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guidelines

CRYOCONSERVATION OF ANIMAL GENETIC RESOURCES

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CRYOCONSERVATION OF ANIMAL GENETIC RESOURCES

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Foreword

Livestock agriculture is in a period of tumultuous change and upheaval. General economic development, and population growth and mobility, have increased demand for livestock products, but have also placed pressures on the sustainability of rural environments and animal production systems. Livestock keepers will need to increase their efficiency to meet the rising demand while continually adapting their animal genetic resources to changing economic and environmental conditions. The genetic diversity necessary to allow this adaptation is in a state of decline, and the genetic resources that remain are not utilized in the most efficient way. *The State of the World's Animal Genetic Resources for Food and Agriculture* (FAO, 2007a) confirmed that a significant proportion of the world's 7 000+ livestock breeds are at risk of extinction and that many countries lack the technical capacity to ensure the proper management and sustainability of their animal genetic resources.

To address these problems, the Member Nations of FAO developed the *Global Plan of Action for Animal Genetic Resources* (FAO, 2007b) (*Global Plan of Action*), which was adopted at the first International Technical Conference on Animal Genetic Resources for Food and Agriculture in Interlaken, Switzerland, in September 2007. The *Global Plan of Action* contains four strategic priorities areas that provide a basis for enhancing the sustainable use, development and conservation of animal genetic resources throughout the world. It calls on FAO to continue to provide technical guidelines and assistance and to coordinate training programmes in order to support countries in their efforts to implement the *Global Plan of Action*.

Conservation of animal genetic resources is the third Strategic Priority Area of the *Global Plan of Action*. Conservation involves both *in vivo* maintenance and management of genetic diversity within livestock populations that are actively contributing to the livelihoods of their keepers or that are maintained in small numbers on research or demonstration farms and *in vitro* storage of genetic material that can be used at a later time to increase diversity in live populations or re-establish a population. A previous FAO publication on conservation – *Secondary guidelines: management of small populations at risk* (FAO, 1998) – covered both types of conservation. However, given the advances in technology and in the availability of information that have occurred during the past decade, the present guidelines will be complemented by a separate publication on *in vivo* conservation.

The development and operation of a gene bank for cryoconservation of animal genetic resources requires technical capacity in genetics, reproductive physiology, cryobiology and data management. Coordination among a wide group of stakeholders is also essential. These guidelines were developed to provide an overview of the fundamental issues involved in developing and operating gene banks as elements in comprehensive national strategies for the management of animal genetic resources.

Acknowledgements

Seven international scientists were responsible for the majority of the technical content of these guidelines: Harvey Blackburn, Robert Godke and Phil Purdy (United States of America); Sipke-Joost Hiemstra and Henri Woelders (Netherlands); Arthur Mariante (Brazil); and Flavia Pizzi (Italy). All of these persons make substantial contributions to the management of animal genetic resources in their respective countries, including the operation of national or regional gene banks.

The guidelines were reviewed, tested and validated at training workshops held at the National Gene Bank of Tunisia, the Escuela Superior Politécnica del Litoral in Ecuador and the Centre for Genetic Resources of the Netherlands, which were organized, respectively, with the local assistance of M'Naouer Djemali and the International Center for Agricultural Research in the Dry Areas (ICARDA), Paul Herrera Samaniego and Paul David Silva, and Sipke-Joost Hiemstra. In addition to FAO, significant financial support was provided by the United States Department of Agriculture, the European Regional Focal Point for Animal Genetic Resources and the various host organizations. More than 120 scientists, technicians and decision-makers attended these workshops.

The guidelines were prepared under the supervision of Paul Boettcher, with the full support of the Chief of FAO's Animal Genetic Resources Branch, Irene Hoffmann, and of officers of the Animal Genetic Resources Branch: Badi Besbes, Beate Scherf, Roswitha Baumung and Dafydd Pilling. Administrative and secretarial support was provided by Kafia Fassi-Fihri and Silvia Ripani.

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The goal and structure of the guidelines

These guidelines are intended to serve as a decision aid with respect to the various cryoconservation options that are available, and to provide technical guidance on the design and establishment of animal gene banks. The guidelines are written under the assumption that a decision has already been taken that cryoconservation will make a valuable contribution to a programme for conserving the animal genetic resources of interest. The advice provided is intended to be relevant to all species of domestic livestock, but species-specific guidance is given where appropriate. Much of the information may also be relevant to cryoconservation of wild relatives of livestock and to other wildlife species. Thus, countries may consider developing joint gene banks for both domestic and wild animals.

The guidelines focus on cryoconservation of animal genetic resources. Matters related specifically to *in vivo* conservation, as well as general issues of conservation, are presented in a separate publication – In vivo *conservation of animal genetic resources* – forthcoming in this series.

The guidelines are intended to provide the technical background information needed by countries wishing to set up, implement and monitor gene banks. Although reading all sections is recommended, certain sections are aimed at specific stakeholders with specific technical interests and responsibilities.

The terms "cryoconservation" and "cryopreservation" are both used frequently throughout the guidelines. Although these words are in some cases interchangeable, an effort has been made to restrict the use of "cryopreservation" to the actual process of freezing biological material for long-term storage. "Cryoconservation" is used to refer to the conservation of animal genetic resources through the use of cryopreserved germplasm.

Section 1 reviews reasons for conserving animal genetic resources and compares the various conservation options that are available. This is intended to help the reader confirm that cryoconservation will be a valuable component in a plan for conserving the animal genetic resource(s) under consideration (assuming a conservation programme of some kind is needed).

Section 2 discusses what must be done before the freezing and storing of germplasm can start, i.e. the preparation, implementation and organization of gene banks.

Section 3 discusses the objectives that can be addressed by gene banking programmes.

Section 4 describes the various types of germplasm and tissue that can be cryopreserved, as well as the uses to which they can be put. This is intended to provide a basis for informed choices regarding the type of material to store.

Section 5 discusses requirements for, and costs of, establishing gene banks of various sizes and degrees of technological sophistication.

Section 6 deals with the genetic issues that need to be considered when designing and implementing a cryoconservation programme, considering in particular the amounts of various types of germplasm that need to be stored in order to capture desired amounts of genetic diversity. Biological material undergoes a number of (sometimes drastic) changes when subject to cryopreservation, and some of these will decrease the viability of the conserved germplasm.

Section 7 describes the process of cryopreservation at cellular level, and the possible effects that the process may have on the stored material. This overview is intended to provide the basic information needed to diagnose and avoid damage to genetic material during the cryopreservation process.

Section 8 describes methods for collecting and cryopreserving various types of genetic material from various species of livestock.

Section 9 addresses the health and sanitary issues that must be considered when establishing and operating gene banks for animal genetic resources in order to help prevent the conservation of potentially dangerous pathogens along with the valuable genetic material.

Section 10 describes documentation and database requirements for storing information on individual animals and on the samples of genetic material stored in the gene bank. To be of use, material stored in the gene bank must eventually be thawed and used to create new animals. Therefore, good organization and annotation of the stored material are essential.

Section 11 addresses the legal issues associated with cryoconservation. Although animal genetic resources can be considered a public good, the animals from which germplasm is taken for cryoconservation are usually privately owned. Ownership may or may not change during the gene banking process, but the terms of agreement between gene banks and the breeders providing germplasm must be explicitly defined.

Section 12 discusses priorities for capacity building and the need to train livestock keepers and extension workers. It also discusses the need for the inclusion of cryoconservation and related topics in higher education curricula.

The main sections are followed by a series of appendices, which provide step-by-step instructions on procedures for collection and cryopreservation of germplasm.

Abbreviations and acronyms

AI	artificial insemination
AnGR	animal genetic resources (for food and agriculture)
AV	artificial vagina
BSA	bovine serum albumin
CASA	computer assisted sperm analysis
DNA	deoxyribonucleic acid
ET	embryo transfer
FAO	Food and Agriculture Organization of the United Nations
FSH	follicle stimulating hormone
ICSI	intracytoplasmic sperm injection
IVF	*in vitro* fertilization
MTA	material transfer agreement
$\mathbf{N_e}$	effective population size
OCM	oocyte collection medium
OIE	World Organisation for Animal Health (Office International des Epizooties)
OMM	oocyte maturation medium
PBS	phosphate-buffered saline
PHE	penicillamine, hypotaurine and epinephrine
SCNT	somatic cell nuclear transfer
TUGA	transvaginal ultrasound-guided oocyte aspiration

Content

Foreword ix
Acknowledgements xi
The goal and structure of the guidelines xiii
Abbreviations and acronyms xv

SECTION 1
Confirming the decision to cryoconserve 1
In situ conservation 3
Ex situ conservation 4
Complementary roles of *in situ* and *ex situ* conservation 4
When is cryoconservation the best option? 5

SECTION 2
Implementation and organization 7
Shaping national strategies and action plans 9
Organization and institutions 9
Participation of stakeholders 10
Funding and attracting support for projects 12

SECTION 3
Objectives of cryoconservation programmes 13
Gene banking 15
Collection goals 17
Collection categories 20
Utilizing the working collection 22
Refreshing the collections 23

SECTION 4
Potential use of different types of germplasm and tissue 25
Semen 28
Embryos 29
Oocytes 31
Somatic cells 32
Cryopreservation of DNA for genetic analyses 34
Choosing the genetic material to store 34
Advanced procedures and their curent potential in cryoconservation 35
Future prospects for cryoconservation 38

SECTION 5
Establishing a gene bank – physical structure and costs **43**
The physical plant 45
Size and capacity requirements for gene banks 53
Recommended equipment and estimated costs 55
Gene bank security 55
Centralization and accessibility 56
Human resources 58
Continuity of operations 58

SECTION 6
Developing gene bank collections **61**
Choice of populations to include in the gene bank 63
Collection targets for reconstituting populations 66
Utilization of gene bank material in live conservation and breeding 75
Selection of individuals for cryopreservation 77
Collection of complementary biological material 80

SECTION 7
Basic principles of cryopreservation **83**
Slow freezing 85
Vitrification 91
Freeze drying 93

SECTION 8
Collection of germplasm and tissues **95**
Semen 97
Embryos 100
Oocytes 109
Somatic cells 114

SECTION 9
Sanitary recommendations **117**
Collection and processing facility 120
Field collection 120
Disease testing 121
Storing samples 121

SECTION 10
Database and documentation **123**
Components of a gene bank information system 125
Database information set 126

SECTION 11
Legal issues – contracts and access **131**

Structure for handling agreements 133

Acquiring germplasm 134

Access to the gene bank's collection 135

International transfers of germplasm 137

SECTION 12
Capacity building and training **139**

References and appendices **143**

References 145

APPENDIX A: Specialized procedures for field collection of germplasm 161

APPENDIX B: List of equipment needed for semen collection and freezing 163

APPENDIX C: Procedures for cryopreservation and thawing of semen from common livestock species 165

APPENDIX D: Guidelines for basic semen analysis 175

APPENDIX E: Commonly used superovulation schemes for donor cattle embryo recovery 177

APPENDIX F: List of equipment and supplies needed for non-surgical embryo collection and transfer in cattle 179

APPENDIX G: Technical procedures for cryopreservation and thawing of livestock embryos 181

APPENDIX H: Bovine oocyte collection procedures 185

APPENDIX I: *In vitro* maturation of bovine oocytes 189

APPENDIX J: Bovine *in vitro* fertilization protocol 191

APPENDIX K: Harvesting tissue samples for cryoconservation of somatic cells 197

APPENDIX L: Collection and freezing of poultry gonadal tissue 201

APPENDIX M: References for the appendices 203

BOXES

1	Maintaining genetic diversity	16
2	Viability of germplasm after long-term storage	22
3	Cryobanking of somatic cells in Viet Nam	33
4	Example animal holding and collection facility	48
5	Liquid nitrogen safety	53
6	Liquid nitrogen tanks	56
7	Use of gene bank semen for revival and support of the breeding programme of the endangered Dutch Friesian Red and White cattle breed	76
8	Reconstituting a research pig line	77
9	Using frozen biological material from at-risk breeds: discrepancy between theory and practice	78
10	Selection of bulls for the National Animal Germplasm Program in the United States of America	80

TABLES

1	Conservation techniques and objectives	5
2	Comparison of non-surgical and surgical artificial insemination in livestock when using frozen–thawed semen	29
3	Non-surgical versus surgical embryo collection	30
4	Non-surgical versus surgical embryo transplantation by species	31
5	An overview of some characteristics of several ways to cryoconserve genetic material	35
6	Estimated equipment costs by gene bank size	57
7	Number of semen doses required to reconstitute a breed of cattle, small ruminant or horse	69
8	Number of doses per male required to reconstitute a breed of cattle, small ruminant or horse	69
9	Number of straws of semen required to reconstitute a breed of pig	70
10	Number of straws per boar required to reconstitute a breed of pig	71
11	Resources required for chicken gene banks to address various conservation objectives	72
12	Number of cryopreserved embryos required to reconstitute a breed population	73
13	Location of the embryo following oestrus	102
14	Donor animal information: recommended minimum and additional database fields	127
15	Sample information: recommended minimum and additional database fields to be associated with animal identification	128

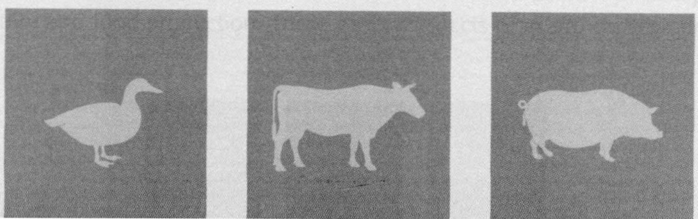
FIGURES

1	Intracytoplasmic sperm injection procedure	38
2	Example design of a germplasm processing and freezing laboratory	52
3	Population reconstitution with cryoconserved semen	67
4	Standard backcrossing plan for breed reconstitution (using cattle as an example)	67

5 Alternative efficient mating plan for breed reconstitution 68
6 Reconstitution using only embryos versus a reduced number of embryos and semen 74
7 Frog erythrocytes in the "unfrozen fraction", which is enclosed by growing masses of ice 86
8 Effect of subzero temperature on salt concentration in the presence and absence of glycerol 88
9 Effect of cooling rate on survival of different kinds of cells 89
10 Example of a programmable freezer 90
11 Examples of freezing curves in programmable and non-programmable freezers 91
12 Commonly used approach for collecting embryos (morulae and blastocysts) from sheep and goats 103
13 Using a forceps to induce ice-crystal formation (seeding) in a plastic straw 108
14 Transvaginal ultrasound-guided oocyte aspiration (TUGA) in the cow 111
15 Ultrasound-guided oocyte aspiration in goats 113
16 Reviewing requests for animal germplasm and/or tissue – an example from the United States of America 137

SECTION 1

Confirming the decision to cryoconserve



Confirming the decision to cryoconserve

Conservation of animal genetic resources for food and agriculture (AnGR) may be undertaken for a number of reasons. In developed countries, traditions and cultural values are important driving forces in the conservation of breeds at risk and the emergence of niche markets for livestock products. However, in developing countries the immediate concerns are food security and economic development.

In general terms, objectives for AnGR conservation fall into the following categories:

- Domestic animal diversity should be maintained for its economic potential in allowing the livestock sector to respond to changes in agro-ecosystems, market demands and associated regulations, availability of external inputs, disease challenges or a combination of these factors.
- Domestic animal diversity plays an important social and cultural role. Loss of typical breeds, therefore, means a loss of cultural identity for the communities concerned and the loss of part of the heritage of humanity.
- Domestic animal diversity is an integral part of the environment in a range of production systems. The loss of this diversity would increase instability and risk in these production systems and reduce their ability to respond to changes. Maintenance and development of adapted breeds are critically important in ensuring that food security can be achieved sustainably without adverse environmental impact.
- Domestic animal diversity should be conserved for research and training. This may include basic biological research in genetics, nutrition, reproduction, immunology and adaptation to climatic and other environmental changes.

The specific objective or objectives for conserving a given AnGR will influence the strategy employed to conserve it. Conservation strategies can be categorized either as *in situ* conservation (in which animals are maintained within the environments or production systems in which they were developed) or as *ex situ* conservation (all other cases). The latter can be further divided into *ex situ – in vivo* conservation and cryoconservation.

IN SITU CONSERVATION

In the context of domestic animal diversity, *in situ* conservation primarily involves the active breeding of animal populations for food and agricultural production in such a way that diversity is optimally utilized in the short term and maintained for the longer term. Activities pertaining to *in situ* conservation include performance recording schemes, development of breeding programmes and management of genetic diversity within populations. *In situ* conservation also includes steps taken to ensure the sustainable management of ecosystems used for agriculture and food production. These various aspects of *in situ* conservation are discussed in detail in the guidelines on In vivo *conservation of animal genetic resources* (forthcoming) and on *Breeding strategies for sustainable management of animal genetic resources* FAO (2010) in this series.

EX SITU CONSERVATION

In the context of domestic animal diversity, *ex situ* conservation means conservation away from the habitat and production systems where the resource developed. This category includes both the maintenance of live animals and cryoconservation.

Ex situ – in vivo conservation

Ex situ – in vivo conservation is *ex situ* conservation in which germplasm is maintained in the form of live animals. As in the case of *in situ* conservation, it is accepted that improvement and natural selection may alter gene frequencies in the conserved population. A key question with respect to this strategy is whether or not long-term finances and commitment are available to maintain generations of animals to the standards required for successful conservation (i.e. with a sufficiently large population size). More details on *ex situ – in vivo* conservation can be found in the forthcoming FAO guidelines In vivo *conservation of animal genetic resources*.

Cryoconservation

Cryoconservation is the collection and deep-freezing of semen, ova, embryos or tissues for potential future use in breeding or regenerating animals. A key question with respect to cryoconservation is whether, in the short term, the facilities and expertise required for collecting the samples can be financed and put in place. The logistics and costs of establishing and maintaining storage facilities will need to be addressed before the cryoconservation scheme is set up.

COMPLEMENTARY ROLES OF *IN SITU* AND *EX SITU* CONSERVATION

The Convention on Biological Diversity[1] emphasizes the importance of *in situ* conservation and considers *ex situ* conservation to be an essential complementary activity.[2] *In situ* and *ex situ* conservation are complementary rather than mutually exclusive. The exact conservation strategy adopted will depend on the specific objectives. *In situ* and *ex situ* strategies differ in their capacity to achieve different objectives.

From a general point of view, *in situ* conservation is often regarded as the preferable method because it ensures that breeds are maintained in a dynamic state. This may be true when the dynamics of a breed are characterized by slow and balanced adaptation to the conditions in which it is maintained. However, commercially important breeds are often subject to high selection pressures and larger than desired levels of inbreeding (a few top sires fathering many offspring), while commercially less-important breeds often have a small population size and are threatened by genetic drift and extinction. Moreover,

1 http://www.cbd.int/convention/articles/?a=cbd-08

2 http://www.cbd.int/convention/articles/?a=cbd-09