheep	1.7	1.3	0.80	0.52	7956	398	10	10
Goat	1.7	1.8	0.80	0.72	5396	270	9	3
Pig	24	6	0.90	2.70	24696	1235	25	123
Rabbit	2	8	0.80	3.20	2004	100	10	10
Horse	50	1	0.80	0.40	285600	14280	90	208
Chicken	2.3	5.1 (e)	0.85	4.34	6544	327	66	153
Turkey	3.5	5.1 (e)	0.80	4.08	9816	491	123	429
Duck	2.3	5.1 (e)	0.80	4.08	6544	327	82	191

(a)   See Table 5.2
(b)   Includes perinatal and pre-pubertal mortality.
(c)   Includes duplicate provision and covers all objectives assigned to the genome-bank; see 5.2.
(d)   For 20 males of proven fertility and with freezable sperm; assumed to be the result from gathering 25 males for testing; see 5.7.
(e)   For an estimate of an egg laid each day for 4 days and followed by a 3 day rest, for a period of 3 weeks.

There is large variation across species in the number of straws per insemination, from 1 in cattle to 8 in horses according to Table 5.2. Doses in the column 'Number of Doses' under 'Requirements per male' is defined as the set of straws needed per insemination.

Table 5.6 Number of doses for frozen embryos required for cryoconservation of an endangered breed

Species	Numbers of male and female parents (a)	Minimum number of embryos in total (b)	Minimum number of recoveries / female (c)	Embryos / dose	Doses required (d)
Cattle	20,20	206	4	1	320
	25,25	206	3	1	300
	30,30	206	3	1	360
Buffalo	20,20	318	10	1	400
	25,25	318	8	1	400
	30,30	318	7	1	420
Sheep	20,20	516	7	2	350
	25,25	516	6	2	375
	30,30	516	5	2	375
Goat	20,20	376	4	2	240
	25,25	376	4	2	300
	30,30	376	3	2	270
Rabbit	20,20	236	2	8	75
	25,25	236	1	8	50
	30,30	236	1	8	60

(a) The number of male and females that are initially gathered for embryo transfer; subsequent infertility in 20% of the males and 15% of the females is expected and accounted for (see **A7.2**). It is recommended that the highest numbers of parents are used whenever possible.

(b) Minimum number of embryos to be 90% certain of obtaining at least 12 breeding individuals of each sex after transferring half of them (to account for duplicate storage). These must be obtained irrespective of the number of recoveries.

(c) Minimum number of recoveries to ensure adequate representation of parents in surviving offspring. These recoveries should be carried out irrespective of the number of embryos.

(d) This is the product of the mean embryos per recovery, the minimum number of recoveries per female and the number of females divided by the number of embryos per dose.

The 'Minimum number of embryos in total' were derived using a function based on the assumptions in Table 5.3, and discounting for the decline in subsequent collections. In applications take care to check that fertility and embryo recovery rates from repeated operations are similar to those being realised in the particular species and field. If the tabular figures are not being achieved, the number of females used must be increased to compensate.

However, the minimum number of recoveries required depends, most closely, upon the minimum of the numbers of parents of each sex and not on the average number (i.e. if 10 males and 30 females are used, then the minimum number of recoveries required will be larger than if 20 males and 20 females are used).

▶· 5.8   Access and use

What are the rules for using the genome-bank?

5.8.1   Legal framework for access to material stored.

There is a need for access to the genome-bank to be placed into a framework that takes account of legislation surrounding the Convention on Biological Diversity and commercial activities. Such aspects include the need to consider the rights of the legal owner, and the country of origin over material stored in the genome-bank, the directive of the CBD that the benefits of the genome-bank be shared. Access and use of the genome-bank may lead to further patent applications and intellectual property and the basic principles regarding their ownership needs to be defined.

Given the anticipated lifetime of the genome-banks in cryoconservation, it may be that the owners of the animals that were used as donors, or their heirs may not be traceable. Even if the samples were owned by a Breeders Association, the breed may have become extinct after the samples were obtained and the Breeders Association will no longer exist. In cases of doubt the ownership will reside with the country of origin as set out in the Convention on Biological Diversity. However it would be advisable for FAO to lay out standard (or default) terms and conditions for the legal rights associated with cryoconserved samples to help clarify the

issues, and to stimulate others to reach appropriate agreements before any problems arise.

5.8.2   Procedures for access to breeding materials.

A request for access to the breeding materials must undergo a process of review. This reviewing procedure should include a population geneticist and reproductive biologist, and where appropriate, (for example in the case of a national breeding programme or a commercial programme) an agronomist and economist. All proposals need to establish:

- why there is a need to access stocks (this may need to include information on the structure, effective size and performance of existing populations);

- where a choice exists, why straws of semen or embryos have been requested; the number of items requested;

- the competence of the technicians to carry out the breeding plan;

- the protocol for replenishing the cryoconserved stock;

- opportunities for identifying genes of large effect (see 5.2.4, this last point is not essential but it is a clear means of equitably sharing the benefits of the genome-bank with donors and with other participating countries).

Longer term proposals must further establish:

- the compatibility of the proposed programme with Chapter 4;

- the predicted environmental impact of the population (for example, any impact upon stocking densities) and the adaptation of the breed to the proposed environment;

- the acceptability of the proposed population to the intended human hosts assuming the project is successful.

Proposals for supporting *in vivo* conservation should also consider the feasibility of obtaining markers for lethal recessives that may be segregating.

The justification for the number of semen straws or embryos requested, or the competence of technicians to carry this out, should be done with reference to the parameters assumed in Tables 5.2 and 5.3 (e.g. if a success rate of 0.3 is assumed for embryo transfer, does the field experience of the technicians show that they can achieve this success rate?) and whether they are approved by some relevant body.

All proposals (commercial or otherwise) will require the agreement of the country of origin and legal agreements on intellectual property, licensing and patenting that may arise from the proposal. It is recommended that a standard agreement is worked out before any usage and agreed by all participating countries.

5.8.3 Procedures for studies using DNA only

The most frequent access to the genome-bank is likely to be for DNA. In this instance the objective is to encourage use in the furtherance of knowledge. However free access is not possible since there are issues of ownership and, as above, this has implications for intellectual and commercially exploitable properties arising from the use of the DNA. A model to achieve this kind of access may be taken from the service for radio-immunoassay materials operated on behalf of National Institute for Health (NIH) in the USA. In this scheme various biological materials required for assays are provided to *bona fide* researchers at low cost. On the first application for use of the materials the researcher is required to give a short CV and brief details of the project for which the material is to be used (including sponsors). The researcher agrees to acknowledge the NIH in any research publication arising from use of the materials. To translate this to the present context the researcher must agree to abide

by certain terms and conditions arising from any intellectual and commercially exploitable property arising from use of the DNA as well as acknowledge the owner and the FAO in any publication. Once again it would be advisable to agree on standard (or default) conditions with participating countries in advance of any usage.

Such an access scheme for DNA could be run on a three year trial and reviewed. This review would have as part of its remit a follow-up on past requests to see the use made of the material and an assessment of the rate of depletion of DNA stocks, and would make recommendations upon any alterations required.

5.8.4   Replenishment and retention of stocks.

Access to the genome-bank will deplete the stock of material. There is a need to consider replenishment of stocks where possible. It might be anticipated that all breeds in the genome-bank will have moved further towards extinction and replacement (at least at the same genetic quality) will not be possible. For this reason there is a requirement for users to replenish stocks as far as possible.

Where embryos have been collected it is unlikely that the circumstances and the economics will allow more embryos to be collected than those required for the re-establishment of one small population and duplicated in two genome-banks. The fact that embryos were obtained is also a measure of the importance attached to the breed. Therefore it seems appropriate that each and every accession to the genome-bank should make plans and budget for the re-instatement of the same number of embryos into the genome-bank (over an agreed period).

In the case of breeds re-established from semen, then replenishment can be requested after the appropriate backcrossing stage has been reached. Where semen is being

used for the creation of composite populations then it is difficult to see how replenishment of the stocks can be achieved. The cryoconservation of the composite population might be undertaken if the composite is perceived to be failing to establish healthy population size, but the reasons for such a failure should be examined before doing so. If the composite is successful then replenishment may be unnecessary.

Where semen is used for supporting live populations then it is possible to replenish stocks. However where support of live populations is required due to the build up of deleterious genes in the live population, care must be taken not to introduce large numbers of these alleles into the genome-bank. The time of replenishment should be after recovery of population fitness.

Given the objectives of the genome-bank there does *not* appear to be any justification for the disposal of stocks that have not been considered for use over a long period of time.

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▶ 6. Organization, Communication and Training in Conservation

- *Organization*
- *Participants in conservation projects*
- *Monitoring and evaluation systems*
- *Communication*
- *Training*

▶ 6.1 Organization

Who owns what and who does what?

An effective conservation programme can only be well managed if the following are clearly defined:

- National interest and community involvement;
- Ownership of the animals and the products derived from them;
- Who is responsible for recording and selection;
- Ownership of, and access to, records;
- Basic standards of husbandry.

6.1.1 National sovereignty and community benefit.

The CBD states that the country of origin has sovereignty over its AnGR and consequently actions by stakeholders in conservation will need to ensure that they are in the national interest of the country of origin. This can be facilitated by sound national policies on AnGR designed to promote a suitable environment for conservation operations by both

public and private sector stakeholders. Successful conservation programmes will need to ensure that the communities owning and associated with the AnGR will perceive benefits both from the present and future generations of the conserved resources.

6.1.2   Ownership of animals and products.

Ownership of animals and products in live-animal conservation schemes raises no new issues. Any community and/or nation will have an existing system for deciding such issues; the fact that the animals are part of a conservation scheme is not in itself a factor. It may be that the keepers of the animals (person or organization) are keeping them in trust for the government. It is possible that, as part of the conservation scheme, owners may have agreed to certain conditions with third parties (governments, NGOs, Breed Associations) and issues arising from such agreements will need to be dealt with according to the contract law that applies. Ownership can have far reaching implications on selection decisions, the size and age structure of the participating herds, and the opportunities for >gene flow= in the live population.

Ownership of cryoconserved samples was dealt with specifically in 5.8, but it will be reiterated here. In some cases the owners of the samples can be identified, either an individual farmer or Breed Association, and in such cases no further issues arise. Agreements for access to and use of samples, and their replenishment will have been entered into at the time of storage and these will apply. In other cases, the breed may no longer exist as live animals or the original owners cannot be traced: in these cases the principle should be applied that ownership resides with the country of origin.

6.1.3   Responsibilities for recording and selection.

The recording and selection is a service which the owners either carry out individually or, by agreement, jointly with other stakeholders. A decision has to be reached regarding who in a country is willing and best placed for the implementation of such a project. Evidence of technical proficiency is important.

Good recording systems are essential for effective genome-banks (so that the genome-bank is correctly maintained and samples are located upon request). The DAD-IS system is developing a country secure databanking system for use in recording and monitoring genome-banks of AnGR.

6.1.4   Ownership and access to records.

The ownership of records will depend on whether they are taken by an agent or the owner. If the latter then ownership is straightforward. If the former, then the terms and conditions under which the agent is able to make use the records for the agent's own purpose should be agreed. An example is given in the box.

National Milk Records plc (NMR) is a milk recording company in the U.K. Farmers pay NMR to record aspects of performance in their herd. In the standard terms and conditions, the farmer retains ownership of records from his own animals and has the right to use this information as he wishes, including the right to give it to a third party. NMR may charge the farmer for any data extraction which is additional to the standard reports. NMR have the right to make use of the data, including charging for access to it by third parties, providing the farm of origin is not identifiable

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Countries will also need to clarify access to data on AnGR, just as they consider policy, based upon the national interest, in relation to the resources themselves.

6.1.5   Basic standards of husbandry.

It is desirable to make clear if there are basic standards of husbandry and management practices that need to be applied. This may be particularly important with *in-situ* conservation schemes where specific adaptations may be valued. It is possible that the gene combinations within the breed conferring the adaptation are not fixed throughout the breed, or the environmental benefit from the breed derives from specific grazing practices. Thus changing management may interfere with conservation objectives. It would be appropriate for the standards and procedures to be documented as an aid to ensuring standards are uniformly applied and understood.

▶·   ## 6.2   Participation in Conservation Projects

What kind of organizations are involved in conservation and what can they do?

6.2.1   Individual or private farmers.

The private farmers are responsible for the day to day care and maintenance of the animals. They may be responsible for recording and for the conduct of much of the conservation scheme. The farmers also control much of the environment of the breed. Individual farmers may provide information about breed origins.

6.2.2   Breed Associations.

Breed Associations such as co-operative breeding and herd-book associations may see it as their responsibility to maintain breeds. Support from breeders organizations is necessary for good survey information and to the general success of conservation schemes. As an organization they are

clearly interested in the well-being of the breed, and the profitability of its products. They may be responsible for much of the conduct of the *in vivo* conservation schemes and may organize cryoconservation. They form an important focus for communication, for maintaining information and preparing regular reports for Government (in order to assist it meet its national and international commitments).

6.2.3   The State

The overall responsibility and control for conservation of AnGR within the Government will generally be with the Ministry of Agriculture. Where the responsibility for biological diversity as a whole is vested in other ministerial portfolios, such as Environment and Natural Resources, then close liaison and co-ordination is necessary to realize effective policy, planning and operations. The Government influences conservation programmes through budget allocation, for example through incentives (see 3.3.6); they can mandate state farms to keep the breeds at risk besides the >money making= commercial flocks or herds in order to maintain national heritage.

Universities and Research Institutes as public institutions could be entrusted with the conservation of endangered breeds maintaining them as control populations and for research and teaching purposes.

Other important responsibilities for the state include developing national policies (as described in 6.1.1 and 6.1.3), co-ordinating national activities involving all stakeholders, providing funding and training, promoting linkages, and providing basic building blocks for regional and international collaboration.

6.2.4   The National Focal Point (NFP).

The NFP for AnGR will be an important partner and should be involved in facilitating the various steps of a conservation project ranging from project identification to the preparation and execution of the project. The NFP will have responsibility for developing and maintaining technical networks of people for each species and resource.

6.2.5   Non-governmental organizations (NGOs).

Non-governmental organizations (NGOs), may be conservationist or grass-root organizations, and are able to utilize added approaches to conservation work beyond those developed by governmental or research bodies. They are more flexible and may be capable of functioning more efficiently than governmental agencies. NGOs work with the grass-roots population, and therefore are effective at assisting farmers in keeping rare breeds, in raising public awareness and funding, the marketing and promoting of products, and in encouraging conservation of the cultural heritage. A Breed Association is one form of NGO. Of course, NGOs should be constituted to promote longer-term conservation capability.

6.2.6   Zoos or farm parks.

These may be a party to conservation projects, but mainly in developed countries, with a tourist industry. They give an opportunity for urban people to get an impression of livestock, and in view of increasing global urbanization, zoos and farm parks may get increasing importance.

6.2.7   Private companies.

Commercial breeding companies, processing companies and agricultural support services may become more interested and increasingly involved in conservation activities

(particularly pig and poultry businesses) in order to maintain the variation of breeds and the possibility to access these breeds easily when producing new founder lines. Private companies continue to seek additional genetic resources outside the company, and are likely to conserve genetic material that may hold future promise, and are responsible for research that directly benefits them.

Table 6.1   Types of organizations involved in conservation and possible contributions

Responsibilities	
State	**NGOs**
- Development of policy and legislation	- Public awareness
- Financial assistance and training	- Fund raising
- Co-ordination	- Technical assistance to breeders
- Research and development	- Maintaining records
- Public awareness	- Marketing of products
Private companies	**Private farmers**
- Research and development	- Provision of information on breeds
- Financial assistance	- Day-to-day care of animals
- Marketing of products	- Maintaining standards

▶· 6.3   Monitoring and evaluation systems

What routine monitoring should be carried out?

Monitoring and evaluation of projects are an integral part of practically every project and encompass two major areas:

- the administration and finance, which will depend of the organization providing the funding and framework;

- the scientific and technical area.

Regular in-depth technical evaluation is considered of critical importance to identify already at an early stage weaknesses in a conservation programme allowing to redress the situation in time. Once important genes or gene combinations are been lost or other factors such as i.e. inbreeding depression have occurred, it will be much more difficult to redress the situation.

There are two levels of monitoring and evaluation that may be considered: firstly regular reports, technical and administrative, which review progress towards the agreed objectives prepared by the organizers of the scheme either for internal use of for the use of sponsors; and secondly evaluation and review missions conducted on behalf of sponsors by internationally renowned experts, in collaboration with national institutions and experts. In internationally funded projects, and often in Government funded projects, regular evaluation missions are integral part of a project.

It is likely that technical reviews and progress reports have to be prepared at least annually to allow for timely implementation of corrective measures and revisions of a given project as new information occurs. The Project Manager Module in DAD-IS will assist in helping to monitor implementation and achievements of a given conservation project, and the Genome-banking Module of DAD-IS will provide important tools to facilitate monitoring and reporting on activities specific to cryoconservation. The review should include topics such as genetic merit and inbreeding as described in 4.5.2, but should also consider wider aspects such as the dynamics of ownership and herd/flock size, market conditions and trends, product development, and marketing. Survey results (2.7 and 2.8) should also be assessed.

An important outcome of the reviews should be the clear identification of tasks and clear time frames for their execution. Measurable parameters for the implementation have to be defined and landmarks for evaluation purposes have to be established. For example, this may involve identifying and obtaining a given number of nucleus animals within a given time; achieving the desired age structure or effective population

size by a given time; management standards (e.g. calving rates) to indicate whether the appropriate level of care is being attained.

It is important that: (i) these tasks and standards are based upon reasonable expectations of the keepers; (ii) for *in-situ* schemes, any management standards are appropriate to the breed and its environment of origin.

▶· **6.4   Communication**

Who needs to know what?

The general goal of communication of AnGR topics is to make a large number of people/organizations with different backgrounds aware of:

- the importance of AnGR for agricultural production;

- the present situation of AnGR and of the breeds at risk;

- what is happening in the field of AnGR management and conservation.

However the need for communication is not restricted to general topics. Any conservation scheme for a specific breed will benefit from the Breed Association or another stakeholder raising the awareness of local farmers, government and the general public as to:

- the benefits of the indigenous breed, and its cultural and agricultural role;

- the progress of the conservation scheme.

Breed Associations involved in conservation schemes would benefit from exchanging information on problems, successes and experiences with other organizations conducting schemes worldwide.

This increased awareness of AnGR, may result in more and more active involvement by a variety of stakeholders in the activities, and programmes for the better management of farm animal genetic resources. There is already evidence that public interest in conservation efforts has considerably increased with the progress of the information age. UNCED and other conferences on protection of the environment and the biodiversity have created and increased the state of awareness of the public about the fact that the breeds which have developed over thousands of years are part of our culture and heritage. The important message of conserving the broad animal gene pool, to be able to respond better to changes in the agriculture production systems, through changing environments and economics, and to meet the demand for food production of an ever growing population is better understood and accepted.

Building awareness of global AnGR encompasses: the principles of sound management; the relevance of AnGR; the status of AnGR worldwide; the strategic directions for improved management; the Global Strategy of FAO and the CBD.

6.4.1   Audiences.

Target audiences may be assigned to three strategic groupings reflecting the communication goal and objectives. These groupings and the target audience within each, are described below:

▶   Primary audience.

National Focal Point (NFP), potential donors and collaborators, Governments and policy makers, scientific and technical professionals, NGOs, mass media;

▶   Secondary.

Specialized media, international organizations (FAO, CBD, UNEP, IUCN etc.);

▶   Tertiary.

The general public.

6.4.2   Communication tools.

There are a large variety of communication tools available which can be used to get the message to the targeted group. Ranging from:

- hard copies such as scientific publications in referenced journals, articles in specialized journals, annual reports, briefing kits and the general press;

- electronic (Internet, Email, ftp, diskette, CD-ROM);

- conferences and workshops;

- virtual conferencing.

International organizations have a key role to play in assisting countries in their communication efforts, and to guarantee that, in particular, developing countries have appropriate access to the communication media. FAO provides countries, through DAD-IS, with an advanced communication tool which can assist countries in their communication efforts.

The NFP co-ordinates networks within countries, is the active link between the countries and the world. Therefore the NFP has the major responsibility for ensuring that information on AnGR in the country are disseminated widely both nationally and internationally. Given this responsibility, it is essential that (i) training is provided for the NFP to develop good communication skills, and (ii) the NFP has good access to the tools for communication.

► · 6.5 Training

What knowledge is required?

The CBD in its Articles 16 to 18 calls for access to and transfer of technology (Article 16); exchange of information relevant to the conservation, management and use of biological diversity; including information on research, training, surveys, and specialized knowledge (Article 17); and technical and scientific cooperation through, where necessary, appropriate international institutions, with special attention to capacity building (Article 18).

The development of sustainable conservation programmes is only possible if they are combined with the development of human resources and the building of institutions. Well-trained researchers and decision makers are critical for creating awareness of the problems and for the implementation of conservation programmes. The National Focal Point and the networks associated with the NFP need training in communication and presentation skills. Further training activities may include students, teachers, breeders, and administrators.

The most important task for the long-term improvement in the knowledge on animal genetic resources, will be to make sure that all major aspects of conservation of animal genetic resources are integrated into the regular university curriculum worldwide. Its natural setting would be in any Animal Science, Agriculture and Zoology course. A holistic view must be taken, covering both the sustainable utilization and the preservation aspects of conservation. More emphasis should be given to global and regional aspects of animal production, considering the importance of interaction effects between different genotypes and environments. A closer collaboration between countries, both developed and less developed, is suggested by extended exchange programmes for students as well as teachers (Malmfors et al., 1994).

It will be necessary to organize training courses for national administrators and heads of departments involved in policy decisions, the future decision-makers and facilitators of conservation programmes. The subject matter should give an appreciation of the importance of AnGR, and explain the major steps in documentation, conservation and improvement as described in these Guidelines.

The first effort, however, has to be made with students. In some developing countries, e.g. in Malaysia and India, Conservation Genetics has been included as one of the courses at undergraduate level. In Brazil (University of Brasilia) and the U.K. (University of Edinburgh and Wye College) have included conservation of AnGR as one of the courses offered to M.Sc. students.

Vangen and Mukherjee (1994) suggested that an integrated approach to teaching animal breeding and the genetics of conservation should be taken at both undergraduate and graduate levels, with graduate level teaching being the most relevant, as the understanding of the integration will be higher at that level. In the traditional teaching of animal breeding theory, assumptions are made which often limit the theory to almost infinite population sizes. It is of mutual interest to these two fields to further develop topics within animal breeding theory that deal particularly with breeding in small populations. The topics which need to be develop further in education are: measures of genetic variation; factors affecting genetic variation; choice of breeds for conservation programmes; measures of genetic distances between breeds; mating strategies in small populations; parameter estimation in small populations and the effects of heterozygosity and inbreeding. It is important to focus on the variability in production environments and the adaptive fitness of different populations to production environments, as they are of vital importance for a sustainable development.

According to Vangen and Mukherjee (1994), conservation genetics has been focussed mainly on preservation and storage of genetic material such as animals, semen, embryos and DNA storage which is more relevant to *ex-situ* conservation. However with emphasis now on *in-situ* as a preferred route of conservation, animal breeding theory, and in

particular aspects of that theory dealing with small populations, has become more relevant to conservation genetics. Nevertheless, it is important to put an emphasis on special aspects of conservation genetics in the teaching of animal breeding theory, otherwise it will become a separate discipline rather than being an integrated and relevant part of animal breeding theory.

6.5.1   Topics to be taught in higher education

Vangen and Mukherjee (1994) and Malmfors et al. (1994) presented two excellent papers on the Conservation Symposium during the 5th World Congress on Genetics Applied to Livestock Production held at Guelph in 1994. The following topics owe much to both papers.

► Global aspects of animal genetic diversity.

This topic should include evolution and the history of domestic species and breeds, the breed and population concepts, animal populations in various parts of the world and present development trends. Livestock production systems in various regions of the world, and the prospects and constraints as regards different animal populations in relation to environmental and socio-economic conditions.

► Measures of genetic variation:

Animal breeding theory normally uses o^2_A as the measure of genetic variation. The term expresses the variation in genetic values of quantitative traits. This term describes the total effect of all alleles on a certain trait. This measure of genetic variation has been a successful parameter in selection work. The relationship of o^2_A with other possible measures should also be examined.

▶   Factors affecting genetic variation.

Conservation genetics *in-situ* is often a matter of breeding work in small populations. Therefore, education in factors affecting the dynamics of genetic variation in small populations is of great importance. Topics such as linkage disequilibrium (Bulmer, 1971; Dempfle, 1989) induced by selection, changed genetic variance due to change in allele frequencies, and changed genetic variance due to limited numbers of animals are all important for all selection in small populations. The concept of rate of inbreeding (and hence effective population size) and its relation to the dynamics of genetic variance (and other measures) over time is important.

▶   Parameter estimation in small populations.

Factors affecting estimates of o^2_A and modifications of formulae predicting genetic progress necessary for small populations. Changes in confidence and interpretation of estimates when they are based upon all information from relatives in addition to the animal itself. Estimation of N_e, including methods where information on pedigrees is incomplete (e.g. Woolliams and Mantysaari, 1994).

▶   Effects of heterozygosity and inbreeding.

Inbreeding depression is a well-established phenomena in animal breeding. However, there has been little quantification of this negative effect of inbreeding (e.g. Weiner *et al.* 1994). Heterosis (hybrid vigour) also has some relevance to conservation (as described in 3).

▶   Risk in selection theory.

Theories of genetic contributions (Woolliams and Thompson, 1994) and the impact of different selection indices on rates of inbreeding, progress and the predictability of response are of critical importance to small populations. How different selection methods and systems of mating may be employed to control rates of inbreeding (i.e. rate of loss of genetic variation) are immediately relevant.

▶   Variability in production environments.

The traditional genotype x environment interaction questions are often neglected in the teaching of animal breeding even though they have played an important role in the pig and poultry breeding programs of developing countries. Variability in production environments on a world scale is much larger than assumed when discussing possibilities for interactions within a country or a region. To obtain sustainable development, the adaptive fitness to a production environment is important. What changes in specific traits associated with fitness take place when selecting in >local= breeds is an important question. The genetic architecture of production and functional traits in different species and breeds, including reproduction and disease resistance, and their relationships with production under different environmental conditions should also be taken into consideration.

▶   Characterization and documentation of animal populations.

In any programme aiming at conservation for future utilization, characterization and documentation of the stored material is extremely important. It is necessary to know the distribution and trait characteristics in relation to defined environments. It is also important to know how to organize and utilize databanks and

descriptors, as well as to monitor population changes and measure genetic distances between breeds (see Secondary Document on Characterization of AnGR).

▶ Methods for conservation of animal genetic resources.

1. *In-situ* conservation; breeding strategies for animal populations at risk under little or no genetic change where genetic drift is serious problem, resulting in the loss of genes; physical structure of the breeding populations.

2. *Ex-situ* conservation, *in vivo* and cryoconservation i.e. storage of frozen semen, embryos, oocytes, cell cultures or DNA, including objectives, methods of collection, sample sizes needed and record keeping.

▶ Reproductive biotechnology.

Training in reproductive biotechnology will allow countries to undertake programmes for *ex-situ* cryoconservation independently. Furthermore it would allow, through technology transfer, to take advantage of the extremely rapid development in the field of advanced biotechnology in developed countries.

▶ International trade with genetic material.

With the need to conserve genetic resources for the future, comes the importance of knowing and taking into account the international developments in trade of genetic material.

▶ Impediments to conservation.

Although techniques for conservation have been available for decades, conservation activity is still low. It is important to understand the barriers to achieving effective conservation programmes of all kinds and approaches towards overcoming them.

6.5.2   Training of farmers and extension workers.

Provision should be made for regular short courses in subjects related to conservation of AnGR for farmers and extension workers due to their critical role in conservation activities at local level. Particular attention should be paid to the unbiased and holistic evaluation of the local breeds and their production characteristics in the prevailing production systems and the environment. This will contribute to a greater acceptance of the conservation of indigenous breeds (see Chapters 2 and 3 where the importance of this is discussed in detail).

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▶ 7. Concluding Remarks

- *A Summary of the Guidelines*
- *Review*

▶· 7.1 A summary of the Guidelines

What are the main themes?

These guidelines stated the importance of the objectives for conservation of animal genetic resources. The objectives are not to conserve for the sake of conservation but for the economic, environmental, scientific, social and cultural benefits arising from the breeds. Further, the conservation activity has an important risk-reducing element in avoiding reliance on just a few breeds.

A step-by-step manual has been provided to assist countries and their stakeholders plan and act to conserve those breeds considered to have the potential for future contributions:

- The first step was to evaluate the present situation of the breed by population censuses and surveys. These reveal the population dynamics of breeds within each agro-ecosystem and aids decisions upon which breeds are at risk.

- Information obtained in the first step is essential for the second step, which aids decisions upon which breeds should be conserved and by which strategy. It is highly recommended that *in-situ* conservation should be the preferred conservation method and that cryoconservation should be used only after having exhausted all other conservation alternatives. *In-situ* conservation has the advantages that simple technologies are required to implement it, and that it allows the animals to adapt to changing environmental conditions and diseases, and the animals are readily available for expanded use.

- A component of the second step which needs to be addressed as part of the decision making process is to make a technical design for conservation strategies; addressing how many animals, how should they be maintained and (in cryoconservation) how many samples are needed. The main difference is between the *in vivo* and cryoconservation strategies which have been examined separately.

- The third step, which must be conducted in parallel with the second step, is to construct a thorough organization, communication and training plan for the activity. This addresses the ownership and responsibilities of stakeholders: private farmers, the state, NGOs and private companies.

- The fourth step is to turn the plan into action!

It may be that organized conservation of a particular breed may not go beyond the second step, since it is unlikely that funding and economics will allow for conservation of all breeds, and choices between breeds will have to be made. It is hoped that the process described here will maximize the chances of successfully conserving any breed and help in making the right choices. The logical step-by-step nature of these guidelines should also help to write well-founded and coherent project proposals that maximize the chances of attracting funding bodies.

►· 7.2   Review

Is this the last word?

No! The guidelines are intended to have sufficient generality to cover all livestock species, all conservation objectives, and options. However the actual implementation of a conservation programme will vary widely from case to case, and the present guidelines can only serve as a manual for planning rather than a blueprint for implementation. Their general nature might miss important points for conserving a breed of a specific

species, which may have wider relevance. Such points should be communicated to FAO.

The Guidelines presented must be viewed as the most recent version of a manual on how to set about managing farm animal genetic resources at risk. As more information accumulates on the conservation of AnGR, the decision aids will improve. Therefore the Guidelines *will* undergo periodic review.

▶ Annex 1 Health Surveillance and Regulations for Collection and Storage of Semen

- ■ *General aspects of cryoconserving semen*

- ■ *The Quarantine Station.*

- ■ *The Semen Collection Centre: General*

- ■ *The Semen Collection Centre: the Processing and Storage Unit.*

- ■ *Approval of the Facilities and Personnel.*

- ■ *Aspects Special to Conservation Programmes*

In commercial embryo transfer and artificial insemination programmes utmost care has to be taken to avoid transmitting of diseases. This is particular important when these techniques are been used as a component of conservation programs and the semen or embryos are to be used after many years of storage.

Cryoconservation of semen is possible for asses, buffaloes, camels, cattle, horses, goat, sheep and pigs. In avian species the technique is not so widespread; it works well and is widely used for turkeys, it may also be used for ducks, guinea fowls and hens (gallus domesticus).

The following text outlines the procedures for mammalian species and differences between mammalian and avian species are highlighted in text boxes. Initially the highest standards of surveillance are described but, in recognition that for some conservation programs these standards may not be attainable, less stringent conditions (and the consequences that follow as a result) are given.

► · A1.1 General Aspects of Cryoconserving Semen

What are the basic principles?

The need to produce disease free semen and to avoid contamination of the inseminated animals, is met if semen is collected:

- in an approved centre;

- in an hygienic way by technically trained and experienced people;

- under full control of the health status of the donor sires;

These requirements are described in detail in the OIE Animal Health Code, Appendices 4.2.1.1, 4.2.1.2, 4.2.2.1 and 4.2.2.2. The following are the key aspects which have to be observed:

- preliminary control of the health status of the donor males through a quarantine period in an adequate station to guarantee that only males with the required health status enter the Semen Collecting Centre (SCC).

- an adequately structured SCC.

- continuous control of the health status of males during the period of semen collection.

- correct management of the donor animals.

- satisfactory procedures of semen collection, dilution and handling in laboratory.

- adequate storage (for details see 5.5)

Principles for avian species.

The sanitary rules for bird semen follow the same logic as the one for mammals, which is to produce semen free of diseases which could be passed on to other females through artificial insemination. Available research data are scarce as compared to mammals. It is therefore recommended to follow the O I E guidelines in terms of exchanges of animals or gametes, section 1.5 of the International Animal Health Code.

Veterinary health control has to be done at three levels:

- flock of origin,

- a quarantine station,

- the Semen Collection Centre.

Veterinary approval starts at the flock of origin: (i) the birds must originate from flocks free of the two major infectious diseases listed in the O I E list A diseases, namely Fowl Plague and Newcastle Disease; (ii) in addition, the selected animals should be healthy and not acutely infected with any of the 13 OIE list B diseases.

▶· A1.2 The Quarantine Station

What are the specifications for the design, and what procedures take place there?

The quarantine station is considered the "interface" between the herd(s) or flocks of origin and the SCC unit. It acts as a recognized pre-entry isolation facility and therefore has to be officially approved by the local official veterinary authorities. A quarantine station must be set up exclusively for semen collection.

A1.2.1 Conditions for approval of the facilities.

The principle of a quarantine station relies on the following:

- it should be fenced and protected to prevent access from other animals. Access is only permitted to the personnel working in the unit such as herdsmen and laboratory personnel.

- the introduction of animals into the quarantine station should be done preferably in an all-in, all-out system, allowing for cleaning and disinfection between the arrival of two batches. If this is not possible and animals enter the quarantine station continuously, the quarantine period starts when the *last* animal has entered the unit.

- only authorized personnel are allowed to enter, and entrance should be through a sluice where they have to change clothes and shoes to avoid introducing pathogens from outside.

- the quarantine station should not be in an area where list A diseases, as listed in the OIE International Animal Health Code, are prevailing (see the definitions of zones according to diseases in the OIE Code, for example a radius of 10 km for Foot and Mouth Disease).

- all individuals should remain in the quarantine station for a period of at least 30 days, during which health testing and sampling should be performed (see Section A1.2.2).

The approval of a quarantine station by the official veterinary authorities must be based on the conditions listed above.

A1.2.2 Health testing.

All animals immediately after entering the quarantine station have to be tested for the diseases listed in Table A1.1. A second test should be performed shortly before moving the animals to the SCC. It is the responsibility of the local veterinary authorities to include other diseases to be tested for if the local situation requires, i.e. in certain areas it might be appropriate to test ruminants also against Blue Tongue. All animals have to be tested with a negative result (twice) before being transferred to the SCC.

Two further consequences arise from this procedure:

- wherever feasible animals should have undergone a preliminary test before entering the quarantine station to avoid contamination of the animals already in the station.

- animals which are tested positive should be culled without delay, and the quarantine period *starts again from day 0*. All the other animals in the quarantine station have to be tested again, once, to make sure that no infection has occurred.

A1.2.3 Domestication and training of the animals.

It is recommended that the quarantine period be used to domesticate animals and to train them for semen collection routine which will take place in the SCC. Training is particularly indicated for animals which were free ranging and which had only limited contact to humans. A training period will also have a positive influence on handling and semen output once the animal are transferred to the SCC.

Table A1.1 Minimum list of diseases for which males of mammalian species have to be tested before semen collection.

Species	Tub	Bruc	Trich	Campyl	Bruc. Ovis	Hog Chol	African Swine Fever	Pseudo Rabies	African Horse Sickness	C. Equ. Metritis
Ass									+	+
Cattle	+	+	+	+						
Buffalo	+	+	+	+						
Camel	+	+								
Goat	+	+								
Horses									+	+
Sheep	+	+			+					
Pigs		+?				+	+	+		

Tub = Tuberculosis, Bruc= Brucellosis, Trich. = Trichomoniasis, Campyl. = Campylobacteriosis, Bruc. Ovis = Brucellosis, contagious epididymitis, Hog Chol = Hog Cholera, Pseudo Rabies = Pseudo Rabies/Aujzesky disease, C. Equ Metritis = Contagious Equine Metritis.

Semen collection should be done with the artificial vagina (AV). After a short training period most of the males will accept the AV. Electro-ejaculation should be limited to very exceptional cases because of the poor quality of the ejaculates obtained and because of animal welfare reasons.

Advantage should be taken of the quarantine period to get sires used to a collection routine and to evaluate the semen quality by their spermiogram so as one can start immediately producing semen for storage purposes once the animal has entered the SCC.

A1.2.4 Movement of animals.

Once the testing and quarantine period is satisfactorily completed animals have to be transferred *directly* to the SCC. Transport should be done in appropriate and correctly disinfected trucks, avoiding any contact with other animals.

Quarantine for avian species.

Selected birds must be taken to a quarantine station. Quarantine period starts when the last bird has entered the station and lasts for at least 30 days. During this period, the animals are tested for list A diseases and at least for pathogenic strains of Salmonellas and Mycoplasmas. Routine semen collection may start after 30 days and once all animals inside the quarantine station have been tested twice negative for these pathogens.

In the event where some particular animals, because of the rarity of their genotype are to be collected but test positive to some of these diseases, they have to be located in a different site for semen collection and semen doses must be clearly marked according to the specific grading system put in place (see **A1.7**).

▶· ## A1.3 The Semen Collection Centre: General

What is a Semen Collection Centre?

As with the quarantine station, the SCC has to be used exclusively for semen production. It should be fenced and adequate provisions should be made to avoid pathogens being brought into the centre (by provision of disinfection mats and baths etc). Particular attention has to be paid when bringing supplies, or feed to the SCC or when removing manure etc. to avoid the introduction of pathogens.

Personnel movement should be limited to authorized people only and subject to special precautions. Movements of animals, goods and people have to be monitored and documented and have to be limited to the absolute necessary.

Only sires for semen collection which have satisfactorily completed the quarantine period are allowed in an SCC. All other animals which may, as an exception, be kept in an SCC have to satisfy the same quarantine requirements. If for one reason or another quarantine is not possible and sires have to be mixed, the grading of all would be automatically according to the lowest graded male in the group (grade 'B' or 'C'; see grading of donors later in this Annexe).

It is internationally agreed that SCC have to be separated in three distinct units which can be adjacent provided that adequate measures are taken to avoid movements from one unit to the other without taking the necessary precautions (an outline of an SCC is given by Thibier, 1993). These are:

- the animal housing and the semen collection room;

- the processing and storage unit (it is recommended to separate also these two parts, although the procedure to their entry is similar);

- administration and offices, which are of free access.

Ideally each unit is independent and is only accessed by people working in the respective unit.

The Semen Collection Centre (SCC) must be officially approved and subject to regular inspections at least twice a year by the Official Veterinary Authority.

Rules for handling and processing of bird semen are not as well developed as for mammals, and should follow those indicated above for Mammalian semen.

▶· **A1.4 The Semen Collection Centre: Animal Housing and the Collection Room**

How are these designed and what procedures take place?

A1.4.1 Animal housing.

To guarantee the production of good quality semen, prospective semen donors require good and balanced feeding, with particular attention to trace elements and vitamins. The preparation for semen collection requires that the animal is perfectly clean and dry to avoid contamination of the semen during the collection. This necessitates frequent changes of litter, regular brushing and, if necessary, regular showering and drying. Hair, particularly around the prepuce has to be kept reasonably short to avoid adherence of soil or dirt. Regular care of hoofs and daily exercise is recommended.

A1.4.2 The collection room.

The collection room should be spacious and exclusively used for this purpose of carrying out semen collections. For example, with bulls, a hexagonal shape with 10m length for each of the six sides is appropriate (seek advice from FAO for best practice in other species). Depending of the climatic condition, it can be open but it should be roofed to protect from direct sun or rain. The quality and type of surface is of extreme importance. It has to be easily to clean and to disinfect for hygienic reasons and should not be slippery as, in particular, bulls refuse to mate if they have no secure stand. Sand, for example, is inappropriate as it is difficult to disinfect and during the mounting process may splash and contaminate the Artificial Vagina (AV), whilst concrete may become slippery after frequent washing etc. One or two "stanchions" should be included to fix teaser bulls and other bulls waiting for collection.

The AV should be prepared in a separate but adjacent room within this sub-unit of the SCC and brought to the collection spot. This room should also be equipped to clean, sterilize and warm the AVs. It is preferable for semen collection to be carried out by the same operator who is well acquainted with the habits of the different sires.

Collected ejaculates have to be protected against cold shock and are transferred through a double window sluice to the semen processing laboratory (see A1.6). This sluice is the only semi-open communication between the collection room and the semen laboratory. The windows should be sliding so as no to aspirate dust too much when opening.

A semen collection record with dates, identification of the donor, and relevant details of the ejaculate accompanies the semen sample from collection to storage and is kept afterwards on file. In modern centres, electronic storage in addition to hard copies is possible, and desirable.

If donor animals could not be trained in the quarantine period and no spermiograms have been done these procedures should precede routine semen collection (see A1.2.4).

A1.4.3 Control of the health status during the semen collection period.

It is imperative that sires in a SCC are under permanent veterinary supervision. In addition to a compulsory clinical examination at collection, the following examinations and controls have to be done at least twice a year:

- clinical examination with particular reference to the reproductive organs;

- microscopic examination of semen and examination for presence of any somatic cells; in the event of any anomalies, a quantitative and qualitative microbacteria analysis should be carried out;

- diagnostic tests, depending on the species and veterinary health situation in the country and according to the recommendations of the OIE Code.

For the third item, the diagnostic tests, the following are required:

▶ Cattle

Intra-dermal tuberculin test; serological test for brucellosis; (where relevant) serological test for Blue Tongue; tests for Campylobacter and trichomoniasis either through direct microscopy or culture of these microorganisms from preputial washes;

►   Small Ruminants

Serological test for Brucellosis (Br. melitensis); intra-dermal tuberculin test for bucks only; serological test and semen culture for Brucellosis (Br. ovis) for rams only;

►   Swine:

Intra-dermal tuberculin test; serological test for Brucellosis (Br. suis); serological tests for Classical swine disease, African swine fever and Swine vesicular disease;

►   Horses:

Serological tests for Infectious Anaemia, African Horse Sickness, Equine Viral Arteritis; culture test for Contagious Equine Metritis.

Should any of the above tests or examinations show positive results, the animal concerned must be isolated and eliminated as quickly as possible from the Centre and the semen collected from it since the date of the last negative examination should be stored separately for further testing by the official veterinary laboratories. After testing of all the other animals semen collection may be resumed.

►·   A1.5 The Semen Collection Centre: the Processing and Storage Unit.

How are these designed and what procedures take place?

The semen processing and storage unit must have its own entrance and changing sluice for the personnel working in this unit.

The personnel of the unit will only work in this unit. Admission is only possible after changing clothing. It consists of four main rooms:

- semen processing laboratory

- glassware washing and disinfection room

- semen pre-storage room

- semen storage room

The design of the Processing and Storage Unit should be carried out after consideration of A1.7 below.

A1.5.1 Semen processing laboratory.

It is connected with the semen collection room through the sluice through which semen from the collection room is passed to the processing laboratory.

The laboratory has to be well lit bit direct sunshine has to be avoided. Furniture, walls and floors should be easy to clean and to disinfect. Regular (at least daily) cleaning and disinfection is a must.

The level of equipment should correspond to the number of doses which are produced in average per day. The market offers a wide range from simple, hand-driven equipment if only a few bulls are collected and only a couple of hundred doses are being produced per day, to sophisticated fully automatic, computer-driven straw printing, filling and sealing machines when more than 10,000 doses a day are being produced.

The personnel should be competent and well trained. Overall supervision should be the responsibility of a veterinarian.

A1.5.2 Glassware washing and disinfection room.

Adjacent to the laboratory it serves to wash the glassware used during the semen processing process. De-ionized water has to be available to rinse the glassware, as well as an oven to sterilize glassware, and a steam sterilizer to sterilize other items which cannot be put into the oven.

This room can also be used for interim storage of supplies needed for semen dilution.

A1.5.3 Semen pre-storage room.

This room is used to freeze the semen and to store it until final biological testing has been done and the required 30 day period has lapsed in which no disease in the donor animal has occurred. Transfer of the semen to the final storage room is only possible if the semen shows satisfactory quality after thawing and if disease has occurred in the donor animal in the 30 days following semen collection.

It is both safer and more convenient to store in one LN_2 container all the doses prepared in a given month for one full month (beware February!) after the end of this month and then to ship the doses together to the storage room. This ensures that there is no contamination of semen from a batch of semen later found to be inadequate. For example, doses collected in June are stored together and are kept throughout July before movement to the storage room; doses collected in July are kept in a separate container. However this requires two sets of containers: one for doses collected in January, March etc, and one for February, April etc.

A1.5.4 Semen storage room.

Room for the final storage of semen doses can be in an adjacent room which should be locked or it could be a separate building. Access to this room is to be restricted to

the authorized personnel only and the room should be at all times locked. Regular control of the containers with regard to LN$_2$ levels is necessary. Modern AI centres have an alarm system once LN$_2$ levels have dropped below a critical level.

Semen Collection Centre for avian species.

Once the birds have satisfactorily completed the quarantine period, they may be transferred to the Semen Collection Centre (SCC) *or* the quarantine station can be declared a SCC, provided that no other birds will be introduced.

Rules for handling and processing of birds's semen are not as well developed as for mammals. Nevertheless some basic rules apply:

- all manipulations have to be done in a perfectly clean environment in a hygienic way;

- manipulations have to be done by trained people with the appropriate equipment;

- facilities have to be designed so that they are easily cleaned and disinfected;

- a physical separation of the various units (similar what has been described for mammals) should be observed (stalls, semen collection, semen processing and storage, general administration);

- access to the various units is limited to authorized people only;

- semen from the pre-storage room may only be transferred to the final storage after 30 days provided that no diseases have occurred during this time;

- it is recommended to use the same labeling of the straws as proposed for mammals.

Quarantine station and SCC have to be officially approved by the local veterinary authorities and further approved by FAO to become officially part of the program. The conditions for such approval follow the same rules as indicated above for Mammal semen.

► · ## A1.6 Approval of the Facilities and Personnel.

Who gives official international approval?

The quality of the semen collection, processing and storage facilities as well as the handling of the animals and the samples are decisive. Future users have to be confident that the samples have been obtained processed and stored according to the before mentioned conditions and that the information given are correct. In order to give full credibility to the proposed system an official accreditation is considered necessary if this is not already done e.g. as in the EU. This should be done by an organization that is appropriately qualified and internationally recognized. The accrediting body carries a very high responsibility.

This official approval has to based upon a well documented report from the official national veterinarian authorities describing the premises and ways of operations, including the personnel involved. The country has to comply with OIE recommendations with regard to notification of disease outbreaks and specific regulations for health testing. A site visit might be necessary to secure that the conditions are met particularly if the reports are not fully satisfactory. Such an approval is a key point for further health grading of semen as described below and consequently for the wide acceptance of the banked semen for use in the future. In addition, the diagnostic tests that will have been performed should comply with the recommendations of the OIE Diagnostic Manual, and such points should be indicated on the report for approval.

► · **A1.7 Aspects Special to Conservation Programmes**

What can be done if the above conditions are difficult to meet?

In conservation programs and particularly in developing countries, compromises in conditions may have to be made particularly:

- when a given individual has been selected because of being one of the few remaining breed representatives, or its own unique genetic characteristics, and yet it does not meet the health criteria;

- when there is a lack of adequate facilities (quarantine station and SCC) in the country or in the vicinity of the donor animal.

To cater for this the FAO recommends the following guidelines:

- *Compulsory requirements* to be fulfilled by each donor animal and take into consideration the clinical status of the donor and the environmental conditions at the moment of semen collection;

- *Individual health testing* to certify donors free of pathogens, particularly those which are transmittable through semen;

- *Grading of donor animals and semen* from A - C for each donor taking into account the prevailing status of veterinary health of the animal and the environment.

A1.7.1 Compulsory requirements.

All semen donors should be clinically examined the day before and just prior to moving the animal to the collection room, to ensure that there are no clinical signs of a disease. This should be done by a veterinarian and certified on an appropriate form. Simultaneously, no infectious disease

should have occurred in the centre for the last 30 days and this should also be certified. Both documents have to accompany the collection and semen evaluation sheet and have to be kept on file.

In addition there should be no report of clinical cases of any disease listed in OIE List A in a 10 km radius around the semen collection site for the last 30 days with a special and explicit attention given to Foot and Mouth disease (FMD), Rinder Pest, Bovine Contagious pleuropneumonia for cattle and (where relevant) for Buffaloes and Camels; Peste des Petits Ruminants for small ruminants; Hog Cholera and African Swine Fever for pigs; and Horse sickness for Horses and asses. Where relevant, vaccination against major diseases should have taken place (FMD, Rinderpest, Peste des Petits Ruminants).

A1.7.2   Individual Health Testing.

Wherever feasible health testing and other procedures should follow as close as possible the procedures described above. As a minimum standard all donors should be tested free from diseases that can be transmitted by semen. Table A1.1 lists only diseases of major importance, upon which the health grading as set out below could be done. Other diseases could be added (provided that the laboratory facilities for testing exist) such as Bovine leucosis, IBR/IPV, BVD/MD in cattle, or else PRRS (Porcine Respiratory, Reproductive Syndrome) in pigs etc. These tests, wherever feasible, should be performed in quarantine stations and only individuals tested negative should be allowed to enter into the SCC. The compromises between the need to conserve the genes of an endangered breed and the health status of its last representatives and/or the availability of appropriate premises in the vicinity, has led the recommendation for the introduction of a grading system for semen and embryos according to the quality of the health monitoring carried out and the health of the donors.

A1.7.3 Grading donors and semen.

This system grades the health status of the males in three categories, grades A (highest), B and C (lowest). This will facilitate storage of animals with similar health status but from different origins in the same genome bank, and promote future access and use.
This is summarized in Table A1.2.

Table A1.2. Grading rules for semen samples.

	Compulsory requirements (A1.7.1)	Individual Health Testing (Table A1.7.2)	Approved quarantine and SCC (A1)
Grade A	X	X	X
Grade B	X	X	
Grade C	X		

► Grade A health status of the sample.

To be graded 'A', the donor animal has to fulfil the entire chain of monitoring, starting with the quarantine station described in A1.2. Before transfer to an officially approved SCC sires have to be tested with negative results for the various diseases. Once in the SCC the sires have to pass the test for semen quality and freezability and will be kept under continuous veterinary control. It should be possible to move Grade A semen freely around the world with no restrictions, as the risk of transmitting diseases with this semen is extremely limited.

Semen doses have to be clearly identified as Grade A, for example by adding >GA= at the end of the normal labelling of the straw (see 5.5.1, JPR4).

▶ Grade 'B' health status of the sample.

To be graded 'B', the donor animal must comply with the compulsory requirements (see A1.7.1) and also to the individual health testing for the major diseases as indicated on Table A1.1.

Semen doses have to be clearly identified as Grade 'B', for example by adding >GB= at the end of the normal labelling of the straw (see 5.5.1, JPR4).

▶ Grade 'C' health status of the donor

All other samples are given Grade 'C'. They should however comply with the compulsory requirements in A1.7.1. It is obvious that semen classified Grade 'C' gives rather low security with regard to the disease risk. In emergency situations, when a given animal has to be immediately collected with no other test or indication, such collected semen is to be categorized 'C'.

If possible and provided sufficient material is available specific tests could be performed at a later stage. Obviously particular precautions will have to be taken when using these semen doses again.

Semen doses have to be clearly identified as Grade 'C', for example by adding >GC= at the end of the normal labelling of the straw (see 5.5.1, Fig. 5.3).

A1.7.4 Consequences of grading on storage.

Grading of semen has consequences on the storage facilities and procedures. Semen doses, once they are packed, are closed tightly and there is very little risk of spreading of disease directly from semen, but there is still a limited risk

which arising from containers and during handling. Therefore it is recommended to store differently graded semen doses in distinct and separate rooms. Each room has to have its own equipment (forceps, basins etc) which should be clearly marked and which should not be moved from one room to the other. Containers, gobelets and viso-tubes have to be thoroughly cleaned and disinfected before being transferred into one of the rooms.

Under practical conditions, one may presume that in countries where a basic AI structure exists semen graded 'A' and 'B' will may need to be stored, while in other countries only semen graded 'B' and 'C' may need to be stored. It is considered quite unlikely that the need will arise to store 'A' and 'C', or 'A', 'B' and 'C'. The design of the SCC must therefore take this into account (see A1.5)

Samples from different countries but of the same Grade can be stored in the same room but always in different containers.

The question becomes somewhat more difficult with regard to the storage of duplicates. There is no problem envisaged with Grade 'A' as this type of semen is almost freely movable. Grade 'B' semen should be transferred to a country with similar general veterinary health conditions as the one prevailing in the country of origin.

More difficulties are envisaged with grade 'C' semen, for which a country with similar (low) veterinary health conditions but good, safe, reliable storage facilities will have to be found which will accept samples of this low grade. One cannot expect that a country with generally higher veterinary health standard will accept samples from countries with lower standards. Facilities may need to be provided by an appropriate international organization.

The grading system should allow the highest flexibility in allowing collection from any donor which has been identified because of its genetic uniqueness, whilst giving on the other hand the maximum information to establish the necessary security measures to avoid spreading diseases when the samples are to be used.

Health grading for avian semen

Similar to what has been proposed for mammals, it is recommended to grade the semen doses according to the health standard of the donor birds.

Grade A health status of the donor.

Semen from birds that originate from flocks free of OIE list A diseases, which have gone through a quarantine station and have been tested free of these diseases and which have been collected in an approved SCC may be qualified as Grade A. Grade A semen from mammals and birds can be stored in the same room but should not be stored in the same container.

Grade B health status of the donor.

Birds that have not passed through a quarantine station and an appropriate SCC but which do test negative to the relevant health tests may be categorized as Grade B provided that their flock of origin was free of the O I E list A diseases. Semen from mammals and birds of the same category may be stored in the same place.

Grade C health status of the donor.

In all other collection conditions semen must be Grade C. Semen from mammals and birds from this category may be stored in the same room also.

Again such information should be clearly stated on the containers (straws) and the accompanying documents with the same code as for mammals GA, GB, GC for grades A, B and C respectively.

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▶ **Annex 2 Health Surveillance and Regulations for Collection and Storage of Embryos**

- ■ *Embryo Production and Storage*

- ■ *The Concept of Officially Approving the Embryo Collection Teams*

- ■ *The Categorization of the Health Status of the Embryos*

▶· **A2.1 Embryo Production And Storage**

The risk of transmitting infectious diseases through embryos is lower than with natural mating or artificial insemination (Stringfellow, 1985; Hare, 1985; Thibier, 1989). However a potential risk still remains particularly when the recommended procedures regarding collection and handling are not precisely followed.

The recommendations made in the following deal only with aspects of embryos of mammalian origin and derived from *in vivo* collection.

Abundant results from world wide research on the risk of disease transmission via embryos are available for the bovine. Less information is available for the ovine, caprine or porcine and is almost non existent for the other species. As a result of these findings, OIE was able to publish in its Animal Health Code special appendices for the bovine (Appendix 4.2.3.1.). Appendices also exist for porcine (Appendix 4.2.3.2.), for ovine and caprine (Appendix 4.2.3.3), for equine (Appendix 4.2.3.7), South American camelids (Appendix 4.2.3.8), cervids (Appendix 4.2.3.9) and laboratory mammals (Appendix 4.2.3.6).

The International Embryo Transfer Society (IETS) has categorized the diseases according to their risk status. In category 1 diseases are listed for which sufficient data are available to show that, provided that embryos are handled according to the recommendations given below, there is almost no risk of any disease transmission. This category 1 list has been accepted by OIE (published in the Bull OIE, 1992, 11, 937-938) and

includes the following diseases: Foot and Mouth disease (bovine), Blue Tongue (bovine), Enzootic Bovine Leucosis, Bovine IBR/IPV (after trypsin treatment of the embryos) and porcine pseudorabies (after trypsin treatment). Research results have shown that trypsin treatment has no negative influence on the embryo and its viability.

The reduced risk however should not lead to carelessness. Any embryo collection has to be preceded by an extensive clinical examination of the donor animal, its herd or flock mates and the general environment in which the animals are kept (see below) for the presence of diseases. This clinical examination may also have a bearing on results obtained from subsequent superovulation and recovery as one can expect only good results from perfectly healthy animals. The disease risk may vary between species but this should not affect the level of attention paid to the animals.

▶· A2.2 The Concept of Officially Approving the Embryo Collection Teams

The direct disease risk related to embryos, although being low, depends very much on the correct handling of the embryos by the embryo transfer team. This entrust a very high responsibility to this group. An embryo transfer team may be defined as a group of technicians, supervised by a team veterinarian, competent to perform the collection, processing and storage of embryos according to the conditions set out below. With the high responsibility carried by this group, and to ensure that the work is always done at the required high standard, it is recommended to introduce a procedure of approving and officially recognizing these ET teams for countries which do not have already such a system of official approval. A team might then be officially approved by FAO following an inspection by a designated expert. The objective is to ensure that embryo collection and processing by this approved team is been done according to the conditions and standards laid down in the present program. This will guarantee the storage of embryos of good biological quality and safe with regard to the risk for transmission of diseases.

The criteria for approval are as follows:

- the competence of the personnel;

- the availability of appropriate equipment;

- the commitment of the team to follow the procedures of handling the donors and embryos according to IETS Manual and OIE recommendations;

- the participation in a quality control programme which includes testing of media and/or degenerated embryos for possible viral, bacterial or mycoplasmas contamination;

All embryo teams to be officially approved, should formally sign an adequate form that commit them to follow these rules.

A2.2.1 Competence of the personnel.

The collection, processing and storage of embryos must be carried out either by a trained veterinarian or by a technician under the responsibility of a veterinarian. Technicians have to undergo special training with regard to technique and hygiene.

A2.2.2 Appropriate equipment.

An ET team must have at its disposal either a permanent laboratory or a mobile laboratory where embryos can be examined, processed and packed. Any laboratory has to be equipped with an appropriate working surface, microscopes and cryogenic equipment. Small equipment items should be either disposable or equipment for sterilizing purposes is necessary.

A2.2.3   A permanent laboratory.

A permanent laboratory must consist of:

- a collection room;

- an embryo handling room which can be adjacent but physically separated from the collection room;

- a room where embryos can be manipulated (splitting, freezing) which is adjacent to the handling room;

- a room or area equipped for cleaning and sterilizing the instruments and equipment used in the collection and manipulation of the embryos;

In the case of a mobile laboratory, a specially equipped part of the vehicle should be used for the examination and manipulation of the embryos, which shall be a "clean" section and the rest used for accommodating equipment and materials which may come in contact with the donor animals. A mobile laboratory should always be associated with a permanent laboratory to ensure correct cleaning and sterilization of the equipment, and the provision of flushing fluids and other media and products necessary for the collection and manipulation of embryos.

Mobile laboratories allow the collection and processing of embryos on the farm. This can, exceptionally, be done in a dust-free room on the farm that is free of animals and has been cleaned for the purpose. For the time of embryo collection and processing no other activity should go on in this room. Needless to say, all the equipment used under these conditions has to be perfectly clean and sterile.

A2.2.4 Health conditions for donor animals.

No OIE list A diseases should occur, or have occurred, during the 30 days before or after collection in an area of 10 km around the site of the donor animals. The herd of origin should not be subjected to veterinary prohibition or quarantine measures.

At the time of collection, donor animals should be clinically inspected by the team veterinarian to confirm that the animal is clinically healthy and free of contagious and infectious diseases.

All shipments of embryos should be accompanied by a statement signed by the responsible veterinarian, and endorsed by the official veterinarian that during the 30 days before and after collection, no contagious and infectious diseases has occurred in the herd. These certificates have to be kept on file and should be easily traceable when the need arises to ship or to use the embryos.

▶∙   A2.3 The Categorization of the Health Status of the Embryos

Just like in the case of semen from mammals, it may be advantageous to grade the embryo according to the health status of the donors. The grading will be A and B only.

A.2.3.1 Grade A health status of the embryo.

Embryos categorized as Grade A will have:

- derive from donors satisfying above health requirements
- have been collected by an FAO approved team, which
  - has expertise and competence,

- has followed the hygienic rules as set in the IETS Manual.

A.2.3.2 Grade B health status of the embryo

Embryos will be categorized Grade B:

- in cases where they have not been collected by an FAO approved team;

- or, if they have been collected by an FAO approved team but where the veterinary health and/or other hygienic rules could not strictly be followed;

Such information should be clearly stated on the containers (straws) (fig. 5.4) and the accompanying documents with the same code as for semen GA, GB, respectively for grades A and B.

References

Hare, W.C.D. (1985). Disease transmissible by semen and embryo transfer techniques. Technical Series, Office International des Epizooties, No. 4., 119 pp. Paris.

Stringfellow, D. S. (1985). In: The round table meeting on sanitary problems related to embryo transfers. Rev. sci. et tech. Office International des Epizooties 4: 843-854.

Thibier M. (1990). Embryo transfer, the safest means, healthwise, of exchanging genes. In: Proc. VI Association Européenne de Transfert Embryonnaire. Lyon, 7-8 September 1990. p. 67-81. Fondation M. Mérieux Ed., Lyon, France.

► Annex 3 Technical Procedures for Cryoconservation of Semen

These procedures require technical expertise. Before implementing them in a conservation programme, the team should test the procedure on a small sample of males of the same species (and where practicable, of the breeds to be conserved). The test should involve (i) collection and cryoconservation, and (ii) thawing and insemination to obtain a successful pregnancy. Substantial evidence of failure to achieve the success rates shown in Table 5.2 should be reported to FAO.

► · A3.1 Cryoconservation of Bull Semen

► Freezing

A3.1.1   Collect sperm (5 to 15 x 10^9 spermatozoa per ejaculate). Avoid temperature changes in semen after collection.

A3.1.2   Evaluate sperm concentration and determine final volume V for a concentration of 200 x 10^6 spz/ml.

A3.1.3   Add half of the final volume with Diluent >1= (milk +10% egg yolk +antibiotics +3% glycerol). Addition must be done progressively over 15 min at 35°C.

A3.1.4   Cool to +5°C within 1 hour.

A3.1.5   Add Diluent >2= up to final volume (Diluent >2= consists of Diluent >1= +11% glycerol).

A3.1.6   Keep at +5°C for 2 hours.

A3.1.7   Fill pre-printed straws of 0.25ml with semen (25-30 million spermatozoa/straw).

A3.1.8   Transfer straws to LN_2 vapour at -70°C/-100°C for 9 min.

A3.1.9   Transfer straws to LN_2 and store.

▶   Thawing

A3.1.10   Thaw a sample to check for quality.

A3.1.11   Thaw straw directly in a water bath at +37°C for 30 seconds.

A3.1.12   Inseminate cows transcervically 12 hours after onset of oestrus.

▶·   **A3.2   Cryoconservation of Boar Semen**

▶   Freezing

A3.2.1   Collect sperm (80 x 10^9 spz per ejaculate). Discard the first emission of sperm; keep only the second one (about 200 ml, rich in spz with 40 x 10^9 total).

A3.2.2   Filter the sperm through gauze to eliminate the bulbo-urethral secretions.

A3.2.3   Dilute one volume of sperm with one volume of Diluent >1= (anhydrous dextrose, 37g; tri-sodium-citrate-$2H_2O$, 6g; sodium bicarbonate, 1.25g; EDTA diNa, 1.25g; KCl, 0.75g in 1 litre of bi-distilled water)

A3.2.4   Cool to 15°C within 2 hours.

A3.2.5   Centrifuge at 800g for 20 min at 15°C. Remove supernatant, which is diluted seminal plasma.

A3.2.6   Resuspend the pellet of sperm with about 10 volumes of Diluent >2= (fructose, 8.5g; sodium bicarbonate, 0.15g; cystein, 0.015g; bi-distilled water, 116 ml; egg yolk, 34 ml; equex STM (Nova Chemicals), 1.69g) to obtain a concentration of 3 x 10^9 spermatozoa per ml.

A3.2.7   Cool the suspension to 5°C over 2 hours.

A3.2.8   Add one volume of the diluted sperm solution to one volume of Diluent >3= (Diluent >3= consists of Diluent >2= +6% glycerol); Diluent >3= *must* be added in 3 steps to give a final concentration of 3% glycerol and 1.5 x 10^9 spermatozoa per ml.

A3.2.9   Keep at 5°C for about 90 minutes during which time the semen is put into 0.5ml. straws.

A3.2.10   Place straws horizontally at 5cm above the level of boiling LN_2 for 15min (this will ensure a freezing rate of about 20°C/min down to -145°C.

A3.2.11   Plunge into LN_2 and store.

▶   Thawing

A3.2.12   Thaw straws in a 38°C water bath for 20 seconds.

A3.2.13   Mix the content of 7 straws (or 5; see Table 5.2) with 95 ml of Diluent >1= at 38°C to obtain one dose for one AI.

A3.2.14   Inseminate the sow within one hour after this dilution (5.3 x 10^9 spermatozoa per AI).

▶·   A3.3   Cryoconservation of Ram Semen

▶   Freezing

A3.3.1   Collect sperm (4 x 10^9 spz per ejaculate); select only those ejaculates with a mass motility >4.5 on a scale of 5.

A3.3.2   Evaluate sperm concentration and determine final volume V for a concentration of 400 x 10^6 spermatozoa/ml.

A3.3.3   Add Diluent >1= (25. 75g lactose in 250ml bi-distilled water +20% egg yolk) at 30°C up to 3/5 of final volume V

A3.3.4   Cool progressively to +4°C over 2 hours (0.2°C/min)

A3.3.5   Prepare Diluent >2=: first proceed to the reconstitution of milk from a non-fat powder source (4g into 100ml bi-distilled water) and adjust pH to 6.6 with a Tris solution (20g of tri-sodium-citrate-5.5H_2O into 70ml H_2O); then mix 9 volumes of the above solution to 1 volume of glycerol.

A3.3.6   Add Diluent >2= in 3 equals parts, over 30 minutes, at 4°C up to the final volume V.

A3.3.7   Keep the semen for 90 minutes at +4°C.

A3.3.8   Fill 0.25ml straws with semen.

A3.3.9   Place straws horizontally in LN_2 vapour at -75°C for 8 minutes.

A3.3.10   Transfer directly into LN_2 at -196°C and store.

▶   Thawing

A3.3.11   Thaw straws in a water bath at 37°C for 30 seconds.

A3.3.12   Assess semen viability. For that mix one volume of sperm to 4 volumes of a sodium citrate solution (20g of Tri-sodium-citrate-$2H_20$ in 70ml bi-distilled water) at 38°C and estimate the proportion of motile spz 5 minutes and 2 hours after: only sperm with more than 30% of living spz at 2 hours can subsequently be used for insemination.

A3.3.13   Proceed to laparoscopic intrauterine insemination of pre-synchronized recipient.

▶·   A3.4   Cryoconservation of Rabbit Semen

▶   Freezing

A3.4.1   Collect sperm.

A3.4.2   Prepare Diluent >1=. For 100ml bi-distilled water dissolve: 3.028g Trishydroxy-methylaminomethane (Tris); 1.25g glucose; 1.67g citric acid-H_20; 5ml Dimethyl-sulfoxide (DMSO); add 1 volume egg yolk for 4 volumes solution.

A3.4.3   Add 4 volumes Diluent >1= to one volume sperm

A3.4.4   Progressively cool the diluted semen to +5°C over 1 to 3 hours.

A3.4.5   Prepare Diluent >2=. For 100ml bi-distilled water dissolve: 8.25g lactose; 1.3ml glycerol; add 20% egg yolk (1 volume egg yolk for 4 volumes of solution).

A3.4.6   Add one volume of Diluent >2= pre-cooled at +5°C to one volume of diluted semen

A3.4.7   Fill 0.5ml straws with semen.

A3.4.8   Keep 10 minutes at +5°C.

A3.4.9   Freeze straws horizontally in LN_2 vapour for 3 minutes at -120°C.

A3.4.10   Plunge directly into LN2 and store.

▶   Thawing

A3.4.11   Thaw straws in a water bath at +37°C for 1 minute.

A3.4.12   Intra-vaginal insemination of does followed by an intra muscular injection of 0.2ml GnRH.

▶·   A3.5   Cryoconservation of Stallion semen

▶   Freezing

A3.5.1   Collect sperm (about 8 x 10^9 spz per ejaculate) and filter on gauze.

A3.5.2   Evaluate sperm volume and concentration.

A3.5.3   Prepare Diluent >1= (1 volume of non-fat milk UHT +1 volume of a solution containing: 50g/l glucose, 3g/l lactose; 3g/l raffinose; 0.6g/l sodium citrate; 0.82g/l potassium citrate; 100 000iu/l penicillin; 0.100iu/l gentamycine).

A3.5.4   Add to Diluent >1= 2% egg yolk and mix three volumes of this solution with one volume of sperm at 32°C.

A3.5.5   Cool to +4°C over about 1 hour (0.4°C/min).

A3.5.6   Centrifuge at 600g during 10 min at 4°C. Remove supernatant.

A3.5.7   Prepare Diluent >2= from Diluent >1= supplemented with 2% egg yolk and 2% glycerol.

A3.5.8   Resuspend the pellet of sperm at 4°C with Diluent >2= to reach a final concentration of 100 x 10^6 spermatozoa/ml (50 x 10^6 spermatozoa/0.5ml straw).

A3.5.9   Keep at 4°C for 30 to 45 min.

A3.5.10   Fill 0.5ml straws with semen.

A3.5.11   Deep freeze straws in a programmable freezer: from +4°C to -140°C at a rate of 60°C/min. Alternatively Freeze straws horizontally in LN_2 vapour by keeping them at 4cm above the level of boiling liquid nitrogen for 4 min.

A3.5.12   Plunge in LN_2 and store.

▶   Thawing

A3.5.13   Thaw eight 0.5ml straws (400×10^6 spz) together in a water bath at +37°C for 30 seconds.

A3.5.14   Sperm should be deposited daily into the uterine body during the oestrus period.

▶·   A3.6   Cryoconservation of Buck Semen

▶   Freezing.

A3.6.1   Collect sperm (4×10^9 spz per ejaculate in season); select only those ejaculates with a mass motility >4.5; keep at 32°C.

A3.6.2   Wash sperm with a Krebs Ringer Phosphate Glucose Solution (0.9%NaCl; 1.15% KCl; 1.22% $CaCl_2$; 2.11% KH_2PO_4; 3.82% $MgSO_4$-$7H_2O$; 5.24% glucose) by mixing one volume sperm with 9 volumes of the washing solution at 28-32°C, followed by a centrifugation at 500g for 15 minutes at 20°C.

A3.6.3   Discard the supernatant, and evaluate semen (wave motion, concentration). Calculate final volume (V). Repeat centrifugation under same conditions at 20°C.

A3.6.4   Prepare Diluent >1=: 80ml of a sodium citrate solution (194mg glucose +3.52g sodium citrate +1.05g streptomycine +50 000 IU penicillin in 100ml distilled water) supplemented with 20 ml egg-yolk.

A3.6.5   Add V/2 of Diluent >1= to the pelleted sperm at 20°C.

A3.6.6   Cool to +4°C within 30 min (at 0.5°C/min).
A3.6.7   Add V/2 Diluent >2= (Diluent >1= +14% v/v glycerol) in
           three successive steps with 10 min intervals.
A3.6.8   Fill 0.25ml straws with semen.
A3.6.9   Freeze straws in LN_2 vapour for 5 min.
A3.6.10   Plunge directly into LN_2 and store.

▶   Thawing

A3.6.11   Thaw straws in a water bath at 37°C for 30 seconds.
A3.6.12   Assess post-thaw motility.
A3.6.13   Proceed to insemination of previously synchronized goats.

▶·   A.3.7   Cryoconservation of Rooster Semen

▶   Freezing

A3.7.1   Collect sperm (1.5 x 10^9 spz per ejaculate).
A3.7.2   Mix three volumes of sperm (an ejaculate is about 300μl
)with four volumes of Diluent >1= (0.7g Magnesium Acetate
           (tetra-hydrated) +19.2g Sodium Glutamate +5.0g Sodium
           Acetate +8.0g Fructose +3.0g P.V.P (MW 10000 to 15000)
           in one litre of bi-distilled water.
A3.7.3   Cool diluted semen immediately over 20-30 minutes to +5°C
           (0.5°C/min).
A3.7.4   At +5°C, add one volume of diluted sperm to one volume of
           Diluent >2= (Diluent >1= +11% glycerol). This gives a final
           concentration of 300 x 10^6 spermatozoa/ml.
A3.7.5   Equilibrate over 30 min at +5°C.
A3.7.6   Fill 0.25ml straws with semen.
A3.7.7   Freeze at a rate of 7°C/min from +5°C to -35°C; and at a
           rate of 8°C/min from -35°C to -140°C.
A3.7.8   Plunge into LN_2 and store.

► Thawing

A3.7.9 Prepare Diluent >3=: 0.8g Magnesium Acetate (tetra-hydrated) +1.28g Potassium Citrate +19.2g Sodium Glutamate +6.0g Fructose +5.1g Sodium Acetate in one litre of bi-distilled water.

A3.7.10 Thaw straws in a water bath at +5°C for 3 minutes. Open and transfer semen in a glass beaker. Mix one volume of sperm with 20 volumes of Diluent >3=, still at 5°C.

A3.7.11 Remove glycerol by centrifugation at 700g at +5°C for 15 min.

A3.7.12 Prepare Diluent >4=: 0.8g Magnesium Acetate (tetra-hydrated) +1.28g Potassium Citrate +15.2g Sodium Glutamate +6.0g Glucose +30.5g B.E.S (N,N-bis-2-hydroxyethyl-2-amino-ethanesulfonic acid) +58ml NaOH (1M/l) in one litre of bi-distilled water.

A3.7.13 Discard supernatant from A3.7.11 and add one volume of sperm pellet to one volume Diluent >4= at +5°C.

A3.7.14 Proceed to insemination of hens immediately with a total dose of 600×10^6 spermatozoa (2 straws) per insemination, with 2 inseminations per week. The insemination volume should be about 60 to 100µl (if higher volumes of sperm are delivered per insemination then sperm will be partly expelled).

►· A3.8 Crypopreservation of Buffalo Semen

► Freezing.

A3.8.1 Collect sperm (5 to 10×10^9 spz per ejaculate) at 35°C. Avoid temperature changes in semen after collection.

A3.8.2 Evaluate sperm concentration and determine final volume V for a concentration of 100×10^6 spz /ml.

A3.8.3   Add half of the final volume with Diluent >1= (milk + 10% egg yolk + antibiotics + 3% glycerol). Addition must be done progressively over 15 min at 35°C.

A3.8.4   Cool to +4°C within 1.5 hours.

A3.8.5   Add Diluent >2= up to final volume (Diluent >2= consists of Diluent >1= + 11% glycerol). The final concentration of glycerol is thus 7%.

A3.8.6   Keep at +4°C for 4 hours.

A3.8.7   Meanwhile fill pre-printed straws of 0.50 ml with semen (about 50-60 million spermatozoa /straw).

A3.8.8   Cool from +4°C to -140°C in 5 minutes, then plunge in liquid nitrogen.

A3.8.9   Transfer straws to LN_2 storage.

►   Thawing.

A3.8.10   Thaw a sample to check for quality.

A3.8.11   Thaw straw directly in a water bath at +35°C for 30 seconds.

A3.8.12   Inseminate females transcervically 12 hours after onset of oestrus.

►·   A3.9 Cryoconservation of Turkey and Duck Semen

For the present it is recommended that Turkey and Duck semen samples are treated as Rooster, but improvements are anticipated. For insemination of Turkeys, 3 straws per insemination are recommended. Please refer to FAO for the latest recommendations.

► ## Annex 4 Technical Procedures for Cryoconservation of Embryos

It is anticipated that the team responsible for the recovery and cryoconservation of embryos will have demonstrated their technical expertise before implementing a conservation programme (see A2.2).

►· ### A.4.1 Cryoconservation of Bovine Embryos

► #### Freezing

A4.1.1   Embryos are collected non-surgically at the compact morulae-blastocysts stage at day 7.

A4.1.2   Wash embryos in 10 consecutive baths of phosphate buffered solution (PBS).

A4.1.3   Equilibrate embryos at room temperature for 10 minutes in phosphate buffered solution + 10% fetal calf serum (FCS) + 10% glycerol.

A4.1.4   Condition embryos between 2 air bubbles in a 0.25ml mini-straw.

A4.1.5   Place straws horizontally in a programmable freezer and cool from room temperature to -7°C at a rate of 5°C/min.

A4.1.6   Induce seeding at -7°C and freeze embryos to -35°C at a rate of 0.5°C/min and plunge directly into LN_2.

A4.1.7   Store straws in LN_2 at -196°C.

► #### Thawing

A4.1.8   Thaw straws rapidly in a water bath at 20°C for 30 seconds.

A4.1.9   De-hydrate embryos in 1M sucrose solution over 10 min followed by one bath of PBS.

A4.1.10   Transfer one embryo per synchronized recipient female

►· **A4.2   Cryoconservation of Ovine and Goat Embryos**

► **Freezing**

A4.2.1   Collect embryos preferably at the compact morulae-blastocysts stage at day 6-7.

A4.2.2   Wash embryos in 10 consecutive baths of PBS.

A4.2.3   Equilibrate embryos at room temperature for 10 min in PBS+10% FCS+10% cryoprotective agent (Ethylene Glycol for Ovine and Glycerol for Goat).

A4.2.4   Condition embryos between 2 air bubbles in a 0.25 ml mini-straw.

A4.2.5   Place straws horizontally in a programmable freezer and cool from room temperature to -7°C at the rate of 5°C/min.

A4.2.6   Induce seeding at -7°C and freeze embryos to -30°C at a rate of 0.3°C/min and plunge straws directly into LN_2

A4.2.7   Store straws in LN_2 at -196°C.

► **Thawing**

A4.2.8   Thaw straws rapidly in a water bath at 20°C for 30 seconds.

A4.2.9   Re-hydrate embryos in 0.5M sucrose solution over 10 min followed by one bath of PBS.

A4.2.10   Transfer two embryos per synchronized recipient female

►· **A4.3 Cryoconservation of Rabbit Embryos**

► **Freezing**

A4.3.1   Collect embryos preferably at compacted morulae stage, 65 h post coitum.

A4.3.2   Wash them in 10 consecutive baths of PBS.

A4.3.3   Equilibrate embryos for 5 minutes in 3 baths of PBS containing respectively 0.5M, 1M and 1.5M DMSO.

A4.3.4   Condition embryos between 2 air bubbles in a 0.25 ml mini-straw.

A4.3.5   Place straws horizontally in a programmable freezer and cool from room temperature to -7°C at a rate of 5°C/min.

A4.3.6   Induce seeding at -7°C and freeze embryos to -35°C at a rate of 0.5°C/min. Plunge straws directly into LN_2.

A4.3.7   Store straws in LN_2 at -196°C.

▶   Thawing

A4.3.8   Thaw straws rapidly in a water bath at 20°C for 30 seconds.

A4.3.9   Re-hydrate embryos progressively over 5 minutes in 3 baths of PBS containing respectively 1M, 0.5M and 0M DMSO.

A4.3.10   Transfer embryos 10 into uterine horns of synchronized recipients.

▶ ## Annex 5 Technical Procedures for DNA Extraction

The procedures for DNA extraction are technically demanding. The procedures should not be used as part of a conservation programme until the capability of the laboratory concerned has been established. This should be done by demonstrating successful extraction of DNA, with the anticipated yields, from samples that are of the same tissue and species as those being obtained as part of the programme.

Some chemicals used in the procedures should be treated with care, and attention should be given to the health and safety information that should accompany all laboratory products. However the procedures given below for DNA extraction can be safely incorporated into a well-managed and technically experienced laboratories.

The DNA from the blood of mammals comes from the white cells only, because the red cells do not nuclei. Birds are different in that they have DNA in both red and white cells which means that far smaller blood volumes are required from birds to obtain good quantities of DNA. The extraction methods covered in the Annexe will be for mammalian blood and semen, and for avian blood and semen. Blood and semen are the most simple samples to obtain (that will give adequate yields) in conservation programmes: semen may be collected in cryoconservation programmes and blood is taken in conjunction with any health testing programme. However the techniques for semen will depend on: the species (spermatozoa density in semen), whether fresh or frozen, and in the latter case the dilution. Therefore only methods for blood will be presented for mammals and avian species.

In Chapter 5 it was recommended that the volume of blood for DNA extraction should be split between two tubes at the time of collection. This served to reduce the risk of the accidental loss of all the sample from an individual. It is recommended that the samples remain split through processing and storage (see 5); furthermore, the processing of the two

halves should be carried out in different batches. Thus, if an unforeseen failure of the extraction technique occurs in one batch, DNA from the individuals affected by the failure can still be obtained from the other half.

Once extracted, DNA should be put into labelled aliquots (see 5.5) of 50 μl with a concentration of 200 μg/ml before long-term storage. This division will avoid repeated freezing and thawing. The DNA may be safely stored at 4°C over 2 months before dividing into aliquots, *provided* the preparation is pure enough, i.e. DNase which may be present in contaminating tissues has been eliminated, otherwise rapid transfer to long-term storage is necessary. For long-term storage, a temperature of -20°C or lower provides a safe storage environment (or at lower temperatures if convenient, i.e. in liquid nitrogen, but this is not necessary).

▶· ## A5.1 DNA extraction from mammalian blood

A5.1.1   Put blood (7 mls) into 30 ml glass Corex tube.

A5.1.2   Add 7 ml of 0.9% NaC1. Centrifuge at 5K rpm for 10 min.

A5.1.3   Remove buffy coat to a 15 ml plastic centrifuge tube.

A5.1.4   Add 9ml H_2O, mix, and then add 1 ml 9% NaC1 and mix again.

A5.1.5   Add 10ml 0.9% NaC1. Centrifuge at 5K rpm for 10 min. Remove supernatant, leaving the white cell pellet.

A5.1.6   Wash the pellet with 0.9% NaC1. Centrifuge at 5K rpm for 15 min and decant supernatant.

A5.1.7   Add 5 ml Lysis buffer to the pellet plus 50μl proteinase K (20 mg/ml in water), mix well to disperse pellet then add 200μl 10% SDS (Sodium Dodecyl Sulphate).

A5.1.8   Incubate at 65°C for 1hr, add a further 50μl proteinase K and incubate for 1hr.

A5.1.9   Add 5 ml phenol and mix continuously for 10 min by gentle inversion. Centrifuge at 8K rpm for 5 min to separate the phases.

A5.1.10   Remove and discard the phenol layer (the lower layer) with a pastette, add 3ml phenol and 3ml chloroform. Mix by gentle inversion for 5 min. Centrifuge at 8K rpm for 5 min.

A5.1.11   Repeat A5.1.10.

A5.1.12.   Remove and discard the organic layer (the lower layer), add 5ml chloroform to upper aqueous phase and mix for 5 min. Centrifuge at 5K rpm for 5 min.

A5.1.13   Remove the aqueous (top) layer (but do not discard it, it contains the DNA!) and add to it 3 volumes ethanol (15ml), spool out DNA by mixing with glass pipette, air dry for 5 min to evaporate ethanol. Dissolve DNA in $500\mu l$ - $1000\mu l$ H_2O. Store at $4^{\circ}C$ for short periods or $-20^{\circ}C$ indefinitely.

▶·   ## A5.2 DNA extraction from avian blood

A5.2.1   Prepare reagents. A: 10mM Tris; 320 mM Sucrose; 5 mM $MgCl_2$; 1% Triton X-100; and adjust to pH 8.0. B: 400 mM Tris (pH 8.0); 60 mM EDTA; 150 mM NaCl; 1%SDS. Both reagents should be autoclaved before using. The SDS in Reagent B should be added *after* autoclaving. Reagent A is required only for chickens.

A5.2.2   If the blood has clotted do **not** wash in Reagent A or else all the DNA will be lost. Spend 5 min homogenizing the clot by hand in Reagent B. Do not use any high speed devices to speed up the process or the yield will contain only low molecular weight DNA. If the blood has not clotted proceed to next step.

A5.2.3   **Chickens only; for other species omit this step!** Put 0.5ml blood in 50ml Falcon tubes and add 20ml Reagent A, mix for 5 minutes at room temperature, then spin at 1300g (about 2500 rpm for a standard rotor) for 3 min. Discard supernatant, taking care not to lose the pellet. (Harder spins make it difficult to re-suspend the pellet).

A5.2.4   Add 30ml Reagent B and use a p1000 pipette tip or a pastette to gently re-suspend the pellet. It is important that the re-suspension is even or else yield will be poor.

A5.2.5   Add 50μl of RNAse (10 mg/ml) and incubate at 37°C for 60 minutes.

A5.2.6   Add 7.5ml of 5M Sodium Perchlorate and shake at room temperature for 25 minutes.

A5.2.7   Make up to 50ml with Chloroform and invert by hand for at least 2 minutes.

A5.2.8   Spin the samples at full speed in a microfuge for 5 min, then remove 25ml of the upper phase (containing the DNA) to a fresh tube, taking care not to carry any middle phase.

A5.2.9   Add 0.8 volumes iso-propanol and invert the tubes until the DNA is precipitated. Spin down the DNA for 5min at full speed. Discard the supernatant, taking care not to lose the pellet.

A5.2.10   Wash the pellet by adding 10ml 70% ethanol (4°C) and shaking. Spin briefly again and discard the supernatant and dry the pellet.

A5.2.11   To re-suspend the DNA, add 10ml TE (50 mM Tris, 10 mM EDTA at pH 7.4) and gently shake overnight at 37°C.

A5.2.12   Put into aliquots. Check quality by running 0.5μg out on a 0.7% agarose gel for 60 mins and check DNA appears at 23000 MW or above. Quantify yield by taking an O.D. reading at 260nm. Check quality again by calculating the 260/280nm ratio and anything between 1.8 and 2.0 is good.

► ## Annex 6 Principles Underlying Simulations to Determine Recommended Sample Sizes

►· ### A6.1 Simulation of semen requirements.

A6.1.1 Objective 5.2.1: re-establishment of a breed using semen.

This requires producing backcrosses containing an expected 92.5% of the cryoconserved breed. Simulation assumed starting from F females from another breed for crossing. Each female had a breeding lifetime of m litters. The value of m was the least integer greater than 2 divided by the fertile offspring per pregnancy given in Table 5.5. The age of the female at the first of the m litters was taken as 2 breeding intervals. Time proceeded in breeding intervals (assumed constant), mating all females of appropriate age. For each female, the number of straws to produce a pregnancy was drawn with a negative binomial distribution with a mean number of doses taken from Table 5.5. The litter size for each mating and sex was assumed to be Binomial with the mean given by fertile offspring per pregnancy in Table 5.5 and a natural maximum litter size.

For each replicate, simulation proceeded until either: (i) failure, i.e. it was clear insufficient numbers of 92.5% crosses would be produced; or, (ii) success, a total of 12 males of 92.5% had been produced over the time course of the simulation, and 12 females of 92.5% have been produced which are simultaneously of breeding age. The males did not need to be of breeding age simultaneously with the females since it was assumed that AI was available.

A total of 1000 replicates were generated for a range of values of F. For values of F that gave >900 successes, the

number of straws required were ranked and the 900th was taken as the required number. The value of F was chosen to minimize the required number (with large F, success is near certain but many females are mated unnecessarily and produce 92.5% crosses well in excess of requirements for the objective).

For species with high reproductive rates the mean number of offspring per litter was also reduced to avoid excessive use of semen. For avian species, if it was desired to reduce the clutch size of the desired genotype, the number of inseminations was also reduced.

A6.1.2 Objective 5.2.2: New breed development.

The above procedure was modified accordingly.

A6.1.3 Objective 5.2.3: Supporting *in vivo* conservation.

The 200 females were multiplied by the number of litters required for an expected replacement rate >1 (m as defined in A6.1.1) and the expected number of straws per pregnancy.

A6.1.4 Objective 5.2.4: Research into identifying single genes of large effect.

The simulations were carried out as described in A6.2.1, but modified accordingly. High reproductive rates were used to their full potential.

► ·   A6.2 Simulation of embryo requirements.

It was assumed that M males and F females were used for embryo recovery. It was assumed that mating to the donor females used AI, and that each male had a probability of 0.2 of being unsuitable. For the F females, each has a probability of 0.15 of never producing an embryo. For those females that are capable of producing embryos it was assumed that each recovery had a probability of 0.2 of failing to produce any transferable embryo, and otherwise the number of embryos was taken as the integer part of $\exp(X)$, where X is a random variable with a log-normal distribution. The mean number of embryos and the coefficient of variation for those females capable of response was matched to the values given in Table 5.3. Recoveries of different recoveries from the same female were considered independent. The conduct of successive recoveries was as recommended in Chapter 5. Transfer of embryos was simulated as binomial trial with the success rate given in Table 5.6.

The minimum number of embryos required to produce 12 males and 12 females was estimated from 1000 replicates of simulated transfer of T embryos. The minimum value of T that gave success >900 times was taken as the estimate.

The number of recoveries required to satisfy the variance conditions after transfer was obtained from a 1000 replicates. For each replicate, success was assessed by: (i) at least 12 males and at least 12 females produced; (ii) for a subset of 12 males and 12 females, selected to have the maximum spread of parents, the sum of squared family sizes of the parents was <70, where each sex of parent was considered separately. The number of recoveries producing 900 successes for particular values of M and F was determined. (The value of 70 was determined from the expected sum of squared family sizes following random selection of 24 individuals produced by 12 parents of each sex.)