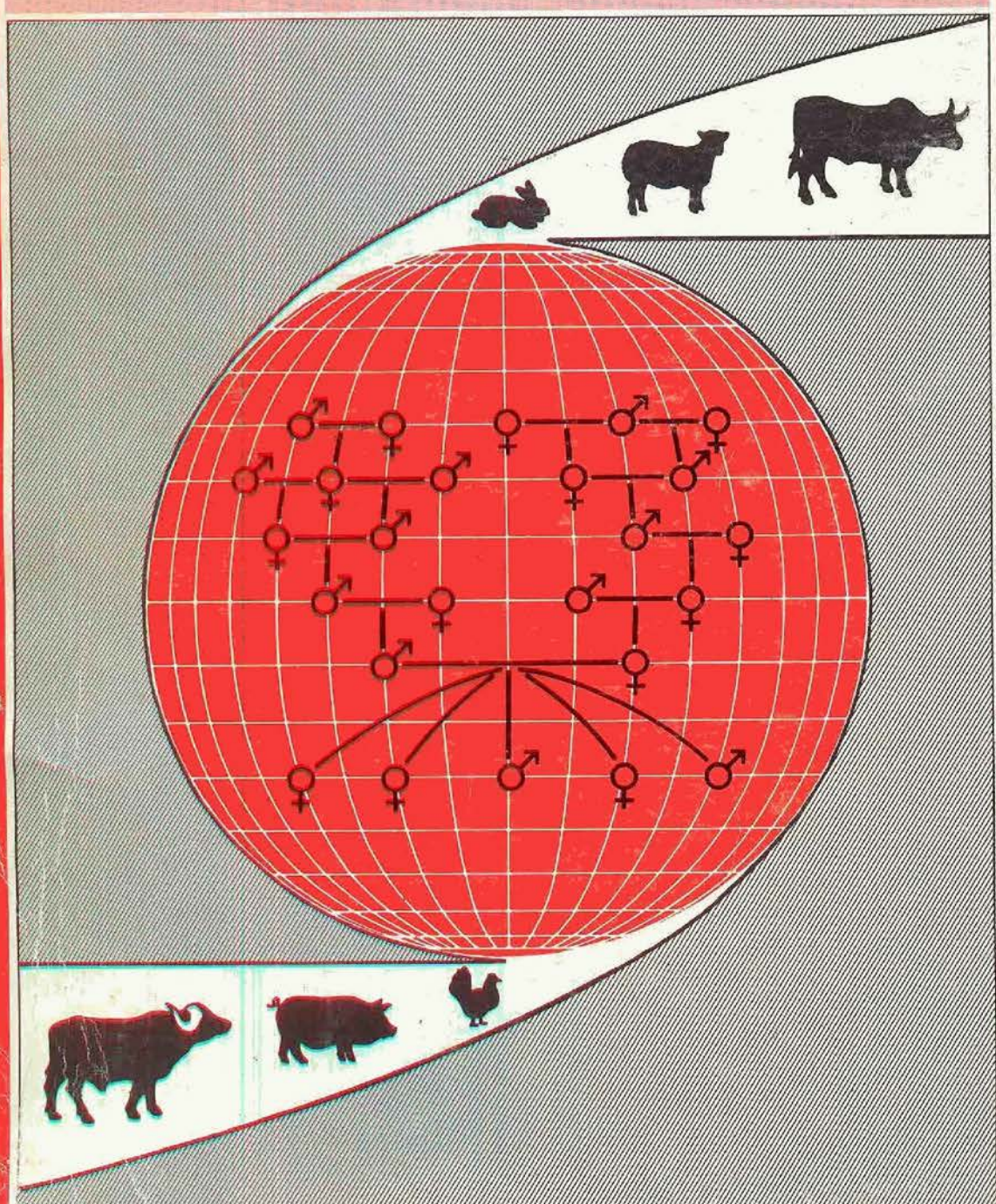
Animal genetic resources: cryogenic storage of germplasm and molecular engineering



FAO ANIMAL PRODUCTION AND HEALTH PAPER

44/2

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Animal genetic resources: cryogenic storage of germplasm and molecular engineering

Proceedings of the Joint FAO/UNEP
Expert Panel Meeting, October 1983
Part 2

FAO
ANIMAL
PRODUCTION
AND HEALTH
PAPER

44/2

FOOD
AND
AGRICULTURE
ORGANIZATION
OF THE
UNITED NATIONS
Rome, 1984

M-22
ISBN 92-5-102111-2

FOREWORD

Animal Genetic Resources are of immense importance to mankind, since they comprise the domestic livestock and birds which provide food, fibre and work, as well as contributing other products and benefits for human welfare throughout the world. The Conservation and Management of Animal Genetic Resources is therefore of concern to the Food and Agriculture Organization of the United Nations (FAO) and the United Nations Environment Programme (UNEP). These two organizations have been working together in recent years to design and apply an appropriate strategy for application at national, regional and global levels which will both improve the immediate productivity of domestic animals and birds and also preserve the valuable, but currently unused breeds for posterity.

In 1983, FAO and UNEP created an Expert Panel of 36 eminent scientists to advise on Animal Genetic Resources Conservation and Management. The first meeting was held in Rome in October 1983, at which work in progress was evaluated, and recommendations made for the next few years. The meeting addressed 4 topics:

Conservation by management
Animal genetic resources data banks
Training methods in animal genetic resources
Cryogenic storage of germplasm and genetic engineering

The Proceedings of the meeting, with the full texts of the working papers presented and subsequently amended by the authors, are now published in two parts. Part 1 contains the papers presented in the first three topic areas. This is Part 2 and contains the papers in the section on Cryogenic storage of germplasm and genetic engineering*. A short Report on the meeting containing summaries of the papers and the recommendations has already been published in English, French and Spanish. Parts 1 and 2 of the Proceedings and the Report are available from FAO Animal Production and Health Division, Rome, or from the official FAO sales agents.

The Proceedings provide an overview of the work currently in progress and visualized for the near future. Those wishing to keep in touch with the ongoing work are invited to send their name and address to FAO at the above address, for regular receipt of Animal Genetic Resources Information (AGRI), a newsletter published twice a year, and sent free of charge to all concerned with the conservation, management or utilization of domestic animals and birds.

* The assistance of Mr. J.D. Turton, Director of the Commonwealth Bureau of Animal Breeding and Genetics, Edinburgh, UK, in editing the papers of Part 2, is acknowledged.

CONTENTS

WELCOME AND INTRODUCTORY REVIEW

ADDRESS OF WELCOME

by

Dr. R.B. Griffiths
Director, Animal Production and Health Division, FAO

I am pleased to welcome you here on behalf of the Director-General of FAO and the Executive Director of UNEP to the First Meeting of the Joint Expert Panel on Animal Genetic Resources Conservation and Management. It is encouraging to see such a distinguished group of scientists, representing, as you do, not only the many separate disciplines of genetics but also different regions of the world. It is noteworthy that although you are here in your personal capacities, and not representing your governments, you are drawn from developing countries in Africa, Asia, Latin America and the Caribbean, as well as from the Middle East; and also from developed countries in Europe, North America and Asia. Such widespread and diverse backgrounds, qualifications and experiences among its members augurs well for the work of the Expert Panel.

Although FAO has been active in animal breeding since its inception, the joint approach since 1974 with UNEP has added a new dimension to the conservation and management of animal genetic resources. A number of joint activities have been undertaken, including such projects as the survey of indigenous sheep breeds in the Middle East, the study of the use and potential of trypanotolerant livestock in Africa, in association with ILCA, and expert consultations on the breeding of Mediterranean cattle and sheep, on the breeding of dairy cattle in the humid tropics, and on the evaluation and conservation of animal genetic resources in Latin America.

The Joint Consultation of FAO and UNEP on Animal Genetic Resources in 1980 provided the first world focus on the objectives, possibilities, limitations, problems and hopes for this important subject. At that Consultation, in which some of you took part, we arrived at a coherent and rational global plan for action for the next few years. Funding for the implementation of the proposals outlined at that time has since been provided by FAO and by UNEP; and the plans have been endorsed by the governing bodies of FAO. Solid foundations are being laid, and although progress may seem to be slow, it is important to know not only where to go, but also how to get there. For this reason, pilot trials and methodological studies have featured prominently in the recent work of FAO and UNEP. This work is directed operationally by the Animal Production Service of FAO. One of the tasks we shall ask you to address during this week is an appraisal of the work which is now in progress. Additionally, we shall be looking to you for new ideas on some of the problems which have been encountered to date.

The subject is a fascinating one, partly because of the unusual combination of objectives. First, we seek not only to harness the animal genetic resources of developing countries in improved management programmes to enhance the production of food, fibre and animal power, but secondly we aim to conserve, in the preservation sense, those genetic resources that are in danger of being lost. The first is capable of yielding immediate results, whereas the second is a means of maintaining the flexibility necessary to respond to future unforeseen changes in animal production and market requirements. Cryogenic storage of fertilized ova and semen constitute appropriate means for the conservation of animal resources, but the mechanics of achieving them in the developing world are yet to be worked through.

Overhanging in this field of interest is the rapid, and to some, terrifying speed of research and development in genetic engineering, which regularly breaks new barriers and opens new scenarios of opportunity. It seems likely that we shall find these affecting both our attempts to increase animal productivity and our options for storing genotypes or even gene segments at risk of extinction. We are maintaining close links with the proposals for the establishment of an International Centre for Genetic Engineering and Technology and we expect to play a lead role in work on animal genetics if and when the Centre materializes.

All of this underscores the need for a wide base of continuing consultation and up-to-date scientific knowledge, combined with concern and commitment to the needs of the developing world. This is another of your roles on this Panel.

The subject of communication is of first rank in every area of human activity and includes as prerequisites the storage of knowledge and the provision of ready access to data needed for competent decision making. In 1980, recommendations were made therefore to create data banks on animal genetic resources and also for a Newsletter. I am glad to tell you that both have been started. We shall be inviting your involvement in facilitating their growth to maturity and their use. Only then will they contribute to the prudent conservation and management of animal genetic resources throughout the world.

We have noted a growing and very encouraging interest throughout the world during the 3 years that have followed the Expert Consultation on Animal Genetic Resource Conservation and Management held in 1980. Several groups of scientists, gathering principally for other purposes, have turned their attention to the matter, and have passed resolutions calling for increased activity at the international level by responsible bodies including FAO, UNEP and CGIAR. We regard the creation of this Joint Expert Panel between FAO and UNEP as a realistic step towards the establishment of an international programme in this field.

We are pleased today that among you are those who, while here in a personal capacity, are nevertheless associated with the work of other organizations in Animal Genetic Resources Conservation and Management. These include the Society for the Advancement of Breeding Researches in Asia and Oceania (SABRAO), The Interafrican Bureau for Animal Resources (IBAR), the Latin American Society for Animal Production (ALPA), the International Livestock Centre for Africa (ILCA) and the Commonwealth Bureau of Animal Breeding and Genetics (CAB). Our working relationships with each of these organizations have been established for many years, and we are pleased that during the last year we have been able to strengthen them further in the pilot trials now under way for data bank establishment.

Finally, may I convey good wishes to you for success in the meeting, not only from myself but also from Dr. D.F.R. Bommer, the Assistant Director-General (Agriculture) of FAO, who would be here welcoming you today but for his unavoidable absence for meetings in the USA. We thank you for coming. We look forward with great interest to your recommendations on this important subject of Animal Genetic Resources Conservation and Management.

REVIEW OF THE FAO/UNEP PROGRAMME ON ANIMAL GENETIC RESOURCES CONSERVATION AND MANAGEMENT

John Hodges[1]

In 1980 FAO and UNEP held a Technical Consultation on Animal Genetic Resources Conservation and Management, which resulted in a number of specific recommendations. The Consultation was global in concept, and was the culmination of earlier work done by FAO and later in cooperation with UNEP, at local, national or regional levels. The Consultation brought together representatives of member countries, scientists and administrators. The recommendations are given in Appendix A to this paper.

Since then, FAO and UNEP have drawn up a cooperative programme of work which is operated by FAO, with funding from the FAO/UNEP project entitled Conservation of Animal Genetic Resources - Phase II and from FAO's Regular Programme. I will deal in turn with the items, giving an outline of the stage reached. Most of the items are also on the agenda for this meeting, and opportunities for detailed discussion of them will therefore arise later.

1. THE JOINT FAO/UNEP EXPERT PANEL ON ANIMAL GENETIC RESOURCES CONSERVATION AND MANAGEMENT

The Terms of Reference are given in Appendix B. The Panel was constituted earlier this year with 36 members being appointed (Appendix C) out of a possible maximum of 40. Members were nominated and agreed by both organizations. Members will serve for four years in a personal capacity and not as representatives of their governments. Consultation will be by meetings such as this, by correspondence with groups or with individuals, depending upon the subject matter. On this occasion 19 of the Panel Members have been invited to attend; in addition 5 invited speakers with special expertise are also invited. Future meetings will be held when needed and will be alternately funded by FAO and UNEP. FAO is funding this meeting from its Regular Programme budget.

The organization of the meeting is in sections each with a rapporteur who has kindly agreed to prepare written summaries of the major points presented and discussed, and also to bring to the meeting on the last afternoon the recommendation of their sections. In preparing these, they will be supported and guided, not only by the general discussion, but also by the speakers in the sections concerned, who with them will form a small consultative group to formulate the recommendations. Following the meeting, a report with the summaries of the meeting and the recommendations will be produced; later, proceedings will be published carrying the papers. Authors will, of course, have the opportunity of revising their working papers before publication, and should return their amended papers to me by 31 December for inclusion in the proceedings.

---

[1] Animal Production Officer (Animal Breeding and Genetic Resources), Animal Production and Health Division, FAO, Rome.

I should add that the proceedings will be published in two parts. Part 1 will carry all papers except those in the section on Cryogenic Storage of Germplasm and Molecular Engineering, which will be in Part 2, and published separately. The authors for this section, at the request of FAO, have produced longer papers and the publication on this topic is intended to contain comprehensive reviews of the subject matter.

2. DATA BANKS

This topic was the subject of a recommendation in 1980. During the last six months pilot trials for one year each have been established in Africa, Asia and Latin America. Their main purpose is to identify appropriate methodologies for the preparation of data for the bank, working with a variety of species in different parts of the world. A more detailed review of the work in progress on this subject will be given later in the meeting.

3. CONSERVATION PROJECTS

Some breeds with economic potential, which are often scattered in several countries, or which are in danger of total loss through having very small population size, are the subject of pilot conservation projects. These are at various stages of progress, and include some of the few dairy breeds suited to the tropics, such as the Sahiwal, Kenana and Butana breeds; also trypanotolerant cattle breeds and sheep breeds in West Africa. Others are being explored in association with the development of data banks. More details of these will be given in the section on this subject later in the meeting.

4. GENE BANKS

The concept of conservation by gene banks is complex. One can think of live animals, being preserved *in situ*, or in some semi-artificial situation; alternatively one may think of cryogenic storage of perm or fertilized ova or other tissues or gene segments. The economic problems are difficult with both live animals and with haploid or diploid cells. Who is to pay? There are also questions of how many to preserve, for how long, and where. These questions are not easy to solve even in the developed countries where cryogenic technology had its origin, but they become especially difficult when applied to the developing countries. At present the plan is to seek the advice and recommendations of this Expert Panel and of other scientists in the field, and as already mentioned to publish their work in a special volume. Then, a feasibility study is planned to study some of the practical problems of costs, health control, movement of semen across national boundaries, safety, etc.

5. TRAINING COURSES

The first training course for animal scientists from developing countries in Animal Genetic Resources Conservation and Management was organized by FAO/UNEP in September 1983. It was held in English and was mounted by the Hungarian University of Veterinary Science in Budapest. This Institution was chosen because of the advanced stage of planning and live animal conservation initiated in Hungary and

because of the body of lecturers available in the country. They were supplemented by lecturers from other countries. Eighteen animal scientists from fifteen countries were present. You will hear more later in the meeting. The intention is to arrange further courses, as the demand exists, from other developing countries and as the technology and experience in the subject matter advances. Other courses may be held in French and Spanish as needed.

6. NEWSLETTER

As requested by the 1980 Consultation, FAO and UNEP have started a newsletter for all concerned with the subject of animal genetic resources conservation and management. It will be published twice yearly. The first issue was in the summer of 1983 and the second will be early in 1984. An initial mailing list of 1000 was created. The newsletter, known as Animal Genetic Resources Information (AGRI) will have 40/50 pages, and will carry articles of up to 3000 words with illustrations, news items, book reviews, descriptions of methodologies, details of breeds in need of conservation and plans for undertaking this work. It will also report on the activities of FAO and UNEP. It is also hoped that there will be a correspondence section which we encourage members of the Expert Panel to initiate and use. We also invite you to nominate others who ought to be on the mailing list.

7. INVENTORIES

The 1980 Consultation drew attention to the lack of information in the West about the extensive livestock resources of the USSR and of China. Initiatives by FAO/UNEP have resulted in a positive response from the USSR in the last month, indicating their willingness to cooperate in creating an inventory of their breeds. This work will be carried out by scientists of the USSR with all the support on techniques and experience that can be offered by FAO and UNEP. The Inventory will be published in Russian and in English. It is also planned to link this inventory with the creation of data banks. We are less advanced in our contact with China, but are glad to be able to announce the intention of FAO to publish, in English, Professor Cheng's book on Chinese Livestock Breeds, already published in Chinese by Chinese Academic Publications. We are fortunate in having the services of Dr. Helen Newton-Turner as editor to prepare the manuscript in readiness for the English publication. Hopefully, this initial work will be extended by further cooperation with out animal geneticist colleagues in China.

These are the principal activities currently underway as a result of the 1980 Consultation. In conclusion, I would like to add that in most of the components of this extensive programme, we are able to cooperate with national and regional organizations as well as with national governments. For example, in Asia, with The Society for the Advancement of Breeding research in Asia and Oceania (SABRAO); in Africa with the Interafrican Bureau for Anima Resources of the Organization of African Unity (IBAR of OAU) and the International Livestock Centre for Africa (ILCA); in Latin America the Latin American Association of Animal Production (ALPA).

APPENDIX A

I. RECOMMENDATIONS OF THE FAO/UNEP TECHNICAL CONSULTATION ON ANIMAL GENETIC RESOURCES CONSERVATION AND MANAGEMENT, ROME 1980

A. Recommendations to FAO/UNEP

1. It is recommended that FAO establish an appropriate coordinating mechanism for the conservation and management of the world's farm animal genetic resources at national, regional and international levels, with the following terms of reference:

   i. To give support and advice to existing activities concerned with breeding programmes, management and conservation of the world's farm animal[1] resources and to find means of providing a framework for cooperation.

   ii. To stimulate the establishment of activities with respect to the conservation of farm animal genetic resources in countries where no such activities exist, but are required.

   iii. To stimulate the establishment of regional activities and laboratories devoted to the documentation, evaluation and conservation of regional livestock resources, including the rationalization of breeding programme development and conservation programmes in each of the countries of each region.

   iv. To stimulate the development of training programmes at regional level for the techniques appropriate to the conservation and management of farm animal genetic resources.

   v. To promote research on the mechanisms of adaptation and disease resistance and tolerance in the genetic stocks in developing countries.

   vi. To facilitate study of health barriers to the international exchange of genetic materials.

2. FAO/UNEP are requested to arrange for the preparation and distribution of an international newsletter on the conservation and management of farm animal genetic resources. The newsletter should provide information about training programmes, techniques, activities and developments; should contain a correspondence section; and should be a means of stimulating cooperation on a worldwide basis.

3. It was agreed that the FAO/UNEP project had brought out a great deal of interesting information on livestock populations and their conservation.

---

[1] The term "farm animals" in this document includes all domesticated mammalian and avian species.

However, it was noted that the information was very incomplete and that in particular, the project did not include two of the major livestock countries of the world, namely China and the USSR, and barely touched on a third, namely the USA. The Consultation therefore recommended that FAO and UNEP, in collaboration with the countries concerned, should try to complete this study.

4. FAO/UNEP should examine the feasibility of establishing one or more centres for the conservation and long-term storage of genetic material - a gene bank. Each gene bank should be designed, health considerations permitting, to serve a region and should be capable of long-term storage of semen, oocytes and embryos (and other types of genetic material where appropriate) of all farm species with which storage is possible. FAO/UNEP should include in the feasibility study the training needs for the establishment, maintenance and use of regional gene banks; the nature (location, size, etc.) and control (health and safety) of stored genetic material; and the circumstances relating to the choice of initial material for storage and the release and replacement of stored material.

B. Recommendations to FAO/UNEP and Member Governments

5. It is recommended that FAO/UNEP assist in the development of a data bank for livestock resources in member countries, and in the coordination of these at regional levels. In this context, it is recommended that FAO/UNEP should investigate:

   i. the development of standardized definitions, nomenclature and data collection and collation systems;

   ii. the provision of assistance to existing regional organizations, and the development of, and subsequent assistance to, necessary new regional organizations in maintaining documentation systems;

   iii. the development of a two-stage data bank system

      a. initially emphasizing enumeration of breed populations, population structure and minimum information on productive and adaptive characters;

      b. to be followed in each country as part of breeding programme development by more extensive documentation of performance and adaptive traits and the environmental conditions under which performance, etc. was measured.

6. In view of the importance of adapted breeds for agricultural development in general and for the promotion of the livestock indutries in particular, it is recommended that FAO should encourage Member Governments and/or participating organizations to include in the agricultural development programmes a component for the development and conservation of local breeds. Such breed development and conservation should take account of economic and genetic considerations appropriate to local conditions.

7. The implementation of breeding programmes at the national level would be greatly facilitated by the introduction of routine recording, evaluation and

selection procedures. FAO should assist in the establishment of a limited number of pilot schemes for selection in local populations which pioneer methods of livestock improvement that make most efficient use of limited resources and infrastructure.

8. Several important breeds in the developing world are spread over a number of countries covering one or more regions. FAO should assist the governments concerned to cooperate in the implementation of a common programme for the genetic improvement and conservation of each such breed.

9. International research projects should be stimulated with a view to (a) the comparison under different environmental conditions, of breeds from different countries, and (b) the clarification of the genetic nature of any differences observed and their implications for breeding programmes. (These might be arranged via AI on the lines of the current dairy cattle strain comparisons in Poland and Bulgaria or suitable modifications of them. Or they might be performed using the technique of reference breeds (control breeds).) Groups of breeds for consideration include prolific sheep, tropical beef cattle and buffaloes.

10. There are several livestock species/breeds which are adapted to very specific environments and which play a major role in rural economies (e.g. the Andean Camelidae, Old World camels, the Himalayan Bovidae, livestock in tsetse-infested areas, etc.). In spite of their importance, too little is known about these species/breeds. It is recommended that international support be given to the governments concerned for studies on their biology, genetic profile, genetic improvement and conservation. Special attention should be paid in this context to endangered as well as genetically unique species/breeds that have particular traits to an exceptional degree and deserve priority treatment.

11. Some livestock breeds which played a significant role in the past in the rural economies of developed countries, and which were adapted to specific environments, are now in danger of disappearing (e.g. seaweed eating sheep, heavy draught horses, breeds of large donkeys). It is recommended that international encouragement be given to the governments concerned, for their conservation, and where not so far available, for their study.

12. The Consultation urged all governments to give full consideration to ways and means of conserving viable populations of wild animal species, including avian, which are the ancestors or close relatives of domestic species and recommended that FAO and UNEP expand their programmes in support of the establishment and improved management of national parks and reserves.

APPENDIX B

EXPERT PANEL ON ANIMAL GENETIC RESOURCES CONSERVATION AND MANAGEMENT

TERMS OF REFERENCE

1. Background and Justification

In the 1930s and 40s the scientific basis for the genetic selection of animals was worked out in institutions in Europe and the United States of America. The application of these findings to practical animal breeding improvement programmes has made possible an unprecedented rate of increase in the production of food and fibre per animal. A few high performance breeds have emerged which are gradually displacing the local breeds in temperate regions. As a result there is growing concern that the latter may disappear altogether unless special efforts are made to conserve them.

The developing countries are likewise increasingly concerned about their livestock resources, especially after the many large scale introductions of high-yielding breeds from the temperate zones which often cause a decline in the numbers of local livestock types. The latter have, through natural and man-selection, developed characteristics which make them well adapted to the often harsh environmental conditions under which livestock have to live and produce in these areas. This valuable genetic material needs to be maintained and improved as the basis for national livestock breeding programmes and policies.

The problems facing the world's animal genetic resources were identified by a high level FAO/UNEP Technical Consultation held in 1980 as being principally of three kinds. The first is a decrease in genetic variability within breeds; this is mainly a problem of the high-yielding breeds maintained in temperate zones and employed in intensive production systems. The second is the rapid disappearance of indigenous breeds and strains of domestic animals through the indiscriminate introduction of exotic breeds. The third concerns the special problem of hot, humid climates and other harsh environments common the developing countries. Only in restricted areas within these environments is it possible to improve animal health protection measures and feeding and management practices to levels that would allow high-yielding animals from the temperate zones to be used. In these circumstances the need is to design and implement appropriate selective breeding programmes based on existing populations of animals adapted to harsh environments.

The emerging awareness of the need for urgent action to conserve and develop the world's animal genetic resources has resulted in a number of limited and mostly uncoordinated efforts in this direction. Regional agricultural and/or animal husbandry organizations in Africa (IBAR of OAU), Europe (EAAP), Asia and the Pacific (SABRAO) and Latin America (ALPA) have set up committees on animal genetic resources and initiated studies on their management. However, there is an obvious need for the coordination of these activities as well as for the continuous exchange of information on experiences, achievements and methodologies for the efficient management and conservation of animal genetic resources for future needs. The future potential use of

a specific animal genetic resource may not necessarily be confined to the country or area where it is at present threatened. Instead, it may well prove its usefulness in some other part of the world. This fact underlines the need for a strong involvement of international bodies like FAO and UNEP.

In recent years techniques for the recovery of embryos of animals and their long term conservation at supra-low temperatures have been developed and the scientific research in this field is at present in a very intensive phase of development. In consequence, new knowledge is being continuously generated on animal genetic resources conservation in vitro, for both short and longer term periods. At present, of course, the development of the embryo transfer/storage techniques is geared mainly toward its immediate use for commercial purposes. But the potential for its use in connection with the conservation of animal genetic resources is great. This would require its continuous study at the global level. There is already information available that embryo banks are being established in some of the industrialized countries.

In the light of the above considerations, it would be desirable to establish an FAO/UNEP Panel of Experts on Animal Genetic Resources Conservation and Management. This would be consistent with the recommendations of the FAO/UNEP Technical Consultation (1980) that FAO and UNEP establish an appropriate coordinating mechanism for the conservation and management of the world's farm animal genetic resources at national, regional and international levels. The work of the Panel will be enhanced by support from UNEP through the FAO/UNEP Project on Conservation of Animal Genetic Resources - Phase II which was recently approved.

II. Objectives and fields of activity

The objectives of the Panel would be to:

- Review periodically ongoing work on animal genetic resources conservation and management in the different parts of the world and delineate future work programmes on a priority basis.
- Identify the principal problems hampering the exploitation and improvement of animal genetic resources at national and regional levels.
- Determine how these problems may be solved, what action programmes and projects may be developed in given situations, and how existing national and regional organizations may be strengthened for this purpose.
- Formulate ways and means of stimulating regional and global cooperation in programmes for promoting animal genetic resources development with special emphasis on mutual assistance among national and regional institutions.
- Advise the Director-General of FAO and the Executive Director of UNEP on critical issues relating to the conservation and management of animal genetic resources.

The Panel activities will cover the following fields:

(i) Genetic resources conservation and management activities at global, regional and subregional levels.

(ii) The design and implementation of selective breeding programmes for animal populations in harsh environments.

(iii) The establishment and operation of data banks on animal genetic resources.

(iv) The development and application of an in situ animal genetic resources conservation methodology.

(v) Public relations and collection and dissemination of information programmes for animal genetic resources conservation in developing countries.

(vi) The development and application of an in vitro conservation methodology on animal genetic material, including disease control aspects.

(vii) The development and maintenance of inventories of animal genetic resources and of a global register of such resources.

III. Membership

The Panel will be a standing and authoritative body of experts, the total number not to exceed 40. The number of participants at specific meetings will depend on the topics dealt with, as well as on the budgetary allocations available.

Half of the members will be nominated by the Director-General of FAO and half by the Executive Director of UNEP. The nominations will be made through consultation between the two agencies to avoid overlapping and to make certain that subject coverage and geographic and linguistic distribution are adequately taken into account.

Responsibility for convening meetings of the Panel would rest with FAO after consultation with UNEP. Secretariat arrangements will be handled by FAO.

In view of the need to obtain the broadest possible involvement in the conservation of animal geneic resources, it is envisaged that other international agencies concerned, such as UNDP and the World Bank, will be encouraged to support the Panel.

IV. Expected duration of the Panel

The problems relating to animal genetic resources conservation and management will require increasing attention over a long period of time. The problems are often complex and are usually not amenable to uniform "one time" solutions. The long generation intervals of the larger species of domestic animals increase the time span required for arriving at viable solutions. Therefore, a long term FAO/UNEP responsibility for the coordination of animal genetic resources conservation has to be accepted. Initially, a six-year duration of the Panel is foreseen, as is an extension, taking into account experiences gained during the initial period.

V. Periodicity of sessions

It is proposed to have a minimum of one panel session every third year. The actual need for panel work is likely to be much higher. FAO and UNEP would, however, make efforts to hold panel meetings more frequently. The parties would also meet the need for expert advice, at least partially, by correspondence with the institutions and/or individuals involved in animal genetic resources conservation work, the world over.

APPENDIX C

MEMBERS OF THE JOINT FAO/UNEP PANEL OF EXPERTS ON ANIMAL GENETIC RESOURCES AND MANAGEMENT

Dr. R.M. Acharya
Deputy Director-General
Indian Council of Agricultural Research
Krishi Bhavan
NEW DELHI 110 011, India

Mr. K.O. Adeniji
Chief, Animal Production Section
Interafrican Bureau for Animal Resources
P.O. Box 30786
NAIROBI, Kenya

Professor J.S.F. Barker
Head, Department of Animal Science
University of New England
ARMIDALE, NSW, Australia

Dr. P.N. Bhat
Director
Central Institute for Research on Goats
P.O. Farah-281122
MATHURA (UP), India

Professor I. Bodó
Associate Professor
Department of Animal Husbandry
University of Veterinary Science
P.O. Box 2
H-1400 BUDAPEST 7, Hungary

Dr. E. Ceballos Bueno
Director, División Ciencias Animales
Instituto Colombiano Agropecuaria
BOGOTA, Colombia

Professor Charan Chantalakhana
Department of Animal Science
Kasetsart University
BANGKOK 10900, Thailand

Professor Pei-Lieu Cheng
Chinese Academy of Agricultural Sciences
West Suburbs
BEIJING, China

Professor R.D. Crawford
Department of Animal and Poultry Science
University of Saskatchewan
SASKATOON S7N 0WO, Canada

Professor E.P. Cunningham
Deputy Director
The Agricultural Institute
19 Sandymount Avenue
DUBLIN 4, Ireland

Dr. J. de Alba
Head, Animal Production
Ganaderia
CATIE
TURRIALBA
Costa Rica

Professor G.E. Dickerson
Research Geneticist, Roman L. Hruska US Meat Animal Research Centre, ARS, USDA;
and Professor of Animal Science, University of Nebraska
225 Marvel Baker Hall
LINCOLN, Nebraska 68583, USA

Dr. H.A. Fitzhugh
Programme Officer
Winrock International Livestock and Training Centre
Route 3
MORRILTON, Arkansas 72110, USA

Dr. G.E. Joandet
Director Nacional Asistente de Investigación Agropecuaria
Instituto Nacional de Tecnología Agropecuaria
Rivadivia 1429
1033 BUENOS AIRES, Argentina

Professor J.W.B. King
Animal Breeding Liaison Group
School of Agriculture
West Mains Road
EDINBURGH EH9 7PQ, UK

Professor A. Lahlou-Kassi
Department of Reproduction
Agricultural and Veterinary Institute
Hassan II
Agdal, RABAT, Morocco

Dr. J.J. Lauvergne
Maître de recherches
Laboratoire de Génétique factorielle
Centre National de recherches Zootechniques
F 78350 JOUY-EN-JOSAS, France

Dr. F.E. Madalena
Animal Breeding Specialist
Centre Nacional de Pesquisa - Gado de Leite
Caixo Postal 151
CORONEL PACHECO 10, MG, Brazil

Dr. Y. Madkour
Agricultural Research Centre
Ministry of Agriculture
DOKKI, CAIRO, Egypt

Professor R.E. McDowell
Department of Animal Science
Frank B. Morrison Hall
Cornell University
ITHACA, NY 14853, USA

Dr. L.O. Ngere
Senior Lecturer
Department of Animal Science
University of Ibadan
IBADAN, Nigeria

Dr. C. Novoa
Instituto Veterinario de Investigaciones Tropicales y de Altura (INVITA)
LIMA, Peru

Professor A.H. Osman
Director
Institute of Animal Production
University of Khartoum
Shambat, KHARTOUM, Sudan

Professor D. Plasse
Facultad de Ciencias Veterinarias
Universidad Central de Venezuela
c/o Apartado 2196, Las Delicias
MARACAY, Venezuela

Dr. C. Polge
Acting Director
Animal Research Station
Agricultural Research Council
307 Huntingdon Road
CAMBRIDGE CB3 0JQ, UK

Professor J. Rendel
Department of Animal Breeding and Genetics
Swedish University of Agriculture
S-750 07 UPPSALA, Sweden

Professor A. Robertson
Institute of Animal Genetics
University of Edinburgh
West Mains Road
EDINBURGH EH9 3JX, UK

Professor Giuseppe Rognoni
Instituto di Zootecnica
Facoltà de Veterinaria
Università degli Studi di Milano
Via Celoria 10
20133 MILANO, Italy

Dr. S. Sivarajasingam
Research Scientist
Animal Science Division
Malaysian Agricultural Research and Development Institute (MARDI)
SERDANG, Selangor, Malaysia

Dr. A. Teixeira Primo
National Centre for Genetic Resources
EMBRAPA-CENARGEN
BRASILIA, Brazil

Dr. J.C.M. Trail
Senior Scientist
International Livestock Centre for Africa (ILCA)
P.O. Box 46847
NAIROBI, Kenya

Mr. J.D. Turton
Director
Commonwealth Bureau of Animal Breeding and Genetics
King's Building
West Mains Road
EDINBURGH EH9 3JX, UK

Professor S. Wierzbowski
Department of Animal Reproduction and Artificial Insemination
Institute of Zootechnics
32-083 BALICE/KRAKOW, Poland

Dr. H. Newton-Turner
Genetic Research Laboratories
Commonwealth Scientific Industrial Research Organization (CSIRO)
P.O. Box 184
NORTH RYDE 2113, NSW, Australia

Professor J.H. Weniger
Institut für Tierzüchtung und Haustiergenetik
Lentzealle 75
1 BERLIN 33 (Dahlem), FR Germany

Professor Yukio Yamada
Professor of Animal Resources
Division of Tropical Medicine
Kyoto University
KYOTO 606, Japan

CRYOGENIC STORAGE OF GERMPLASM AND GENETIC ENGINEERING

GENETIC ASPECTS OF GERMPLASM STORAGE AND GENETIC ENGINEERING

H.A. Fitzhugh[1]

A 19th century American author, Washington Irving, wrote a popular story about Rip Van Winkle, who awoke after a 20 year nap to find that his world had greatly changed. Animal breeders trained in the Sixties or earlier can be pardoned for feeling somewhat like Mr. Van Winkle as they ponder the new technologies available to implement genetic change. Directed genetic improvements are still largely accomplished through the fundamental methods of selection and migration. But breakthroughs in reproductive and genetic technology over the past few decades now make possible directed combination of individual gametes and even interspecies transfer of genetic material. Technologies such as artificial insemination and embryo transfer greatly increase the intensity of selection, especially if combined with other technologies, such as sex control. These, and other technologies, have come to be known under the broad definition of genetic engineering.

Before proceeding with a review of these newer technologies and their potential impact, it serves a useful purpose to quote from a recent paper by Dickerson and Willham (1983):

> "... Application of knowledge to useful ends is engineering. Application of quantitative genetics to improve animal production certainly qualifies as genetic engineering, but has the journalistic disadvantage of a longer history compared with the novelty of DNA transfer."

Rutledge and Seidel (1983) make much the same point:

> "Genetic engineering is defined as those technologies that make possible directed changes in gene or genotype frequencies of a population or that substantially increase the fecundity of individuals so these make a greater than normal contribution to the genetic or zygotic pool of that population."

The terms of reference followed in the development of this paper included:

i. examine the impact that genetic engineering may have on the traditional approaches of classical and quantitative genetics to improve livestock;

ii. review published literature and interpret the effect of genetic engineering on the rate of genetic improvement and design of breeding programmes;

iii. examine genetic implications of storing animal cells for breeds and species that are at risk of elimination and provide guidelines on numbers of cells and animals to be involved in such preservation.

---

[1] Winrock International Livestock Research & Training Center, Morrilton, Arkansas, USA.

Brief descriptions of technologies will be given to set the stage for discussion of their application to livestock improvement. More detailed descriptions are given by the other authors of papers in this publication. The bulk of the discussion has been derived from a review of literature.

1. REPRODUCTIVE TECHNOLOGIES

Regulation of the reproductive rates of individuals is the principal process by which nature and man effect genetic change. The single exception is mutation. This usually has only transient impact on the population, unless it confers an advantage which translates to a higher reproductive rate for carriers.

Until recently, man's regulation of the reproductive process of livestock was largely limited to determining which sires mated with which dams. Major improvements in rate of genetic change became possible through artificial insemination and embryo transfer, by which means man more directly intervened in the harvest, preservation, and transfer of gametes.

1.1 Artificial Insemination

Early use of AI was promoted as a means of controlling venereal disease and utilizing sires unable to mate successfully (e.g. crippled bulls, broad-breasted turkeys). However, with the development of successful semen extenders, a single collection of semen could be diluted by a factor of 100 or more to increase greatly the number of progeny a single male could sire. Successful freezing and storage of semen was the next major technological innovation which stimulated usage of AI, especially for cattle. Although the freezing process kills approximately half the spermatozoa, the subsequent preservation of sperm viability over long storage periods, and the convenience of frozen semen, more than compensate for losses (Foote 1981).

AI has had a marked impact on genetic improvement, with the most dramatic improvements occurring for dairy cattle over the last three decades. Several factors combined to produce these improvements. Through AI, sires could produce daughters in many different herds. Comparison of daughter performance against contemporaries in different herds provided raw material for more accurate estimates of the sire breeding value, using statistical procedures made feasible by advent of high speed computers. Examples of the potential for genetic improvement from AI, either alone or in combination with other technologies, are given in the later section on estimated genetic change.

1.2 Embryo Transfer

The similarities of this technology to AI are obvious. However, the principal differences from AI lie in the greater complexities of embryo harvest and transfer, and the markedly fewer potential numbers of progeny per dam per year.

As Seidel and Seidel (1981) point out, embryo transfer is supported by a number of discrete technologies which in combination make it a practical process. These include oestrus synchronization, superovulation, embryo harvest, and short term *in vitro* culture of ova. Longer-term preservation, while not yet highly successful,

will substantially reduce costs and improve the practicality of embryo transfer. Many of the micromanipulation technologies discussed in a subsequent section also depend on these same support technologies.

1.3 Oestrus Synchronization

The survival probability drops rapidly for embryos transferred to recipients asynchronous with the donor. Thus, superovulation, in which the donor may be providing 5-10 embryos, has practical consequences only when linked with oestrus synchronization of recipients. Even without embryo transfer, oestrus synchronization may also have genetic consequence by improving the feasibility of AI to superior sires, especially under extensive management conditions. Options and future possibilities for controlling oestrus of livestock are discussed by Dzuik and Bellows (1983).

1.4 Superovulation

The genetic and economic value of embryo transfer rose sharply with the development of effective technologies for superovulation. For example, without these technologies, five was the biological upper limit on the number of calves a cow could produce per year. This limit was calculated using values given by Seidel (1981) as follows:

Probability of non-surgical recovery of ovum	= 0.70
Probability that the ovum is not fertilized	= 0.10
Probability that the ovum is abnormal	= 0.10

Thus, the probability of recovering a normal embryo is 0.56. The probability that transfer of this embryo will produce a live calf is 0.7 so (0.56 x 0.7) = 0.39 is probability of obtaining a live calf. Approximately 15 detected oestruses per year per cow can be expected; therefore, (0.39 x 15) = 5-6 calves can be produced per cow per year without superovulation.

Hormonally promoted superovulation may not be appropriate for lactating dairy females because commercial milk yields would be affected; however, most donor females for commercial embryo transfer are superovulated.

1.5 Harvest and Transfer

The process of harvesting and transferring embryos is rather more complicated than that for harvesting semen. Recovery of embryos from donors involving surgery has been largely replaced by non-surgical recovery of 5- to 9-day old embryos from the uterus (Sreenan 1983), especially for commercial transfer where the donor is highly valued. Skilled technicians can recover approximately 60 percent of ova following superovulation.

Many research and commercial laboratories continue to use surgical transfer of embryos to recipients because of the higher pregnancy rates achieved. However, the simpler, non-surgical technique, which is quite similar to AI (e.g. eggs stored in straws), has more general applicability, especially under field conditions. Most

practitioners anticipate that pregnancy rates for the non-surgical method can be substantially improved.

1.6 *In vitro* Fertilization

Brackett (1981) indicated that such fertilization had been reported for 14 mammalian species, but that normal gestational development had been reported only for the rabbit, mouse, rat, cow and human. Reasonably high rates of fertilization yielding viable embryos have been achieved only for the laboratory species. The birth of test-tube babies has been widely publicized; however, Brackett cautioned that the few successes were accompanied by many failures, often due to abnormal development of the foetus, including chromosomal aberrations. With respect to livestock improvement, *in vitro* fertilization will primarily facilitate research to develop and, subsequently, apply many genetic engineering technologies to commercial livestock production.

At present, applications of *in vitro* fertilization include testing the fertilizing ability of male gametes *in vitro* as a replacement for the relatively subjective assessment of motility and morphology of spermatozoa. Possible future applications to livestock production (Brackett 1981) include microinjection of individual spermatozoa into the ovum to conserve the small stores of gametes available from especially superior sires, rare breeds, or stocks of sex-chromosome enhanced semen (e.g. techniques of sexing semen may markedly reduce the number of gametes available for insemination). *In vitro* techniques will allow controlled fertilization of multiple ova from a superovulation using spermatozoa from different sires. *In vitro* fertilization may increase advantages derived from long-term storage of oocytes, perhaps from prepubertal females.

1.7 Germplasm Storage

Rutledge and Seidel (1983) called long-term *in vitro* frozen storage of spermatozoa the "cornerstone of the cattle artificial insemination industry". Similarly successful techniques for freezing and long-term storage of embryos have yet to be worked out. Seidel (1981) stated that about one-third of embryos frozen and stored at -196°C were killed or severely damaged; but after thawing and transferring the remaining two-thirds their pregnancy rate were similar to rates for fresh embryos. He felt that reducing embryo losses below 20 percent would be adequate for commercially successful storage.

Embryos have been transported long distances, both within and between continents, stored in rabbit oviducts (Betteridge 1981) or *in vitro* (Bedirian *et al.* 1979) and have produced pregnancies. However, freezing and storing embryos greatly facilitates the logistics of embryo transfer, reducing costs primarily by reducing the numbers of synchronized recipients required. Any substantial growth in interchange of embryos across large geographical distances needs frozen embryo storage. Finally, freezing embryos will simplify the task of preserving valuable and/or rare genotypes indefinitely.

2. GENETIC TECHNOLOGIES

The interventions described in this section include micromanipulation of gametes

and embryos, and recombinant DNA procedures which directly alter the genotype of individuals and/or breeding populations. So far, these interventions remain at the experimental stage, and have not yet had significant impact on livestock productivity, but the potential for such impact is considerable. Recent reports of experimental breakthroughs lead to optimism that several of these interventions could have substantial application by the end of the decade.

2.1 Micromanipulation

Technologies considered under this heading involve microsurgery of gametes or embryos.

2.1.1 Cloning

Early enthusiasm about the possibilities for cloning somatic cells from mature individuals of proven genetic value now appears unfounded. Mature body cells appear to have lost totipotency, and this loss is probably irreversible (Markert and Seidel 1981). Only spermatogonia from mature males offer theotrical promise for cloning cells from mature animals (Hoppe and Illmensee 1977).

Cloning of immature cells is feasible. Indeed, it routinely occurs in nature, yielding monozygotic siblings for a variety of species, including livestock. Experimental production of identical siblings has been accomplished. The experimental technique which has produced live monozygotes (rabbits, mice, cattle, sheep, horses) involves cleaving the embryo at the 2-, 4- or 8-cell stage, separating the blastomeres, and placing them in surrogate zonae pellucidae, which are transferred to recipients for gestation (Willadsen *et al.* 1981; Ozil *et al.* 1982).

The applications of this technique are many (Markert and Seidel 1981). All but one of the siblings can be frozen. Biopsy of the remaining identical sibling can be used to determine sex and possibly other genetic characteristics of the clone. Alternatively, one sibling can be developed to evaluate performance traits such as growth rate or milk yield. Once this information is available, more objective decisions can be made about the advisability of developing the remaining siblings.

Another substantial application is to increase the total number of progeny which might be produced from a single collection of embryos, assuming cleavage produces more embryos than are destroyed (Table 1). Using these identical siblings

Table 1 RESULTS FROM NON-SURGICAL TRANSFER OF PAIRS OF MONOZYGOTIC EMBRYOS

Starting no. embryos	16
No. half embryos obtained	30
No. monozygotic pairs transferred	14
Twin pregnancies obtained	6
Single pregnancies obtained	3
No. foetuses obtained	15
as % of half embryos transferred	54

Source: Ozil *et al.* (1982)

should have major benefits in reducing costs and increasing the efficiency of research to improve nutrition, health, reproduction and genetics of livestock. For example, environmental trends could be evaluated by allowing identical siblings to develop in different years. At a more fundamental level, these techniques should supply sufficient numbers and types of experimental animals to facilitate research on inbreeding depression and hybrid vigour.

2.1.2 Homozygous diploids

Procedures for producing homozygous diploids were described in reviews by Markert and Seidel (1981) and Seidel (1982). The starting point is to remove either the male or female pronucleus from a fertilized one-cell egg. The first cell division is prevented by treatment with cytochalasin B; however, the remaining pronucleus does divide, producing a homozygous diploid cell. Individuals produced by this procedure are female, because the YY sex chromosome combination is lethal. Individuals are 100 percent inbred, so losses due to deleterious genes and inbreeding depression are expected. Mice have been produced by this technique (Hoppe and Illmensee 1977).

Lines of homozygous diploids could be established by superovulating homozygous diploid females, followed by the process of fertilization, removal of the male pronuclei, cytochalasin B treatment, and so on. Markert and Seidel (1981) suggested the possibility of microsurgical substitution of a Y-chromosome for one of the X-chromosomes in embryos from homozygous diploid females to produce males. These males would be homozygous and genetically identical to females, except for the Y-chromosome.

2.1.3 Androgenesis and gynogenesis

These technologies involve combining a male gamete with another male gamete, or a female gamete with another female gamete. If the two gametes are from the same individual, the result is analogous to selfing in plants. Alternatively, progeny could be produced from "mating" two outstanding males or two females. Neither of these technologies has yet produced live progeny; however, the process appears feasible (Seidel 1982).

Androgenesis involves dispermy, i.e. the fertilization of an egg by more than one spermatozoa, which happens occasionally in nature or can be induced. Following dispermy, the female pronucleus is removed microsurgically. The remaining two male pronuclei can then combine to produce a viable embryo, except when both carry Y-chromosomes. Thus, the expected sex ratio for progeny from androgenesis would be two-thirds male and one-third female, a significant change in sex ratio. For progeny from the initial selfing, the level of inbreeding would be 50 percent.

Two techniques for gynogenesis have been suggested. One involves removal of the zona pellucidae to allow fusion of ova, and has analogies in tissue culture work. Another (Seidel 1982) involves normal fertilization followed by removal of the male pronucleus from a one-cell ovum exposed to cytochalasin B to prevent extrusion of the second polar body, which would then provide the set of chromosomes to produce a selfed diploid. Both techniques would yield only female progeny.

2.1.4 Sex selection

Altering the sex ratio of commercial livestock populations has several benefits. The genetic influence of highly selected bull mothers could be augmented by producing a higher proportion of sons for evaluation and potential use as sires. Also, a smaller and presumably superior proportion of breeding females would be needed to produce replacement females. In dairy or dual-purpose herds, the remainder of the females could be bred with sexed semen from meat strains to produce males for slaughter. Increasing the proportion of males in the population could improve average growth rate and feed efficiency for meat production.

At present, none of the processes for sexing semen of livestock species has been consistently effective in altering sex ratio (Amann and Seidel 1982). Physical techniques, such as sedimentation, centrifugation and electrophoresis of spermatozoa, have been tried, but perhaps not adequately explored. Expression of the haploid genotype on the phenotype of the gamete could produce exploitable differences; however, Ohno (1983) is not optimistic about a functional sex difference between spermatozoa. An important development has been a process for sorting X- and Y-bearing spermatozoa (Gledhill *et al.* 1982). Probes such as the technique for marking Y-bearing human spermatozoa with a fluorescent dye also show promise. At the present stage of development, these techniques destroy the gametes. Foote (1982) was optimistic that "within the next decade one or more reliable methods for producing populations of sperm greatly enriched in X- or Y-bearing sperm will be available".

The sexing of embryos is currently feasible through biopsy and examination of chromosomes from cells in metaphase (Betteridge *et al.* 1981). Results summarized in Table 2 indicate a 68 percent success rate in sexing 12- to 15-day old embryos, and a 33 percent pregnancy rate for sexed and transferred embryos. Other workers, cited by Betteridge *et al.*, had sexed 6- to 7-day old embryos with success rates ranging from 33 to 59 percent. The earlier sexing would be advantageous because 6- to 7-day old embryos survive freezing better than those aged 12-15 days. The potential value of sexing one of a set of identical siblings to determine if the remainder should be used to establish pregnancies has been previously mentioned, as were the effects of androgenesis (two-thirds males, one-third females) and gynogenesis (all females) on sex ratio.

Table 2 PREGNANCY RATE FOR 12- TO 15-DAY SEXED BOVINE EMBRYOS

Sample	No. embryos	No. sexed	%	No. sexed embryos transferred	No. pregnancies	%
1	26	15	58	7	1	14
2	117	87	74	4	2	50
3	40	26	65	6	2	33
4	31	23	74	25	10	40
5	69	41	59	29	12	41
6	21	20	95	20	3	15
7	43	25	58	19	6	32
Total	347	237	68	110	36	33

Source: Betteridge *et al.* (1981)

Other techniques discussed by Betteridge *et al.* (1981) include assay of the H-Y antigen found on the surface of male cells of several species (mice, rats, guinea-pigs,

humans, cattle and dogs). Assay of foetal fluids obtained by amniocentesis may detect hormones such as foetal testicular androgen. If reasonably consistent diagnostic procedures could be developed, a foetus of the undesired sex might be aborted. Genetic and economic consequences of sex control are treated in more detail in a later section of this paper.

2.1.5 Chimaeras

The fusion of embryos has been accomplished to produce chimaeras which have four (or more) parents. In at least one case, (sheep-goat chimaeras) pairs of parents were of different species (Polge, personal communication). In the similar technique of somatic cell fusion involving tissue culture, a single nucleus is formed with a mixture of chromosomes from the different parental cells (Solter 1981); however, the integrity of the individual nuclei of fused embryos appears to be maintained. A consequence is that the relative contributions of each embryo to the chimaera are unequal. Seidel (1982) speculated that directed control of these contributions might be attained so that (for example) the mammary system of a dairy strain might be combined with muscling of a beef strain. However, it seems likely that a principal use of chimaeras will be for research in developmental biology.

2.1.6 Transfer of chromosomes

Genes which directly affect the same trait tend to be linked. If technology were developed to transfer individual chromosomes and if chromosomes of livestock species are mapped, it may become feasible to transfer individual chromosomes or pairs of chromosomes. This technology would facilitate introducing only selected alleles into a line without large-scale and long-term breeding programmes. Other possibilities include the development of reproducing homozygous diploid lines, mentioned previously. Seidel (1982) pointed out that transfer of chromosomes has been accomplished with plants and invertebrates, and that there seemed to be no theoretical barriers to this technology for higher animals.

2.2 Recombinant DNA

Animal breeders have tended to discount the possibility of recombinant DNA procedures as a means of improving performance of livestock. One reason has been pessimism that interspecies transfer of genes would be feasible for higher animals. In addition, most important traits appear to be influenced by many different genes so that transfer of one or a few genes, even if feasible, would have relatively little impact. While nothing has changed the premise on which this latter belief was based, recent dramatic breakthroughs in gene transfer between mammalian species challenge the original pessimism. Also, it has become apparent that recombinant DNA holds considerable promise as a means for economically and efficiently producing vaccines and other biologicals which can be used indirectly to improve the health and performance of commercial livestock.

2.2.1 Transfer to microorganisms

The development of a "safe, stable, and effective polypeptide vaccine" for

foot-and-mouth disease has particular significance to livestock production (Kleid *et al.* 1981). The basis for this development was the discovery that one of the four polypeptides which constitute the coat of the foot-and-mouth disease virus serves as an antigen to stimulate resistance to the virus itself. This coat protein is not itself virulent, so its antigenic effect is the basis of an effective and harmless vaccine.

Because there are many strains of the virus, the vaccine is not comprehensive. However, the techniques for developing variants are now established.

This is but one example of using rapidly reproducing microorganisms as 'living factories'. Others involve production of insulin, interferon and growth hormone.

2.2.2 Intermammalian species transfer

First reports of transfer of genes to mammalian species included transfer of the rabbit gene for betaglobin and viral gene for thymidine kinase to mice (Wagner *et al.* 1981). Regulated expression of the transferred viral gene for thymidine kinase was achieved by fusing it to the mouse metallothionein-I (MT-I) promoter gene, the expression of which is affected by heavy metals such as zinc (Palmiter *et al.* 1982).

Subsequently, however, the dramatic increase in growth of mice to which the rat gene for growth hormone had been transferred brought widespread attention to this technology. The details of gene transfer are reviewed in this publication by Frankham and Gillings. The approach involved fusing the rat gene to the moise MT-I gene. Approximately 600 copies of this fusion gene (MGH) were microinjected into male pronuclei in fertilized eggs. One hundred and seventy of these eggs were transferred to foster dams; 21 mice were produced.

After weaning, total nucleic acids were extracted from tail cells and tested for evidence of MGH. Seven of the mice showed evidence of carrying the gene (Table 3). One of the mice (no. 10) transmitted MGH genes to 10 of 19 offspring produced.

Table 3 EFFECTS OF TRANSFERRED RAT GROWTH HORMONE GENES ON GROWTH OF MICE

Individual	No. transferred growth genes/cell	Growth hormone (µg/ml)	74-day weight g	Ratio[1]
Females				
2	20	57.0	41.2	1.87
3	1	0.9	22.5	1.02
21	35	112.0	39.3	1.78
Avg. for transgenic females	19	56.6	34.3	1.56
Avg. for untreated female littermates	0	0.16	22.0	1.00
Males				
10	8	0.3	34.4	1.32
14	2	0.3	30.6	1.17
16	2	17.9	36.4	1.40
19	10	32.0	44.0	1.69
Avg. for transgenic males	5	12.6	36.3	1.39
Avg. for untreated male littermates	0	0.15	26.0	1.00

[1] Ratio of individual's 74-day weight to average weight of untreated littermates of same sex.

Source: Palmiter *et al.* (1982)

Comparison of the 74-day weights of transgenic mice with means for their untreated littermates indicated a distinct advantage of the former for growth rate. With the exception of mouse 16, there appeared to be a relationship between number of MGH genes inserted per cell and growth rate.

Based on results from earlier experiments, it was expected that addition of zinc to the diet would initiate or accelerate expression of the MGH fusion gene. Results were ambiguous for this experiment. Most transgenic mice were already larger than littermates before they were weaned, and zinc was added to the diet at 33 days of age. One mouse (no. 19) was removed from the zinc diet at day 56, but continued accelerated growth.

Tissue analyses indicated that the principal MGH activity was in the liver and other large organs. The much greater accumulation of growth hormone was at least partially attributed to functioning of the gene in these large organs rather than in the pituitary.

A major effect of growth hormone is to stimulate somatomedin production in the liver. Thus, production of growth hormone in liver cells may have been sufficient to affect sharply growth even for individuals such as nos. 10, 14 and 16, in which the level of growth hormone was not particularly high, but growth response was strong.

The authors speculated on practical applications of transfer in addition to the increase in growth rate. These included the homology of growth hormone and prolactin, with potential for increasing milk yield. They also suggested that mice (or other higher animals) might serve as living factories to produce biologicals that require cellular processing not possible by bacteria. Although the effect of zinc on gene regulation was not clear, the possibility exists of regulating gene expression to fit the environment; e.g. seasonal flux in feed supplies.

3. CONSERVATION OF RARE GENOTYPES

Concern for the loss of irreplaceable gene resources has stimulated action by numerous local, national and international agencies, with FAO in the forefront of many of the international efforts. Gene banks for rare and unusual plant genotypes are fairly common, with their implementation simplified in many cases by the ability to store seed or vegetatively propagated tissue. Preservation of animal genotypes has been more difficult.

3.1 Justification

Effective breeding strategies to improve genotype for performance traits of major economic merit are a principal factor leading to loss of genotypic variants. Selection may improve performance at the expense of fitness or adaptability, especially where the environment is concomitantly improved to buffer livestock from climatic, nutritional, disease and other stresses. The danger is that this improved environment may not be sustainable. Relative costs of basic inputs (such as energy) may change over time, disease organisms may develop resistance to drugs, and so on. Alternatively, selection goals may change to meet different market requirements.

A problem of special concern is that relatively few breeds and lines of

livestock in developing countries have been adequately characterized for production and fitness traits. Thus, introduction of 'improved' stocks from developed countries may be at the expense of local strains that have adapted to local conditions through centuries of natural selection. Often, the effects of hybrid vigour for the initial crossbreds in grading-up programmes masks the loss of these genetic adaptations. Priority should be given to the conservation of these probably valuable, perhaps unchangeable, but largely undefined sources of genetic variation.

Mason (1974) reviewed the issues involved in conservation of breeds whose numbers are declining to the point where they are at risk of extinction. He gave as a principal justification for conservation, a point already noted. Production environments and market requirements can and do change. The expanding human population wants and needs animal products, but the better production environments must be increasingly devoted to crop agriculture. Thus, the role of animals, particularly ruminants, adapted to conversion of low-cost, non-competitive feed resources becomes increasingly important (Fitzhugh *et al.* 1978). Local hardy strains may contribute genes, for tolerance to environmental stresses, which will be useful in both developing and developed regions. Conversely, economic pressures to intensify production systems have created opportunities for breeds which are unusually prolific (Finnish Landrace sheep; pigs from the Lake region of China).

Rare breeds often have particular value for research on mechanisms which support adaptation to such factors as disease. Resistance to trypanosomiasis was cited by Mason as being of particular interest. Rare breeds may also provide the raw material for evaluating the mechanism of natural selection and documenting the history of domestication.

3.2 Methods

Three methods of conserving rare breeds were listed by Mason (1974):

i. Gene pools in which several breeds are combined to preserve genes.

ii. Stores of frozen gametes or embryos.

iii. Flocks or herds of pure breeds kept in public parks or on private farms by enthusiasts.

Smith (1984) distinguishes between two situations in which the objectives are: (i) to preserve individual genes, and (ii) the conservation of combinations of genetically influenced traits. Gene pools serve as the most efficient means of preserving genes for future utilization. Once established, the gene pools can be maintained in stores of frozen gametes or embryos. The probabilities of failing to include a given gene in frozen stores depends on frequency of the gene (p) and the number of individuals contributing gametes to the store. Smith (1984) showed that the probability of failing to include a gene in a frozen semen store is $(1-p)^{2N}$ where N is the number of sires sampled. Assuming 10 or more embryos are stored per mating, the probability that none of the embryos carry a given gene is $(1-p)^{4N}$.

Mason concluded that "preservation without exploitation cannot be recommended". Choices of which breeds should be preserved should be based on unusual adaptations such as the ability to thrive in difficult habitats or high levels of hybrid

vigour when crossed with other breeds. Genetically unusual breeds should be kept for research purposes. Attractive and historically important breeds should be kept for their cultural value.

Economic or technical factors will generally limit the total number of individuals sampled and/or the number of gametes or embryos preserved per individual. Smith (1984) identified three principles to be applied when these limits prevail:

a. Sample more stocks keeping fewer individuals per stock, instead of more individuals from fewer different stocks.

b. Conserve stocks which are genetically and phenotypically dissimilar rather than those which are similar.

c. Conserve stocks as pure lines rather than as gene pools in order to maintain diverse combinations of genetically influenced traits and to provide future flexibility in recombination of conserved stocks.

3.3 Storage of Gametes and Embryos

Technological developments reviewed in this paper offer some optimism for efficient long-term storage of genotypes. Substantial differences between species in tolerance to freezing must be considered; however, as noted by Leibo (1981), these problems should be solvable through scientific application of cryobiological principles if sufficient resources are brought to bear on the research.

Maintenance of sufficiently large effective population size to avoid loss of genetic variation from inbreeding is the principal criterion for determining number of individuals to be sampled and the number of cells to be stored. Factors to be considered include:

- size of population available for sampling,
- losses during freezing and thawing,
- losses during fertilization and gestation,
- losses prior to entry into breeding population.

The simulation results of Yamada (1981) provide guidance to the type of mating system to be followed to minimize inbreeding when using stored gametes or to produce embryos for storage. A mating strategy for fixed population sizes in which each random pair contributed one male and one female progeny to the next generation, increased effective population size two-fold over the case of each pair producing a random number of progeny.

Using 2 percent as the maximum acceptable level of inbreeding resulting from the sampling and conservation process, Smith (1984a) estimated that the required effective population size is only 25. This could be provided by preserving semen from 25 unrelated sires or embryos from 25 unrelated pairs of parents. The inbreeding level of 2 percent was suggested as a rate commonly occurring in approximately 4 generations for many domestic livestock breeds.

3.4 Benefits and Costs of Conservation

The principal costs of genetic conservation were identified by Smith (1984b). These included: identifying and characterizing representative examples of stocks to be conserved; maintenance of live gene pools and/or collection and preservation of gametes and embryos; administration of the conservation programme; and lower economic performance of conserved stocks. Initial costs of collection are lowest for a living stock, but the annual costs of maintenance of living stock accumulate over time. Initial costs for collecting and preserving embryos and semen are relatively high, but occur only once.

The lower economic performance of conserved stocks and their failure to be commercially competitive is often the reason they are at risk of being displaced by superior stocks. Presumably, these superior commercial stocks will continue to be improved further, widening the disparity in economic competitiveness of the conserved stocks. Thus, the likelihood that the conserved stock will have commercial value in the future largely rests on major shifts in the production environment or market requirements. Smith (1984b) considered that only if gains of 5-10 percent in economic efficiency could be anticipated from future use of conserved stocks would conservation be a worthwhile strategy.

This economic argument against the value of conserving currently non-competitive stocks has greatest merit with regard to well characterized breeds in developed countries, but less so with respect to adapted strains of livestock in developing regions for which neither current nor future production requirements have been adequately characterized. Smith (1984b) concluded that the potential economic benefits and, thus, probability of future use of these adapted stocks (e.g. in new synthetic breeds) justified their preservation as "...the main area for input of international resources in conservation".

4. EXPECTED GENETIC AND ECONOMIC GAINS

A number of papers have considered the potential impact of various types of genetic engineering on rate of change in additive genetic merit, e.g. sex control (Cunningham 1975; Van Vleck and Everett 1976) and superovulation and embryo transfer (Land and Hill 1975; Powell 1981; McDaniel and Cassell 1981). The following discussion is largely derived from two recent publications which estimated potential genetic change from combinations of technologies including AI, superovulation, embryo transfer, embryo splitting, sex control, and selfing (Van Vleck 1981; Nicholas and Smith 1983).

As background to this discussion, both Van Vleck (1981) and Nicholas and Smith (1983) restated fundamental relationships involved in predicting genetic change.

The expected additive genetic difference between a selected group and the base population is $\Delta G = \hat{r}_{gg} I \sigma_g$, where $\hat{r}_{gg}$ is the correlation between actual and predicted additive genetic value, I is the standardized selection differential, and σ_g is the additive genetic standard deviation. Progress per year can be estimated by $\Delta G/L$, where L is the generation interval. Contribution to ΔG can occur through four pathways:

Selected Parents	Genetic Difference	Generation Interval
Sires of sons	Δ SS	L_{ss}
Sires of daughters	Δ SD	L_{sd}
Dams of sons	Δ DS	L_{ds}
Dams of daughters	Δ DD	L_{dd}

Genetic gain per year is approximated from the formula (Rendel and Robertson 1950):

$$\Delta G = \frac{\Delta SS + \Delta SD + \Delta DS + \Delta DD}{L_{ss} + L_{sd} + L_{ds} + L_{dd}}$$

which illustrates the potential for different contributions from each pathway and for differences in average time required for genetic change in the population mean to be effected through different pathways.

Van Vleck (1981) stated that within-herd selection of dairy cattle for milk yield with natural service could at best yield an annual ΔG of 0.5-0.6 percent. Theoretically, with optimum selection strategies incorporating AI, annual ΔG could be 2-3 percent. AI allows more intense selection through the sire-son, sire-daughter, and dam-sire pathways. Accuracy of selection is also improved by comparison with contemporaries in several environments. That this potential of 2-3 percent has not been achieved (Van Vleck 1977) was attributed to a number of factors, such as including other traits (e.g. type) in the selection criteria, and non-random management of cows. In the latter case, the example was given of dairy producers managing the cow according to their expectations of her production potential.

The extent of the difference in genetic progress actually achieved by using AI, compared to potential progress, was striking (Van Vleck 1977), with achieved rates of progress actually below 1 percent and for some cases below the 0.5 percent available through natural mating. These results are worth emphasis, because they demonstrate that it is application of the appropriate genetic strategy, not the reproductive technology, which produces genetic change.

4.1 Superovulation and Embryo Transfer

Nicholas and Smith (1983) discussed potential for increasing rates of genetic change through embryo transfer and the related technologies of embryo splitting and sexing. First, they considered two strategies involving superovulation (multiple ovulation, in their terminology) and embryo transfer. One strategy (the juvenile scheme) involved selection among transferred sons and daughters based on available records of their genetic dam and other relatives (granddam and dam's full- and half-sisters). The other strategy, called the adult scheme, delayed selection until transferred sibs were three years old, at which time three records would be available for the dam and first-lactation records would be available for transferred females. Both strategies shorten the generation from the 6.3 years in a conventional progeny testing scheme to 1.8 years for the juvenile and 3.7 for the adult scheme.

The assumed values for genetic and phenotypic parameters, the effects of inbreeding, and other details which affect the absolute values for estimated rates of change are given in the original paper. Comparisons of the values in Table 4 indicate the relative effects of different strategies incorporating superovulation and embryo transfer in the selection programme. The juvenile scheme provided a more rapid rate of response because of the shorter generation interval even though accuracy ($\hat{r}_{gg}$) was greater for the adult schemes because of the greater numbers of individuals and records included in the index. Increasing the number of progeny per donor female and the number of donors per sire increased rate of genetic change to as high as 189 (for this example), compared to 100 for traditional progeny testing schemes without embryo transfer. However, for a fixed number of transfers (1000 in this example), both increasing number of progeny per donor and number of donors per sire sharply increased the rate of inbreeding, as would be expected because of the resulting decrease in effective population size. Inbreeding rates were highest for the juvenile scheme, because of the shorter generation interval coupled with culling of a proportion of the donors and all their progeny. For the adult scheme, the generation interval is longer and donors have already been selected.

Table 4 ANNUAL GENETIC CHANGE AND INBREEDING RATE FROM DIFFERENT SELECTION STRATEGIES INVOLVING SUPEROVULATION AND EMBRYO TRANSFER

Strategy[1]	Donor dams per sire											
	8				16				32			
	Transferred progeny per donor				Transferred progeny per donor				Transferred progeny per donor			
	4	8	12	16	4	8	12	16	4	8	12	16
	Annual genetic change (s.d. x 10^3)[2]											
Juvenile	110	136	150	158	127	153	166	175	142	168	181	189
Adult	99	127	141	150	116	143	156	165	130	156	170	179
	Annual inbreeding rate (x 10^4) per 1000 transfers per year[3]											
Juvenile	30	72	125	191	54	119	197	287	102	215	340	478
Adult	7	13	20	27	13	25	38	51	25	49	74	99

[1] Only one male selected per full siblings.

[2] For comparison, the possible rate for traditional progeny testing system is 100 (i.e. 0.1 s.d. x 10^3).

[3] For comparison, the inbreeding rate in a national progeny testing system is about 10 to 20 (i.e. 0.001 to 0.002 x 10^4).

s.d. = standard deviation

Source: Nicholas and Smith (1983)

Additional results were given for expected genetic changes when embryo sexing, or splitting, were incorporated. Sexing embryos would allow fewer male embryos to be transferred per group of siblings and thus, with the same total number of transfers (1000 in the example), reduce the rate of inbreeding. Splitting embryos would produce genetically identical sibs (R = 100 percent vs 50 percent for full sibs) which would increase accuracy of selection and rate of genetic change over values in Table 5 approximately 30–40 units for the juvenile scheme and 20–30 units for the adult scheme. However, these increases would also increase inbreeding unless the total

number of transfers were increased by the same amount as the splitting factor (e.g. 2, 4, 8, or 16).

Projections for rate of genetic change presented by Nicholas and Smith indicated that a relatively small-scale programme involving 1024 transfers combined with annual milk recording of 512 females per year would actually yield 30 percent more genetic progress than a conventional national progeny testing programme involving milk recording of several thousand cows. For many developing countries in which the organization and operation of a national progeny testing programme is unlikely, this scheme involving superovulation and embryo transfer might have especially practical relevance as a means of improving, as well as conserving, indigenous stocks. The infrastructure required for collection and preservation of the local stocks would also support the genetic improvement programme.

4.2 AI, Sexed Semen and Embryo Transfer

Van Vleck (1981) compared the potential genetic gains per year in kg milk (Table 5) possible through use of various reproductive technologies with the approximately 23 kg attainable per year from selection and natural service. A principal consequence of these technologies is the increased selection differential operating through the four pathways. For example, use of sexed semen could markedly reduce the percentages of dams required to produce sons and daughters (in the example, from 90 percent for AI alone to 45 percent for AI with sexed semen). In the example, the top 3 percent of dams would be mated to produce sons destined for testing and sire selection for the next generation; the next 45 percent of best dams would be mated to produce daughters which would become the dams of the next generation. Progeny from the remaining 52 percent of dams would presumably be slaughtered for beef. Van Vleck (1981) estimated that cost for sexed semen would have to be less than US $20 per breeding unit to be economic.

Table 5 PREDICTED EFFECTS OF AI, SEXED SEMEN AND EMBRYO TRANSFER ON SELECTION INTENSITY OF SIRES AND DAMS AND ANNUAL GENETIC GAINS IN MILK YIELD[1]

Intervention	Percent selected				Gain/year
	SS	SD	DS	DD	kg
Artificial insemination	4	20	6	90	100
Sexed semen	4	20	3	45	115
AI with embryo transfer	4	20	1	10	135
Embryo transfer, intense sire selection	2	2	1	10	159
Embryo transfer, intense sire selection, sexed semen	2	2	0.5	5	167

[1] Genetic standard deviation/sum of generation intervals = 568 kg/24.

Source: Van Vleck (1981)

Combining AI with embryo transfer further increased selection differentials through the dam pathways, allowing the best cows to produce sons and daughters for the next generation. Estimates of annual gain were also made for combining embryo transfer with more intense selection or sires and(or) use of sexed semen (Table 5).

Net present economic value (at 10 percent interest rate and US$ 0.11/kg milk profit over feed cost) was estimated for the additional annual gain of 35 kg milk from using embryo transfer compared with AI alone. These estimates indicated that 79 years of accumulated gains in milk yield would be required to balance a $300 cost of embryo transfer per cow. With more intense sire selection, the additional 59 kg gain over AI alone would have to accumulate for 46 years to cover a $300 cost of embryo transfer. These economic comparisons depend on the assumed interest rates and profit over feed costs. However, Seidel (1981) quoted charges for embryo transfer of about $2000 per pregnant recipient, considerably above Van Vleck's estimate of $300 as the cost which might be justified by genetic improvement. Thus, economic justification of embryo transfer on genetic change in milk production seems unlikely until embryo transfer becomes much less expensive.

4.3 Twinning

The potential of fecundity as a means of increasing offtake and income is well recognized. For example, cows weaning twins produced 171 kg more calf than did cows weaning singles (Turman *et al.* 1971). The success of increasing litter size in Australian Merino sheep through selection and the effect of a major gene (Piper *et al.* 1980) have stimulated hopes that similar opportunities might be discovered in cattle (Rutledge 1975). However, heritability for twinning in cattle is low, probably less than 5 percent (Maijala 1964; Bowman *et al.* 1970), as are breed averages for twinning (Table 6). Successful selection for twinning in cattle would be a long-term process at best.

Table 6 SUMMARY OF REPORTED INCIDENCE OF TWINNING FOR DAIRY AND BEEF CATTLE BREEDS IN USA

Breed		Total no. births	Twins	
			No.	%
Dairy Breeds:	Holstein	25 397	857	3.4
	Jersey	3 537	45	1.3
	Guernsey	3 263	44	1.3
	Ayrshire	889	25	2.8
	Brown Swiss	305	27	8.9
Beef Breeds:	Hereford	8 857	35	0.4
	Angus	1 722	19	1.1
	Shorthorn	1 755	12	0.7

Source: Rutledge (1975)

The alternative of contralateral transfer of embryos does show promise for more rapid attainment of the benefits of twinning (Sreenan 1983). A reasonably practical approach involves insemination of cows, followed by non-surgical, contralateral transfer of an embryo. Results in Table 7 indicate that about 64 percent (43/67) of pregnant cows carry the transferred embryo to parturition with about 50 percent (35/67) of cows producing twins (i.e. one contralateral and one ipsilateral). Survival of the transferred embryo is positively affected by survival of the ipsilateral embryo. Under field conditions, pregnancy of recipients following insemination will not be known with certainty at the time of embryo transfer, but as results in Table 8 indicate, the increase in weaning rate can be substantial. Sreenan (1983) quoted Cunningham's (1977) calculations that induced twin production could increase revenue from diary herds by 12 percent and from beef herds by 60 percent.

Table 7 SURVIVAL OF EMBRYOS TRANSFERRED TO PREGNANT RECIPIENTS

Trial	No. pregnant recipients	Surviving transfers			
		As singles[1]	As twins	Total	%
1	15	2	9	11	73
2	32	4	15	19	59
3	20	2	11	13	65
Total	67	8	35	43	64

[1] Native or ipsilateral embryo did not survive.

Source: Sreenan (1983)

Table 8 EFFECTS ON CALF CROP OF NON-SURGICAL CONTRALATERAL EMBRYO TRANSFER TO INSEMINATED BEEF COWS

	Inseminated only	Inseminated + embryo transfer
No. cows	104	125
Calves born/cow calving	1.0	1.45
Calves weaned/cow calving	0.9	1.25
% increase in weaning rate		+39

Source: Sreenan (1983)

5. CONCLUSIONS

Recent major, often unexpected, research breakthroughs in reproduction and genetics, combined with the rapid evolution of support technologies, give promise of more exciting developments to come. The value of support for basic research as a means of eventual improvement of livestock production has never been more evident.

All concerned with livestock improvement should become familiar with the results of basic research in reproduction and genetics. But this is not a simple matter for those not involved in this research on a day-to-day basis. Clearly, there is need for interpreters to bridge the gap between the laboratory and application. It is tempting to leave this job to new generations of students. But when the concern is for application to livestock in developing countries, are there students with the necessary knowledge and firsthand experience of the relevant problems? Certainly this is an argument for training more students from developing countries, but it is a valid argument only if these students have the requisite practical knowledge and experience with livestock production. Perhaps, nothing will do as well as to send the experienced 'old hands' back to school, either literally or figuratively, to update them on applications of these new technologies.

The reproductive and genetic technologies reviewed in this paper offer significant opportunities to improve livestock production through conservation of genetic resources, increased selection intensity, shortening generation intervals, and producing new genetic combinations to take advantage of complementarity and hybrid vigour. On the horizon are very real possibilities for tailoring genotypes through transfer of chromosomes and genes into different genetic backgrounds. Rare and unusual genotypes can be stored indefinitely at relatively low cost to provide

insurance against changes in the environment or in market requirements. These technologies are particularly relevant to the preservation of representative samples from breeds which have evolved adaptations to the environmental conditions of developing countries. These breeds are at particular risk of loss because of rapid displacement by so-called 'improved' breeds imported from temperate, developed countries.

Much of the power of these new genetic and reproductive technologies stems from their current and potential synergism. However, much work remains to be done before the full benefits of these new technologies can be realized. Foremost is the need to characterize production systems, resources and constraints to determine which genotypes are best suited to each system. Before embryo transfer is used to conserve rare breeds or to move exotic genotypes between continents, there is need to measure objectively performance and fitness of the genotype to be transferred. Before genes are transferred between species, there is need to determine which genes (or alleles) are worth transfer. Even more limiting to gene transfer is lack of knowledge of gene action and location for livestock species.

Thus, while exciting breakthroughs have been achieved, much research needs to be done at both basic and applied levels before the full benefits of germplasm storage and genetic engineering will be realized.

REFERENCES

Amann R.P. and Seidel G.E. Jr. (eds). Prospects for Sexing Mammalian Sperm. 1982 Colorado Associated University Press, Boulder, Colorado.

Bedirian K.N., Mills K.S., Bligh P.J., Geroldi R. and Kilmer B.A. Commercial export 1979 of bovine embryos from Canada to Europe. Theriogenology 11:3-4.

Betteridge K.J. An historical look at embryo transfer. J. Reprod. Fertil. 62:1-13. 1981

Betteridge K.J., Hare W.C.D. and Singh E.L. Approaches to sex selection in farm 1981 animals. In: New Technologies in Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 109-125.

Bowman J.C., Frood I.J.M. and Wood P.D.P. A note on the variation and heritability 1970 of twinning in British Friesian cattle. Anim. Prod. 12:531-533.

Brackett B.G. Applications of *in vitro* fertilization. In: New Technologies in Animal 1981 Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 141-162.

Cunningham E.P. The effect of changing the sex ratio on the efficiency of cattle 1975 breeding operations. Livest. Prod. Sci. 2:29-38.

Cunningham E.P. Genetic applications of technical developments in bovine repro- 1977 duction. Jahrestagung der deutschen Gesellschaft für Züchtungskunde, Bonn. September 1977. p. 19.

Dickerson G.E. and Willham R.L. Quantitative genetic engineering of more efficient
1983 animal production. J. Anim. Sci. 57 (Suppl. 2):248-264.

Dzuik P.J. and Bellows R.A. Management of reproduction of beef cattle, sheep and
1983 pigs. J. Anim. Sci. 57 (Suppl. 2):355-379.

Fitzhugh H.A., Hodgson H.J., Scoville O.J., Nguyen T.D. and Byerly T.C. The Role of
1978 Ruminants in Support of Man. Winrock International, Morrilton, Arizona.

Foote R.H. The artificial insemination industry. In: New Technologies in Animal
1981 Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 13-39.

Foote R.H. Prospects for sexing: present status, future prospects and overall
1982 conclusions. In: Prospects for Sexing Mammalian Sperm. R.P. Amman and G.E. Seidel (eds). Colorado Associated University Press, Boulder, Colorado. pp. 285-288.

Frankham R. and Gillings M.R. Molecular biology and its applications to domestic
1984 animals. In: Cryogenic Storage of Germplasm and Genetic Engineering. Animal Production and Health Paper No. 44/2. FAO, Rome.

Gledhill B.L., Pinkel D., Garner D.L. and Van Dilla M.A. Identifying X- and
1982 Y-chromosome-bearing sperm by DNA content: Retrospective perspectives and prospective opinions. In: Prospects for Sexing mammalian Sperm. R.P Amman and G.E. Seidel Jr. (eds). Colorado Associated University Press, Boulder, Colorado. pp. 177-191.

Hoppe P.C. and Illmensee K. Microsurgically produced homyzygous-diploid uniparental
1977 mice. Proc. Nat. Acad. Sci. USA 74:5657-5661.

Kleid D.G., Yansura D., Small B., Dowbenko D., Moore D.M., Grubman M.J., McKercher
1981 P.D., Morgan D.O., Robertson B.H. and Bachrach H.L. Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. Science 214:1125-1128.

Land R.B. and Hill W.G. The possible use of superovulation and embryo transfer in
1975 cattle to increase response to selection. Anim. Prod. 21:1-12.

Leibo S.P. Preservation of ova and embryos by freezing. In: New Technologies in
1981 Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 127-140.

Maijala K. Fertility as a breeding problem in artificially bred populations of dairy
1964 cattle. 1. Registration and heritability of female fertility. Ann. Agr. Fenn. 3 (Suppl.). 94 p.

Markert C.L. and Seidel G.E. Jr. Parthenogenesis, identical twins and cloning in
1981 mammals. In: New Technologies in Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 181-200.

Mason I.L. The conservation of animal genetic resources - introduction. In: 1st World
1974 Congress on Genetics Applied to Livestock Production. Vol. 2. Round Tables. pp. 13-22.

McDaniel B.T. and Cassell B.G. Effects of embryo transfer on genetic change in dairy
1981 cattle. J.Dairy Sci. 64:2484-2492.

Nicholas F.W. and Smith C. Increased rates of genetic change in dairy cattle by
1983 embryo transfer and splitting. Anim. Prod. 36:341-353.

Ohno S. Sex control in mammals. Proceedings of the International Symposium on Beef
1983 Production: Strategies for the most efficient beef production. Kyoto, Japan.
pp. 263-274.

Ozil J.P., Heyman Y. and Renard J.P. Production of monozygotic twins by micro-
1982 manipulation and cervical transfer in the cow. Vet. Rec. 110:126-127.

Palmiter R.D., Brinster R.L., Hammer R.E., Trumbauer M.E., Rosenfeld M.G., Birnberg
1982 N.C. and Evans R.M. Dramatic growth of mice that develop from eggs
microinjected with metallothionein-growth hormone fusion genes. Nature
300:611-615.

Piper L.R., Bindon B.M. and Nethery R.D. (eds). The Booroola Merino. Proceedings of
1980 a Workshop, Armidale, NSW, Australia. August 1980. Commonwealth Scientific
and Industrial Research Organization. Melbourne.

Powell R.L. Possible effects of embryo transfer on evaluation of cows and bulls J.
1981 Dairy Sci. 64:2476-2483.

Rendel J.M. and Robertson A. Estimation of genetic gain in milk yield by selection in
1950 a closed herd of dairy cattle. J. Genet. 50:1-8.

Rutledge J.J. Twinning in cattle. J. Anim. Sci. 40:803-815.
1975

Rutledge J.J. and Seidel G.E. Jr. Genetic engineering and animal production. J. Anim.
1983 Sci. 57 (Suppl. 2):265-272.

Seidel G.E. Jr. Superovulation and embryo transfer in cattle. Science 211:351-358.
1981

Seidel G.E. Jr. Applications of microsurgery to mammalian embryos. Theriogenology
1982 17:23-24.

Seidel G.E. Jr. Mammalian oocytes and preimplantation embryos as methodological
1983 components. Biol. Reprod. 28:36-49.

Seidel G.E. Jr. and Seidel S.M. The embryo transfer industry. In: New Technologies in
1981 Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds).
Academic Press, New York. pp. 41-80.

Smith C. Genetic aspects of conservation in farm livestock. Livest. Pro. Sci. 11:37-48.
1984a

Smith C. Estimated costs of genetic conservation in farm animals. In: Animal Genetic
1984b Resources Conservation and Management. Proceedings of the FAO/UNEP
Technical Consultation. Animal Production and Health Paper No. 44/1. FAO,
Rome.

Solter D. Gene transfer in mammalian cells. In: New Technologies in Animal Breeding. 1981 B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 201-221.

Sreenan J.M. Methods of consistent supply, recovery and transfer of embryos in cattle. 1983 Proceedings of the International Symposium on Beef Production: Strategies for the most efficient beef production. Kyoto, Japan. pp. 197-212.

Turman E.J., Laster D.B., Renbarger R.E. and Stephens D.L. Multiple births in beef 1971 cows treated with equine gonadotropin (PMS) and chorionic gonadotropin (HCG). J. Anim. Sci. 32:962-967.

Van Vleck L.D. Theoretical and actual genetic progress in dairy cattle. In: 1977 Proceedings of the International Conference on Quantitative Genetics. August 1976. Iowa State University Press. pp. 543-568.

Van Vleck L.D. Potential genetic impact of artificial insemination, sex selection, 1981 embryo transfer, cloning and selfing in dairy cattle. In: New Technologies in Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 222-243.

Van Vleck L.D. and Everett R.W. Genetic value of sexed semen to produce dairy 1976 heifers. J. Dairy Sci. 59:1802-1807.

Wagner E., Stewart T. and Mintz B. β-globin gene and a functional viral thymidine 1981 kinase gene in developing mice. Proc. Nat. Acad. Sci. USA 78:5016-5020.

Willadsen S.M., Lehn-Jensen H., Fehilly C.B. and Newcomb R. The production of 1981 monozygotic twins of preselected parentage by micromanipulation of non-surgically collected cow embryos. Theriogenology 15:23-29.

Yamada Y. The importance of mating systems in the conservation of animal genetic 1981 resources. In: Animal Genetic resources Conservation and Management. Animal Production and Health Paper No. 24. FAO, Rome. pp. 268-278.

CRYOGENIC STORAGE OF MAMMALIAN CELLS

C. Polge[1]

Remarkable progress has been made in the last 35 years in the science of low temperature biology and it has now become possible to store a wide variety of living cells for prolonged periods of time at very low temperatures. Some of the earliest successes in the preservation of cells were achieved with the spermatozoa of various farm animals. More recently, considerable progress has also been made in techniques for freezing eggs and embryos. The widespread application of artificial insemination to animal breeding has stimulated the adoption of these techniques on a very large scale. This is particularly true of cattle breeding in which the majority of semen now used for artificial insemination is stored in the frozen state. Semen banks have been established and the export and import of semen between countries throughout the world is commonplace. Both AI and the use of frozen semen are applied to a lesser extent in sheep, goats, pigs and horses, but nevertheless the techniques are perfectly feasible.

Embryo transplantation in farm animals has made considerable progress in the last 10 years. Again, the greatest application has been in cattle in which the economic advantages are greatest due to the low reproductive rate and high intrinsic value of animals of this species. Nevertheless, it has been demonstrated that embryo transplantation is equally possible in other species. In some countries the freezing of cattle embryos is now applied on a commercial basis.

The ability to store spermatozoa and embryos at very low temperatures for prolonged periods of time opens up the possibility of establishing 'gene banks'. Techniques such as these must therefore be considered more and more seriously today, particularly in relation to the conservation of endangered breeds of farm livestock. It is of much concern that the genetic base of farm animals throughout the world may be becoming severely constricted due to modern breeding methods and economic pressures. At last the potential value of a wide variety of breeds should be determined before the opportunity to do so has been lost forever.

The objective of this paper is to review the technical aspects involved in the preservation of mammalian gametes and embryos.

1. BASIC PRINCIPLES IN THE PRESERVATION OF MAMMALIAN CELLS

There are two main factors which cause the death of cells during freezing and thawing. These are, first, the formation and growth of ice crystals within the cytoplasm and, secondly, the exposure of cells to increasing concentrations of solutes resulting from the formation of pure ice in the medium in which they are suspended. In order to counteract these factors, the rate of cooling and rewarming must be adjusted according to the nature of the cells. An optimal rate of cooling is generally one which is slow enough to permit cells to lose water and shrink in response to the

[1] ARC Institute of Animal Physiology, Animal Research Station, 307 Huntingdon Road, Cambridge, UK.

formation of ice in the external medium. If cooling is too rapid the cells may not be able to lose water fast enough to maintain equilibrium and this will lead eventually to internal crystallization. After partial dehydration, however, cells can often be cooled very rapidly, such as by plunging into liquid nitrogen, without the induction of internal crystallization. Remaining water under these circumstances becomes vitrified. An optimal rate of rewarming should be such as to avoid recrystallization of remaining vitreous water within the cells. Injury due to exposure of cells to increased salt concentrations is generally most pronounced during slow cooling and it is in this situation that cryoprotective additives play an important role. Neutral, non-toxic, solutes such as glycerol or dimethylsulphoxide (DMSO) have been very widely used as cryoprotectants for mammalian cells. Non-permeating solutes such as sugars and polymers have also been used in some circumstances. These compounds have the ability to reduce the amount of ice formed in aqueous solutions at sub-zero temperatures and maintain the salts in the liquid phase. It is thus possible to cool slowly enough to allow water to diffuse out of the cells and avoid formation of intracellular crystals and at the same time to minimize damage related to hypertonic conditions. Thus, in all techniques for the successful preservation of spermatozoa or embryos, a suitable choice of diluent composition, freezing and thawing rate is required. The stability of biological systems at the temperature of liquid nitrogen (-196°C) is considered to be very prolonged.

2. SPERMATOZOA

2.1 Harvesting

Simple techniques for the collection of ejaculated spermatozoa by means of an artificial vagina have been developed for most species. Alternatively electro-ejaculation may be used. The quality of semen may be affected by age of animals, season and environment, and it is preferable to collect it when fertility is normally highest. In animals that have been slaughtered, it is also possible to harvest semen from the epididymis.

Numerous tests *in vitro* on the quality of semen have been devised. Few of these have a very strong correlation with fertility by themselves, but probably sperm morphology and motility are the most important.

2.2 Dilution

There are complex interactions between diluent components and their effects on spermatozoa during freezing and thawing. Osmolarity, ionic strength and pH are important and spermatozoa generally tolerate hypertonic media rather better than hypotonic ones. Media buffered to a pH of 6.5-7.2 have been commonly used. A significant protective action may be provided by components of egg yolk or milk and very simple medium such as egg yolk-citrate have given good results in some species. Alternatively, better results might be obtained in media of low ionic strength and in which the osmolarity is increased by non-permeating solution such as sugars, e.g. egg yolk-lactose. Other diluents that have been examined have been ones in which TRIS forms an important osmotic component of the medium in combination with sugars. By far the most commonly used cryoprotectant for the preservation of spermatozoa has been glycerol.

2.3 Freezing and Thawing

Today the most widespread method for freezing cattle semen is in plastic straws. Since spermatozoa are relatively small cells, they can generally be frozen quite rapidly and the straws are suspended in liquid nitrogen vapours before storage in liquid nitrogen. "Pelleting" is another method for freezing semen. Small drops of semen are placed on the surface of solid CO_2 and after about 10 minutes are transferred to liquid nitrogen. Pelleting has generally been the method of choice for boar semen, but it can also be used for the semen of other species. Pellets are less easy to identify than straws and they are also directly exposed to the liquid nitrogen. For long term preservation it is essential that the temperature is maintained below $-150^{\circ}C$.

Before insemination the straws are thawed in warm water. Pellets are thawed either in dry test tubes or in warm diluent.

2.4 Fertility

Conception rates following normal insemination of frozen-thawed cattle semen have been found to be almost equivalent to those of fresh semen (around 75 percent) although there are individual variations between bulls. With ram and boar semen, the conception rate is somewhat lower (around 50 percent) and individual variations are more pronounced. Results with horse semen are generally still lower. Selection of males providing semen of good motility and longevity after thawing may increase conception rates. Increased numbers of inseminations during oestrus may also be advantageous. In special circumstances, surgical insemination of semen directly into the uterus or fallopian tubes can be carried out.

3. EMBRYOS

3.1 Harvesting

The supply of embryos from donor animals can be increased by using techniques for superovulation. In most species superovulation can be induced by administration of gonadotrophic hormones either in the form of anterior pituitary extracts (FSH) or, more commonly, pregnant mare's serum gonadotrophin (PMSG). There are generally quite large variations in response between individual animals, between different strains, and between animals of different ages. Nevertheless, these variations do nct detract from the advantages gained by using exogenous gonadotrophins and the eggs produced following superovulation have generally been found to be normal. Gonadotrophins should be given close to the start of the follicular phase and accurate knowledge of the stage of the cycle is therefore required. However, in species in which luteolysis can be induced by prostaglandins the gonadotrophins can be given at almost any time during the luteal phase so long as luteolysis is induced shortly afterwards. For routine superovulation in cattle, optimal response is obtained when PMSG is administered during the mid-luteal phase and the prostaglandin given two days later. Insemination, sometimes with increased doses of semen, is carried out during the ensuing oestrus. A dose of 2000-3000 i.u. PMSG has been found to provide an average of 5 to 6 embryos of suitable quality for transfer. Excessive superovulation may result in reduced fertility and poor embryo quality. In sheep, induced to superovulate a large number of eggs, fertility has been increased by means

of intrauterine surgical insemination. The horse is one species in which it has not been possible to induce superovulation with any degree of success. In most species superovulation can be induced during periods of anoestrus but results are more variable.

Embryos can be collected from the reproductive tract by relatively simple surgical techniques involving laparotomy in the anaesthetised animal and flushing them from the oviducts or uterus. Surgical techniques are mandatory in sheep, goats and pigs, but in cattle and horses non-surgical techniques can be used. By means of a suitable catheter introduced via the cervix, good recovery of uterine embryos (6-8 days after ovulation) can be obtained. The advantage of non-surgical techniques is that they can be applied repeatedly in the same animal without the disadvantage of adhesions associated with surgery. Embryos have also been collected from the reproductive tracts of animals shortly after slaughter and used successfully in transfer. The flushing method used for recovery and handling of embryos is generally a phosphate buffered saline enriched with bovine serum albumin (PBS).

3.2 Freezing and Thawing

In some species, notably the cow, the survival of embryos during cooling and freezing is critically dependent on the stage of embryonic development. Cow embryos less advanced than the fully compacted late morula or early blastocyst stage (Day 6-7 after the onset of oestrus) do not generally survive cooling below +10°C. Such early stages are therefore not suitable for freezing. Early and expanded blastocysts, however, will survive cooling, and this is the stage of development most commonly used for freezing (Day 7-8 after onset of oestrus). Most of the experiments with sheep and goat embryos have also been done on early blastocysts, although earlier stages of development may not be so critically sensitive to cooling as those of the cow. In the pig, all stages of early embryonic development have been found to be adversely affected by cooling to temperatures below +10°C.

The most commonly used cryoprotectants for embryos are DMSO or glycerol in concentrations of 1 to 2 M in PBS. The concentration of the cryoprotectant in the medium is raised at room temperature in 0.5 M steps every 10 minutes until the final concentration is reached. After a further period of time to allow full equilibration, the embryos are cooled to a temperature slightly below the freezing point of the medium. Ice crystal formation is then induced by 'seeding' and cooling is continued at a very slow rate (0.3-0.5°C/minute). Two methods of freezing have been applied successfully. The first involves continued slow cooling to a temperature of about -60°C before plunging into liquid nitrogen. The second involves cooling to only an intermediate sub-zero temperature (-30 to -40°C) before plunging into liquid nitrogen. The important difference between the two methods is that survival is critically dependent upon thawing rate. Embryos frozen by the former method must be rewarmed slowly (about 20°C/min) whereas embryos frozen by the latter method must be thawed rapidly (about 300°C/min). This difference is due to the fact that embryos plunged into liquid nitrogen from temperatures as high as -30 to -40°C still contain appreciable quantities of residual water. This vitrifies on plunging into liquid nitrogen, but will recrystallize during slow warming causing severe intracellular damage. Recrystalliztion is avoided by rapid thawing. By contrast, embryos cooled slowly to -60°C are far more dehydrated and slow warming does not result in recrystallization. Rapid thawing, however, can lead to damage caused by osmotic effects.

In practice, glycerol is now the cryoprotectant of choice in most instances and the method of freezing is that of slow cooling to -30 to -40°C before plunging into liquid nitrogen.

Embryos are generally frozen in small glass ampoules or plastic straws. After thawing, the concentration of cryoprotectant must be reduced and this is again done in a stepwise manner. More recently a method has been devised for rapid reduction of cryoprotectant concentration by dilution with hypertonic media containing sucrose. The whole procedure can, in fact, be carried out in one step within a plastic straw.

The dependence of embryo survival on controlled slow cooling generally requires automatic apparatus which is more complicated and expensive than that needed for freezing spermatozoa.

3.3 Pregnancy

The successful use of deep frozen embryos in practice depends on the assessment of embryo survival after thawing. Purely morphological assessment of survival has been found to be just as accurate as more complicated procedures based on dye-exclusion tests. A small proportion of embryos (10-20 percent) will usually be discarded as not having survived the freezing and thawing procedure. Recent results from a commercial embryo transfer unit in Great Britain, in which frozen embryos were used, gave a pregnancy rate of 65 percent from over 400 embryos. Results from other countries have been somewhat lower. Some centres use a surgical method of transfer, but the majority are now turning over to non-surgical techniques.

Far fewer results are available from the use of deep frozen sheep or goat embryos, but there is evidence that pregnancy rates equivalent to those obtained in cattle can be achieved. From the few experiments done in horses, very few live foals have resulted from transfer of frozen-thawed embryos. In pigs, no live offspring have been produced.

4. APPLICATIONS IN ANIMAL CONSERVATION

The technology for freezing semen is relatively simple and it has been developed and applied in most countries. In estimating needs relative to conservation, it is perhaps wise to assume that about twice the number of spermatozoa will be required for insemination with frozen semen than with fresh semen.

Preservation of spermatozoa is obviously not as effective a means of breed conservation as preservation of embryos. However, the technology of embryo transplantation is far more complicated and expensive and it has been developed in relatively few countries. In addition the succes of embryo transfer (collection of high quality embryos and pregnancy rate) is critically dependent on the 'condition' of the animals. Poor results have been obtained under conditions in which it is not possible to ensure a relatively high management standard.

CRYO-PRESERVATION OF GENETIC MATERIAL: DISEASE RISK AND CONTROL

Stefan Wierzbowski[1]

1. INTRODUCTION

Since the introduction of liquid nitrogen as a refrigerant during the mid-fifties, the cryo-preservation of genetic material has expanded dramatically.

Artificial insemination in cattle, estimated to be about 150 million first inseminations annually (Bonadonna and Succi 1980), is now carried out almost exclusively with frozen semen. The advantages of its practical use, combined with the possibility of its application in modern methods of genetic improvement boosted its rapid development. The unlimited preservation time of frozen semen resulted also in the development of international semen exchange and trade. Frozen semen transfer is used to carry out numerous programmes aimed at improving cattle productivity in developing countries. All the leading cattle breeding countries are involved in semen exports with the USA and Canada heading the list. Semen exports are expanding markedly. In 1981 2 408 030 doses of semen were exported from the USA. During the first three quarters of 1982 30 percent more doses than in the same period of 1981 were exported (George 1983).

The next development in the exchange of genetic material was made in the seventies, when embryo transfer became an accepted practice. Though the results of embryo transfer are largely dependent on the skill and experience of the operator and embryo freezing needs further improvement, its use in several countries is expanding. In the 1982 fiscal year 533 cattle and 160 pig embryos were exported from the USA (George 1983).

Storage of frozen mouse embryos is already a well established procedure to maintain special stocks as a source of genetically defined mice for research. According to Mobraaten (1981) the programme to freeze embryos from stocks maintained at the Jackson Laboratory (Barharbor, Maine, USA), for long-term preservation in a repository was started in late 1978. In May 1980 33 554 embryos from 106 stocks of mice were frozen. Thirty-nine of these stocks each have over 500 stored embryos and 24 stocks have been successfully reconstituted from frozen embryos. Mice maintained at the Jackson Laboratory are routinely monitored with regard to pathogenic organisms (viruses and aerobic bacteria). Due to such preventive measures, it is assumed that embryos held in the repository are free from pathogenic microorganisms.

Cryo-preservation has been used as a method of indefinite storage of genetic material in some domestic animal breeds threatened by extinction (Alderson 1982; Bodo *et al.* 1982; Whittingham 1974). Furthermore, it has been suggested that semen and embryo freezing can be used to overcome inbreeding problems resulting from several species of deer being represented in several zoos by only a few individuals

---

[1] Institute of Zootechnics, Department of Animal Reproduction and AI, 32-083 Balice/Krakow, Poland.

(Krzywinski 1982). Cryo-preservation in this respect will be used on a relatively small scale. However, this usage has to be mentioned as several diseases are common to cattle and deer.

2. LONG-TERM STORAGE

Practical experience in long-term storage of genetic material goes back about 20 years. It has been shown that calves can be born as a result of using semen kept in a frozen state for about 20 years (HL 1975). It can be expected that from time to time such experiments will be repeated with semen stored for increasingly long periods.

In view of the fact that at temperatures below -130°C chemical reactions virtually cease, and that genetic material is normally stored in liquid nitrogen, i.e. at -196°C, it can be expected that the duration of viability of biological materials preserved at such a temperature will be unlimited. However, there remains the question of damage caused by physical processes, such as ionizing radiation. Lyon *et al.* (1981) suggest that the lowest dose of radiation to result in deterioration of viability of embryos is about 4.5 rad, and this dose would be accumulated in 40-50 years of normal storage. The highest dose used in the experiment, equivalent to 400-500 years of normal background radiation, gave only a slight loss of viability (Lyon *et al.* 1981). A period of about 32 000 years is estimated to be required for cells stored in liquid nitrogen to accumulate the same degree of lethal and chromosomal damage as cells exposed to an acute dose of X-rays at 22°C (Ashwood-Smith and Grant 1977). This means that in practical terms the duration of safe storage of genetic material at -196°C can be considered unlimited.

3. RISK OF TRANSMITTING A DISEASE

Frozen semen, embryo transfer, and to some extent embryo freezing, are methods already well established in cattle breeding. Therefore sanitary control and disease prevention are of basic importance as far as cattle are concerned. In sheep, goats, pigs and horses, the use of frozen semen is still on an experimental or semi-practical scale, and embryo freezing in sheep and goats is not common. Probably the practical application will be the creation of embryo banks for some endangered breeds of sheep (Wierzbowski *et al.* 1983). In this respect, sanitary procedures developed for cattle may help to evolve future principles for other species.

The risk of transmitting a disease agent by semen is well known and became evident when artificial insemination began to be put into practical use. It is very well understood that the risk of spreading infection has been increased by AI in comparison to natural service. Long-term preservation of semen also increased the risk by virtue of unlimited time factor. It has now been established that all aetiological agents of infectious diseases transmittable by semen can survive freezing and low temperature preservation as effectively as spermatozoa.

The risk of transferring infectious diseases via frozen embryos was reviewed (Bowen 1979; Mitchell and Betteridge 1977; Parez 1978; 1981; Whittingham 1974). Numerous investigations done on laboratory animals showed that some viruses may infect the gametes and preimplantation embryos. Thus the risk of transmitting disease through embryo transfer seemed to be formidable. A new approach to the problem was

introduced by Day (1979), Eaglesome *et al.* (1980) and Waters (1981). These authors suggested using embryo transfer as a means of controlling or eliminating an infectious disease in a herd or flock. The general suggestion was made that it is necessary to determine if the aetiological agent of each disease under consideration can infect the embryo. Another review of the risk of transmitting a disease by embryo transfer was made by Jolivet and Parez (1982), taking into consideration the new information available.

Before evaluating in detail the present situation as regards the risk of spreading disease and methods of disease control, the following three aspects of semen and embryo deep-freeze preservation must be considered:

i. The methods of semen and embryo freezing protect to a similar degree infectious disease agents present in semen, embryos and media. Opportunist pathogens and ubiquitous microflora are also conserved effectively.

ii. At the present time there are no safe methods of neutralizing microorganisms present in semen.

iii. Storage in the deep-frozen state appears to have no time limit, at least in practical application, for breeding methods that are now in use.

4. DISEASES OF MAIN CONCERN

The high pathogenicity and economic risk of foot and mouth disease, rinderpest and contagious pleuropneumonia suggest that it is not necessary to consider details of possible transmission of infectious agents of these diseases by genetic material. It seems justifiable to assume that production of frozen semen and embryos for international trade will not be carried out in countries not declared officially free of these diseases.

The methods of testing bulls in AI centres for brucellosis, tuberculosis, paratuberculosis, leptospirosis, and trichomoniasis are well established and effective. As suggested by Bartlett (1981), it "can be coped with anywhere in the world on a Specific Pathogen Free (SPF) basis". Undoubtedly, uniformity of diagnostic methods will facilitate obtaining comparable results in different countries.

At the moment, the main concern seems to be the group comprising campylobacteriosis and virus diseases (Table 1). Mycoplasma, ureaplasma, chlamydia, rickettsia, salmonella and a number of opportunist pathogen organisms which are occasionally or periodically present in male and female genitals and semen, create a complication. Although their pathogenic significance has been pointed out many times, there is a lack of evidence of their possible role as a major epizootic factor. Basic sanitation and prophylactic measures and an individual approach, if necessary, seem to be at present the most acceptable solutions.

4.1 Campylobacteriosis genitalis bovis

Streptomycin and penicillin are a regular component of nearly all semen extenders. Sometimes other anitbiotics and/or sulphonamides are also added. Justification of such a procedure goes back to the beginning of AI when some increase

Table 1 THE PRINCIPAL DISEASES OF CONCERN FOR AI AND ET - SHEDDING OF INFECTIOUS AGENTS BY THE GENITAL ROUTE

Disease	Aetiological agent	Abbreviations and contractions	Genital shedding of agents*	
			Semen	Ova and day 608 embryos
Brucellosis	Brucella abortus	Br. abortus	+	- (Stringfellow *et al.* 1982) - (Voelkel *et al.* 1982)
Tuberculosis	Mycobacterium tuberculosis	Mycobact. tuberculosis	+	
Paratuberculosis	Mycobacterium paratuberculosis	Mycobact. paratuberculosis	+	
Bovine trichomoniasis	Trichomonas foetus	T. foetus	+	
Campylobacteriosis genitalis bovis	Campylobacter foetus subspec. foetus	C. foetus	+	
Leptospirosis	Leptospira pomona		+	
Enzootic bovine leukosis	Bovine leucosis virus (BLV)	BLV	+ (Lucas *et al* 1980) - (Kaja and Olson 1982) - (Schultz *et al.* 1982)	- (Bouillant *et al.* 1981) - (Singh *et al.* 1982a; c) - (Eaglesome *et al.* 1982)
Infectious bovine rhinotracheitis	Infectious bovine rhinotracheitis virus (IBRV)	IBRV		- (Singh *et al.* 1982a; c; d)
Bluetongue	Bluetongue virus	BTV		- (Bowen and Howard 1981) - (Singh *et al.* 1982a; c; d) - (Thomas *et al.* 1982) - (Bowen *et al.* 1983)
Bovine viral diarrhoea	Bovine viral diarrhoea virus	BVDV	+	- (Singh *et al.* 1982a; b)

* + = positive; - = negative

of Non-Return Rate was demonstrated after the addition of antibiotics to the semen of some bulls of rather low fertility. Later, when wide dissemination of campylobacteriosis in cattle was recognized, the addition of certain antibiotics, particularly streptomycin, was considered to be an effective means of controlling *C. fetus* spread by semen. The addition of antibiotics became routine and was stimulated by the necessity of keeping the number of contaminating bacteria as low as possible. However, the effectiveness of antibiotics to control *C. fetus* depends on the composition of the extender. As a result, it was suggested that polymycin and streptomycin should be added to raw semen before extension (Certified Semen Services 1980). However, it was indicated by Howard *et al.* (1982) that the procedure specified by CSS was ineffective in controlling *C. fetus* in semen extended in complete egg-yolk-Tris. Besides, about 7 percent of *C. fetus* strains are resistant to streptomycin (Losinski *et al.* 1974).

From consideration of the programme of the meeting in Wels, Austria (32 Intern. Fachtagung für Fortpflanzung und Besamung, 22-23 September 1983) the conclusion can be drawn that campylobacteriosis still presents a problem for AI organizations in several countries. In addition to preventive measures, vaccination may be useful and does not interfere with diagnostic methods used at present.

Furthermore the addition of antibiotics to semen cannot be accepted as a safe method of controlling the spread of *C. fetus* by semen. It can be considered as a supplementary method only, after preventive elimination of *C. fetus* carriers and after all sanitary measures have been applied to obviate semen contamination.

Existing diagnostic methods applied with proper care offer high accuracy in controlling *C. fetus* in bulls. It has been shown in several countries or AI organizations that it is possible to keep whole bull populations free of *C. fetus*.

4.1.1 Status quo

- Campylobacteriosis still presents a problem in several countries or AI organizations.
- Treatment of semen with antibiotics cannot be considered a safe method of controlling the spread of *C. fetus* by semen.

4.1.2 Observed or proposed security measures

- Bull populations in AI centres producing semen for export should be free of *C. fetus*.

4.2 Enzootic bovine leukosis

Enzootic bovine leukosis (EBL) is a disease of mature cattle. The mechanisms of virus transmission are still not fully understood. Cell-free virus in infected cattle has not yet been demonstrated but there is ample information about virus production of lymphocytes. It is assumed that infection usually occurs after blood cell transfer between animals (Miller and Van der Maaten 1982). Although several attempts have been made to determine the possibility of transferring the virus via semen from bovine leukosis virus (BLV)-infected bulls, results have been negative (Baumgartner *et al.*

1978; Kaja and Olson 1982). So far, there is only one report of such an experiment with positive results (Lucas *et al.* 1980). However, since the semen was obtained by massage and was not examined for the presence of lymphocytes, these results may be explained by the presence of infected lymphocytes in the semen due to massage trauma.

The "Cornell Semen Test" was used for four years to determine the viral contamination in semen. Approximately 11 percent of the tested bulls had BLV antibodies but none of the 40 000 examined ejaculates contained BLV (Schultz *et al.* 1982). BLV was not found in unfertilized ova and zona pellucida of intact embryos collected from BLV infected cattle (Bouillant *et al.* 1981). Transfer of embryos collected from cattle infected with BLV did not change the health status of the BLV-negative recipients. The resulting calves were also serologically negative (Eaglesome *et al.* 1982; Singh *et al.* 1982a).

4.2.1 Status quo

- Transmission of BLV via lymphocyte-free semen from BLV infected bulls does not occur.

- Transmission of BLV via oocytes or embryos does not occur.

4.2.2 Observed or proposed security measures

- Routine semen examination for the presence of lymphocytes.

4.3 Infectious Bovine Rhinotracheitis (IBR)

The group of viruses responsible for IBR/IPV/IPB creates a rather complicated situation. In some regions these viruses are ubiquitous (Bartlett 1981), while in other areas there are strong and successful attempts to keep AI centres on the SPF level in respect to those agents (Autrup and Bitsch 1971; Bitsch 1973; Müller 1971; Romanowski *et al.* 1975; Romanowski 1981; Steck 1971).

Vaccination of cattle against IBR with modified live vaccine is widely used in the Western Hemisphere, apparently with satisfactory results. In approximately 50 000 ejaculates from serum positive healthy bulls submitted to tissue culture for IBR virus presence the results were negative (Bartlett 1981).

The risk of virus in semen of bulls with negative antibody titres seems to be rather limited. In Romanoswki's (1981) report based on about 700 bulls regularly examined during a five-year period, virus was not detected in semen. Also, Schultz *et al.* (1982) reported that semen was not infected while bulls were serologically positive to IBR. However, during active infection, virus has been traced in many cases in semen, and transmission by sexual contact or artificial insemination is possible (Bartlett 1981) and has been described several times.

Khars *et al.* (1980) suggest the existence of some special situation in respect to IBR-virus evidence in bull semen. The following quote describes some of the paradoxes.

"The lack of IBR virus isolation in North American studies contrasts with the apparent frequency of IBR virus isolation from semen in Australia, Africa and Europe. Until 1977 the North American experience has been failure to isolate IBR virus from semen unless virus was added experimentally or bulls were given intravenous inoculations with IBR virus. Workers on other continents have found virus in semen of bulls with clinical balanoposthitis, bulls with latent infection reactivated by corticosteroid treatment and in bulls with latent infection that apparently reactivated spontaneously.

"Speculation has been advanced that North American IBR virus strains replicate largely in respiratory mucosae with minimal reproductive involvement and that conversae may prevail elsewhere. If this hypothesis is correct, bulls experiencing primary infection with IBR virus strains with predilection for respiratory mucosa would rarely shed virus from the prepuce in quantities great enough to produce detectable semen contamination. This would be especially true where hygienic semen collection is practised as in the large artificial insemination units that produce most semen examined for export. This hypothesis is tenable but difficult to test. The alternative, that of poor test sensitivity, must be explored."

The in vitro susceptibility of bovine embryos to IBR virus was investigated by Singh *et al.* (1982a; 1982d). It has been found that most probably the virus cannot penetrate the zona pellucida but merely sticks to the surface of this structure. It can be "washed out" using IBR antiserum, or removed more effectively after trypsin treatment. Further development of embryos in vitro was not affected either by exposure to the virus or by the "disinfection". The same authors also observed that eggs and embryos from seropositive donors for IBR were not infected with virus. The transfer of day 7-8 embryos collected from cattle shedding IBR virus did not infect the recipients (Singh *et al.* 1982d; Singh *et al.* 1983).

4.3.1 Status quo

- In regions with ubiquitous appearance of IBR virus and the widespread use of modified live vaccine, the status of bulls with antibody titres to this virus is ignored.
- The opposite tendency prevails in several European countries where SPF semen production and SPF status of bulls used in AI is a goal.
- Embryos collected from seropositive donors are not infected with virus.
- In laboratory experiments it has been shown that virus may adhere to the zona pellucida, but after proper treatment, may be removed.

4.3.2 Observed or proposed security measures

- Acquisition of bull calves from IBR-free dams and herds only.
- Serological testing of bulls from calfhood.
- Strict isolation of bulls in semen production units.
- Semen testing for the presence of IBR virus.

4.4 Bluetongue

Bluetongue (BT) is a viral disease. Its occurrence seems to be related to the range of *Culicoides varipennis*, which is considered to be the main vector. BT virus has been found in the semen of some infected bulls (Luedke *et al.* 1975). Virus-like particles in spermatozoa from bulls infected with BT virus have also been found (Foster *et al.* 1980). Seroconversion of females subsequent to coitus with an infected bull has been demonstrated experimentally (Bartlett 1981). However, up to now there is no evidence that the disease is transmitted by sexual contact in natural conditions or by artificial insemination. The Cornell Semen Test on 40 000 ejaculates showed that they were not contaminated with BT virus. But the semen donors were also negative for antibody to BT-virus (Schultz *et al.* 1982).

Incubation of bovine embryos in vitro with BT virus has shown that they did not become infected with the virus (Singh *et al.* 1982a; 1982b). Embryos collected from cattle with viremias were transferred to recipients. All recipients and the calves subsequently born, remain serologically negative for antibodies to BT virus (Singh *et al.* 1982a; 1983c; Thomas *et al.* 1983). Virus antigen was not detected in oocytes and embryos recovered from viremic donors. BT virus was also not isolated from any of the recipients nor did any recipient seroconvert to the virus (Bowen *et al.* 1983).

4.4.1 Status quo

- There is evidence of the presence of BT virus in the semen of the infected bulls.
- Eggs and embryos collected from viremic donors were not BT virus carriers.

4.4.2 Observed or proposed security measures

- Semen testing for the presence of BT virus.
- Bulls in semen production units should be serologically negative to BT and free of the virus.

4.5 Bovine Viral Diarrhoea

Bovine viral diarrhoea (BVD) is fairly common in the Western Hemisphere. The use of BVD vaccine is routine in many herds and this determines the frequency of occurrence of BVD antibody in North American cattle, and regardless of BVD antibody titre (Bartlett 1981). The status of BVD in European herds is not clear.

The presence of the BVD virus in semen of naturally infected bulls has been demonstrated by Coria and McClurkin (1978), after experimental infection by Whitmore *et al.* (1978), and in routine semen testing by Schultz *et al.* (1982). Approximately 50 percent of the tested bulls had antibodies to BVD virus (Schultz *et al.* 1982).

Laboratory experiments carried out by Singh *et al.* (1982a) have shown that day 6-8 bovine embryos are resistant to BVD infection. Similarly eggs and embryos collected from donors that were seropositive to BVDV were found to be uninfected with

this virus (Singh *et al.* 1982b), but it has also been shown by Archbald *et al.* (1979) that BVD virus inoculation of the uterine horn on day 7 of pregnancy may interfere with normal development of preimplantation embryos.

4.5.1 Status quo

- There is evidence of the presence of BVD virus in the semen of the infected bulls.

- Eggs and embryos collected from seropositive donors are uninfected with BVD virus, but BVDV infection *in utero* seems to have a negative influence on embryo development.

4.5.2 Observed or proposed security measures

- Semen testing for the presence of BVD virus.

- BVD virus-free bulls in semen production units.

5. THE ROLE OF SEMEN AND EMBRYOS IN DISEASE TRANSMISSION

One may conclude that there are basic differences between the role of semen and embryos in transmitting infectious agents. Specific microorganisms have been demonstrated in several diseases (Table 1). Bovine leukosis virus, although demonstrated in semen by Lucas *et al* (1980) may gain entry by genital trauma, resulting in the presence of BLV containing lymphocytes in semen, as suggested by Kaja and Olson (1982). In BLV virus-infected cattle there is no evidence for the occurrence of cell-free virus.

The opposite situation occurs with bovine day 6-9 embryos. None of the infectious agents investigated to date (Table 1) was found to penetrate the zona pellucida and to infect the embryo. Only in the case of IBRV has it been found that the virus attached to the zona pellucida, but it was removable with the application of proper treatment (Singh *et al.* 1982a; 1982d). However, Archbald *et al.* (1979) in the first experiment on bovine embryo resistance to BVDV infection showed that when a pregnant uterus was inoculated with BVD virus culture, about 66 percent of the embryos were retarded and a structure resembling BVDV was seen beneath the zona pellucida.

Recognition of the specific embryo resistance to infection resulted in the suggestion of embryo transfer as a method of infectious disease control (Eaglesome *et al.* 1980). Practical application of this idea materialized in an attempt to save a herd of pigs infected with pseudo-rabies (James 1980), and in cattle, EBL-free progeny from serologically positive dams were obtained (Eaglesome *et al.* 1982). Similarly, BTV-free calves were obtained from infected dams (Thomas *et al.* 1983). However, embryos resistant to infection may exist only until hatching.

6. PRODUCTION OF SPECIFIC PATHOGEN-FREE (SPF) SEMEN AND EMBRYOS

Infectious agents which may contaminate semen and be present in secretions

collected with embryos can be deep-frozen and preserved for long periods. The methods of cryoprotection of gametes and embryos are similarly effective in respect to the disease agents. In this situation prevention of contamination is the basic solution. In practical terms, it means production of SPF semen and embryos.

The concept of SPF semen was strongly suggested by Bartlett (1976; 1980; 1981). The idea was brought into practice in some countries and AI organizations during the last decade (Adler 1973; Kuperschmied 1982; Nicolet 1981; Romanowski 1975; 1981).

It is proposed that SPF status of semen and embryos be based on two official statements issued by national or state veterinary health authorities with copies attached to every package of genetic material when exported.

i. A statement of the absence of infection in the country of origin of the semen or embryos:

Foot and mouth disease
Rinderpest
Contagious pleuropneumonia

ii(a). For semen only: a statement of the specific disease-free (SDF) status of the semen production unit (licence for production of semen eligible for exportation), based on the health status of bulls. The list of diseases amenable to testing periodically to validate the SDF status (condition of obtaining the licence), is as follows:

Brucellosis
Tuberculosis
Paratuberculosis
Bovine trichomoniasis
Campylobacteriosis genitalis bovis
Leptospirosis
Enzootic bovine leukosis
Infectious bovine rhinotracheitis
Bluetongue
Bovine viral diarrhoea

Continental, territorial or regional differences in the incidence of epizootic diseases occur. Wherever a given disease is unknown in a country testing for it is unnecessary.

ii(b). For embryos only: a statement of the location of embryo transfer (ET) units operating in accordance with animal health control rules (licence for production of embryos eligible for exportation). The conditions to be fulfilled to obtain the licence:

- Semen from SDF licensed semen production units only is used for embryo production.

- Donor animals fulfil requirements in respect to health status.

- Components of animal origin used in media for embryo flushing and preservation are of guaranteed SPF status.

iii. General: complementary conditions to be considered by the authorities before issuing the licence:

- Permanent veterinary supervision of semen production and embryo transfer units.

- Maintenance of the highest standard of laboratory work from the sanitary and technical points of view.

7. INFECTION PREVENTIVE MEASURES

Permanent isolation of semen production units seems to be one of the basic conditions for SDF status of animals. The recruitment of bulls for semen production must be adapted to the concept of SDF status.

i. Calves should originate from dams and herds officially free from listed infectious diseases.

ii. Bull calf rearing centres should be isolated in the same way as semen production units.

iii. Pre-entry tests of bulls covering listed diseases should be obligatory with every change of location by the animal.

iv. Frozen semen storage units should be kept in strict isolation.

v. Semen should be kept in quarantine for 28 days after production. Only after such a period, if there is no disease outbreak in the semen production unit, can semen be issued for export. The certificate of semen origin should be stamped "Quarantined".

7.1 Supplementary Means of Protection

7.1.1 Antibiotics

These are a routine component of most semen extenders. However, their bactericidal and bacteriostatic efficiency in protecting frozen semen cannot be considered absolute. In respect to viruses their efficiency is nil. The addition of antibiotics to embryo media is of questionable use.

7.1.2 Antibodies

The addition of specific antibodies to semen extenders and embryo media may be the future solution to eliminating the risk of transmitting infection in this way (Bartlett 1981).

7.1.3 Lytic enzymes

These may be of practical value to wash out viruses attached to the zona pellucida of embryos. Such treatment was effectively used by Singh *et al.* (1982a) to remove IBR viruses from the surface of bovine embryos.

8. DIFFERENCES IN THE RULES GOVERNING THE INTERNATIONAL MOVEMENT OF FROZEN SEMEN AND EMBRYOS

Due to the differences between semen and embryos as vehicles for contaminating agents, some differences in applying preventive measures may be considered in respect to male and female donors.

8.1 Males

The most effective way of producing specific pathogen-free semen seems to be by accepting obligatory SDF status for all semen donors. Such animals have to be kept in semen production units in accordance with the semen production licensing system.

8.2 Females

An individual approach towards embryo donors seems to be justified on the basis that bovine embryos seem not to serve as carriers of infectious agents. However, licensing embryo transfer units and strict veterinary health control is advisable to enforce satisfactory sanitary standards.

It seems reasonable to consider quarantine for embryo recipients. As long as embryo export is limited as it is today, facilities and all necessary sanitary measures for a limited number of recipients are reasonably easy to arrange. However, if breeders wish to import hundreds or maybe thousands of embryos from some exotic breeds, quarantine may be unpracticable for such a large number of recipients. Therefore, the most practicable course will be to apply preventive measures at source thereby enabling the transfer of embryos guaranteed free of infectious agents. Licensing of embryo transfer units, combined with control measures, seems to be the solution.

9. CONCLUSIONS

i. It can be expected that live animal trade (on an international scale) in cattle will be gradually replaced by trade in frozen semen and frozen embryos. In this way the transfer of genetic material will be cheaper and easier. At the same time, the method gives access to the top genetic material at comparative low or even very low prices. In the case of climatic differences between the environments of donors and recipients, animals born in the recipients' environment are better able to withstand conditions than animals transferred from one environment to the other.

ii. The volume of trade in frozen semen is growing and most probably its annual level now exceeds previous live cattle exports. The export of embryos is still

on a very low level but proposed simplification of the thawing procedure of frozen embryos (Leibo *et al.* 1982; Renard *et al.* 1982) may change this situation.

iii. The risk of transmitting diseases by semen and embryo transfer is obviously lower in comparison with the export of live animals. For example, all parasites are excluded. The risk of transmission of microbial diseases is very limited considering the high level of sanitary preventive measures practised in all top cattle breeding countries exporting genetic material. However, some more steps must be considered to achieve SPF status of semen and embryos as this will minimize the risk of transmitting disease.

iv. The production of SPF semen and embryos seems to be the only option for all parties interested in semen and embryo trade on an international scale.

v. Licensing of AI centres and ET units which produce genetic material for export should be the responsibility of the national or state veterinary health authorities. The fulfilment of all the health requirements established for semen and embryo trade will result in an AI organization or ET unit being given official recognition as an organization producing genetic material suitable for international trade.

vi. Information on the health status in AI centres on a country, state or breeding organization levels would be useful for all interested in buying semen. FAO, as an international organization interested in animal health control on a worldwide scale, might collect data. A list of such AI organizations and ET units published annually would be of help to interested parties.

REFERENCES

Adler H.C. 1973. Legislative systems for the prevention and control of disease transmission through AI in cattle. Proc. Actual Biol. Hyg. Probl. AI in Cattle. Danish-Polish Conf. Pawlowice. 4-6 June 1973. p. 36-40.

Alderson G.L.H. 1982. Semen bank. Ark 9(9):328-329. (ABA 50(12): 7069)

Archbald L.F., Fulton R.W., Segar C.L., Al-Bagdadi F. and Godke R.A. 1979. Effect of the bovine viral diarrhea (BVD) virus on preimplantation bovine embryos: a preliminary study. Theriogenology 11(1):81-89.

Ashwood-Smith M.J. and Grant Elizabeth. 1977. Genetic stability in cellular systems stored in the frozen state. The Freezing of Mammalian Embryos. Ciba Foundation Symp. 52. Elsevier Excerpta Medica. North Holland. p. 251-272.

Autrup E.H. and Bitsch V. 1971. The IBR/IPV situation in Denmark. Proc. Tagung: Infektionen mit dem IBR/IPV Virus und künstliche Besamung. Bern. 19 October 1971. p. 3-6.

Bartlett D.E., Larson L.L., Parker W.G. and Howard T.H. 1976. Specific pathogen free (SPF) frozen bovine semen: a goal? Proc. 6th Tech. Conf. on AI and Reprod. National Association of Animal Breeders, USA. p. 11-22.

Bartlett D.E. Health management of bulls used in AI. In: Current Therapy in
1980 Theriogenology. D.A. Morrow (ed). Section V. W.B. Saunders Co., Philadelphia, London, Toronto. p. 405-410.

Bartlett D.E. Bull semen: specific microorganisms. In: Disease Control in Semen and
1981 Embryos. FAO Animal Production and Health Paper No. 23, p. 29-48. Rome.

Baumgartner L.E., Crowley J., Entine S., Olson C., Hugoson G., Hansen H-J. and
1978 Dreher W.H. Influence of sire on BVL infection in progeny. Zbl. Vet. Med. B. 25(3):202-210.

Bitsch V. Control of the IBR-IPV infection in AI centres in Denmark. Proc. Actual Biol.
1973 Hyg. Probl. AI in Cattle. Danish-Polish Conf., Pawlowice, 4-6 June 1973. p. 22-25.

Bodo I., Dohy J. and Takàcs E. Theoretical aspects and practical methods for
1982 maintaining gene frequency in domestic animal breeds which are exposed to the danger of becoming extinct. Proc. Int. Conf. Gene Reserves, Debrecen, Hungary. 6-9 September 1982.

Bonadonna T. and Succi G. Artificial insemination in the world. Proc. 9th Int. Congr.
1980 Animal Reprod. and AI Gen. Rep. Vol. 1, Suppl. p. 1-15.

Bouillant A.M.P., Ruckenbrauer G.M., Eaglesome M.D., Samagh B.S., Singh Elizabeth
1981 L., Hare W.C.D. and Randall G.C.B. Attempts to isolate bovine leukemia and bovine syncytial viruses from blood, uterine flush fluid, unfertilized ova and embryos from infected donor cattle. Ann. Rech. Vet. 12:385-395.

Bowen R.A. Viral infections of mammalian preimplantation embryos. Theriogenology
1979 11(1):5-15.

Bowen R.A. and Howard T.H. Cited by Waters H.A. 1981. Theriogenology 15(1):57-66.
1981

Bowen R.A., Howard T.H., Elsden R.P. and Seidel G.E. Jr. Transfer of embryos from
1983 cattle infected with bluetongue virus. Theriogenology 19(1):115.

Certified Semen Services. CSS minimum requirements for health of bulls producing
1980 semen for AI. Columbia, Missouri.

Coria M.F. and McClurkin A.W. Specific immune tolerance in an apparently healthy
1978 bull persistently infected with Bovine Viral Diarrhea Virus. JAVMA 172(4):449-451.

Day B.N. Embryo transfer in swine. Theriogenology 11(1):27-31.
1979

Eaglesome M.D., Hare W.C.D. and Singh Elizabeth L. Embryo transfer: a discussion on
1980 its potential for infectious disease control based on a review of studies of infection of gametes and early embryos by various agents. Can. Vet. J. 21:106-112.

Eaglesome M.D., Mitchell D., Betteridge K.J., Randall G.C.B., Singh Elizabeth L., 1982 Samagh B.S. and Hare W.C.D. Transfer of embryos from bovine leukaemia virus infected cattle to uninfected recipients: preliminary results. Vet. Rec. 111(6):122-123.

Foster N.M., Adlers M.A., Leudke A.J. and Walton T.E. Abnormalities and virus-like 1980 particles in spermatozoa from bulls latently infected with bluetongue virus. Am. J. Vet. Res. 42:1045-1048.

George A.E. The international movement of livestock. Theriogenology 19(22):285-291. 1983

H.L. Nach fast zwanzig Jahren wieder erweckt. {After almost 20 years it can be 1975 reanimated} K.B. Mitteilungen des Schweizerischen Verbandes für künstliche Besamung. 13(4):105.

Howard T.H., Vasquez L.A. and Amann R.P. Antibiotic control of campylobacter foetus 1982 by three extenders of bovine semen. J. Dairy Sci. 65(8):1596-1600.

James E.J. Cited by Waters H.A. 1981. Theriogenology 15(1): 57-66. 1981

Jolivet G. and Parez M. Reflections sur les conditions d'une réglementation sanitaire 1982 française en matière de transferts embryonnaires. {Reflections on the stipulations of a French sanitary regulation on embryo transfers} Proc. Congr. Int. Transfert d'embryons chez les mammifères. Annecy, France. 20-23 September 1982. p. 1-13.

Kaja R.W. and Olson C. Noninfectivity of semen from bulls infected with bovine 1982 leucosis virus. Theriogenology 18(1):107-112.

Khars R.F., Gibbs E.P.J. and Larsen R.E. The search for viruses in bovine semen, a 1980 review. Theriogenology 14(2):151-165.

Krzywinski A. Personal communication. 1982

Kuperferschmied H. Praktische Beteutung der theoretischen Informationen für den 1982 Leiter einer Besamungsstation. {The practical importance of theoretical data for the Director of an AI Station} Proc. Tagung: Mykoplasmen und Ureaplasmen im Sperma und ihre Bedeutung in der kB. Zollikofen 11-12 November 1982. p. 18-19.

Leibo S.P., West A.W. III. and Perry B. A one step method for direct nonsurgical 1982 transfer of frozen-thawed bovine embryos. I. Basic Studies, II. Applied Studies. Cryobiology 19(6):673-674.

Lucas M.H., Dawson M., Chasey D., Wiberley G., Roberts D.H. and Saunders R. 1980 Enzootic bovine leucosis virus in semen. Vet. Rec. 106(6):128.

Luedke A.J., Walton T.E. and Jones R.H. Detection of bluetongue virus in bovine 1975 semen. Proc. 20th World Vet. Congr. Vol. 3. p. 2039-2042.

Lyon Mary F., Glenister P.H. and Wittingham D.G. Long-term viability of embryos
1981 stored under irradiation. In: Frozen Storage of Laboratory Animals. G.H. Zeilmaker (ed.). Gustaw Fischer Verlag, Stuttgart, New York. p. 139-147.

Łosinski T., Rosłanowski K. i Wyszanowski J. Praktyczne aspekty rozpoznawania i
1974 leczenia vibriozy buhajów. {Practical aspects of diagnosis and therapy of vibriosis in bulls} Przeglad Prac ZHW Poznan, Vol. I. p. 139-160.

Miller Janice M. and Van der Maaten M.J. Bovine leucosis - its importance to the
1982 dairy industry in the United States. J. Dairy Sci.. 65(11):2194-2203.

Mitchell D., and Betteridge K.J. Embryo transfer for import and export and possible
1977 dangers of transmitting infectious disease. In: Embryo Transfer in Farm Animals: A Review of Techniques and Applications. Canada Dept. of Agric. Monograph 16. p. 66-68.

Mobraaten L.E. The Jackson Laboratory Genetics stocks resource repository. In: Frozen
1981 Storage of Laboratory Animals. G.H. Zeilmaker (ed.). Gustaw Fischer Verlag, Stuttgart, New York. p. 165-177.

Müller E. IBR/IPV Situationsbericht Osterreich. proc. Tagung: Infektionen mit dem
1971 IBR/IPV-Virus und künstliche Besamung. Bern, 19 October 1971. p. 13-14.

Nicolet J. Probleme der bakteriologischen Ueberwachung von Besamungsstationen.
1981 {Problems of bacterial monitoring at AI Stations} Proc. 30 Int. Fachtagung f. Fortpflanzung und Besamung. Wells. p. 1-6.

Parez M. La transplantation d'oeufs fécondés chez les mammifères. Problèmes sani-
1978 taires. {Transplantation of fertilized ova in mammals. Sanitary problems} Bull. Off. Int. Epiz. 89(1-2):1-12.

Parez M. Risks of infection due to embryo transfer in cattle. In: Disease Control in
1981 Semen and Embryos. FAO Animal Production and Health Paper No. 23, p. 89-96. Rome.

Renard J-P., Heyman V., and Ozil J.P. Simplified procedure for freezing cow embryos
1982 for cervical transfer. Annales Med. Vet. 126:23-32.

Romanowski W., Marré H. und Pfeilsticker J. Ein Weg zur IBR/IPV-Sanierung des
1975 Bullenbestandes einer grossen Besamungsorganisazion. {A method of IBR/IPV treatment of bulls at a large AI organization} I. Tierzüchter Nr 9:379-381, II. Tierzüchter Nr 10.429-432.

Romanowski W. Bullenbestand IBR/IPV-frei. Der Bullenbestand der RPN, der z. Zt. 373
1981 Bullen bzw. Bullenkälber umfast, ist IBR/IPV-frei. Rinderproduktion, Nr 48:4-5.

Schultz R.D., Adams L.S., Letchworth G., Sheffy B.E, Manning T. and Bean B. A
1982 method to test large numbers of bovine semen samples for viral contamination and results of a study using this method. Theriogenology 17(2):115-123.

Singh Elizabeth L., Thomas F.C., Hare W.C.D., Mitchell D., Eaglesome M.D., Randall
1982a G.C.B., Betteridge K.J., Dulac G.C., Samagh B.C. and Papp-Vid G. Embryo transfer in disease control. Theriogenology 17(1):108.

Singh Elizabeth L., Eaglesome M.D., Thomas F.C., Papp-Vid G. and Hare W.C.D.
1982b Embryo transfer as a means of controlling viral infections. I. The in vitro susceptibility of preimplantation bovine embryos to akabane, bluetongue and bovine viral diarrhea viruses. Theriogenology 17(4):437-444.

Singh Elizabeth L., Hare W.C.D., Thomas F.C., Mitchell T.D., Eaglesome M.D.,
1982c Randall G.C.B., Bouillant A.B.P., Betteridge K.J., Samagh B.S. and Bielanski A. Disease control through embryo transfer. Proc. 2nd Congr. Embryo Transf. in Mammals, Annecy 1982. In press.

Singh Elizabeth L., Thomas F.C., Papp-Vid G., Eaglesome M.D. and Hare W.C.D.
1982d Embryo-transfer as a means of controlling the transmission of viral infections. II. The in vitro exposure of preimplantation bovine embryos to infectious bovine rhinotracheitis virus. Theriogenology 18(2):133-140.

Singh Elizabeth L., Hare W.C.D., Thomas F.C., Eaglesome M.D. and Bielanski A.
1983 Embryo transfer as a means of controlling the transmission of viral infections. IV. Non-transmission of infectious bovine rhinotracheitis (infectious pustular vulvovaginitis virus following trypsin treatment). Preprint. Accepted for publication in Theriogenology.

Steck F. Serologische und virologische Oberwachung von Besamungsstieren. {Serological
1971 and virological monitoring of AI bulls} Proc. Tagung: Infektionen mit dem IBR/IPV-Virus und künstliche Besamung. Bern, 19 October 1971. p. 23-27.

Stringfellow D.A., Howell V.L. and Schnurrenberger P.R. Investigations into the
1982 potential for embryo transfer from brucella abortus infected cows without transmission of infection. Theriogenology 18(6):733-743.

Thomas F.C., Singh Elizabeth L. and Hare W.C.D. Embryo transfer as a means of
1983 controlling viral infections. III. Non transmission of bluetongue virus from viremic cattle. Theriogenology 19(3):425-431.

Voelkel S.A., Stuckey K.W., Looney C.R., Enright F.M., Humes P.E. and Godke R.A.
1982 An attempt to isolate brucella abortus from uterine flushings of superovulated donor cattle. Theriogenology 17(1):111.

Waters H.A. Health certification for livestock embryo transfer. Theriogenology
1981 15(1):57-66.

Whitmore H.L., Gustafson B.K., Havareshti P., Duchateau A.B. and Mather E.C.
1978 Inoculation of bulls with bovine virus diarrhea virus: Excretion of virus in semen and effects on semen quality. Theriogenology 9(2): 153-163.

Whittingham D.G. Embryo banks in the future of developmental genetics. Gentics
1974 78:395-402.

Wierzbowski S., Wierzchos E., Smorag Z., Kareta W., Gajda B., Krupinski J. and
1983 Zukowski K. The practical application of embryo freezing and transfer for preservation of endangered Polish Red cattle and longwool primitive sheep. (Submitted for 10th Int. Congr. Animal Reprod. and AI, Urbana.)

HARVESTING, PROCESSING, STORAGE AND SUBSEQUENT USE OF ANIMAL CELLS IN DEVELOPING COUNTRIES

M. Parez[1]

New reproductive technologies (AI, oestrus control, superovulation, embryo transfer, cell micromanipulation) become more widely used every day (Brackett, Seidel and Seidel 1981). They do not always improve fecundity, but they offer solutions to problems concerning the creation and spread of genetic improvement. With the help of cryobiology, they enable, in space and time, the transfer of advantageous genes. To make the most of this potential, it is necessary to create gene stocks or banks, as gametes or embryos.

1. GENE BANKS FOR ANIMAL GENETIC MATERIAL

1.1 Concept

The establishment and maintenance of a bank of animal genetic material to be preserved at low temperature (-196°C; liquid nitrogen), employing techniques so far developed, encompass:

- harvesting of the genetic material,
- processing of the material for storage,
- monitoring of the fertilizing capacity/viability of gametes and embryos,
- maintenance of the store.

An important component of the procedure for cryopreservation of genetic material is the disease control aspect, which has been dealt with by Wierzbowski (1984). The genetic material for which preservation technology has been developed so far encompass:

- spermatozoa (cattle, goats, pigs, horses and poultry),
- blastocysts (cattle, sheep and goats).

Table 1 illustrates the present stage of utilization of cryopreserved animal genetic material. For the future, there are indications that preovulatory and/or postovulatory oocytes may also be stored.

It should be noted that experience of harvesting, processing and storage of genetic material has so far been gained almost exclusively in developed countries with well-established laboratory facilities and well-trained technicians and scientists. Experience from (tropical) developing countries, where genetic material of interest for cryogenic storage may be identified, is at present available only on a limited scale. Therefore, procedures for cryopreservation in such circumstances will have to be based on experience gained in developed countries.

---

[1] Laboratoire pour le Contrôle des Reproducteurs, 13 rue Jouët,94704 Maisons-Alfort, France.

Table 1 COMPARISON OF THE USE OF SPERM AND EMBRYO FREEZING IN VARIOUS SPECIES (From Cole and Cupps 1977; Foote 1981; Gomes 1977; Hafez 1968)

Species	Average mean conception rate to 1st service (natural mating) %	Practical use of frozen semen at present	Number of females inseminated per ejaculate	Average mean conception rate to 1st service (frozen semen)	Embryo freez-ability	Mean birth rates from frozen embryos %
Cattle	60-65	extensive	200-400	50-60	yes	35-45
Goat	65-75	limited to certain areas	5-30	50-65	yes	?
Sheep*	75-85	occasionally	4-6	40-50	yes	?
Pig*	65-75	occasionally	5-10	45-55	no	
Horse*	50-65	occasionally	10-20	25-50	no	
Poultry	90	occasionally	10-15	30-55	no	

* Frozen semen almost exclusively used for import-export of valuable genetic material.

1.2 Aims of Cryogenic Storage

These can be summarized as follows for semen banks:

- Genetic progress, by widespread use of progeny-tested, improver bulls.
- Dissemination of this genetic process in purebreeding or crossbreeding.
- Maintenance of reference sires against which genetic change can be measured.
- Storage of genes to reconstitute a disappearing strain or breed.

The aims of embryo storage are:

- To obtain offspring from cows with high genetic potential.
- To develop a herd from a few cows with high genetic potential.
- To use with efficiency the genetic material available.
- To shorten the generation interval.
- To make the transfer of animals easier (import-export).
- To store genetic material for reconstituting a disappearing strain or breed.
- To control disease.
- Experiments in physiology and genetics.

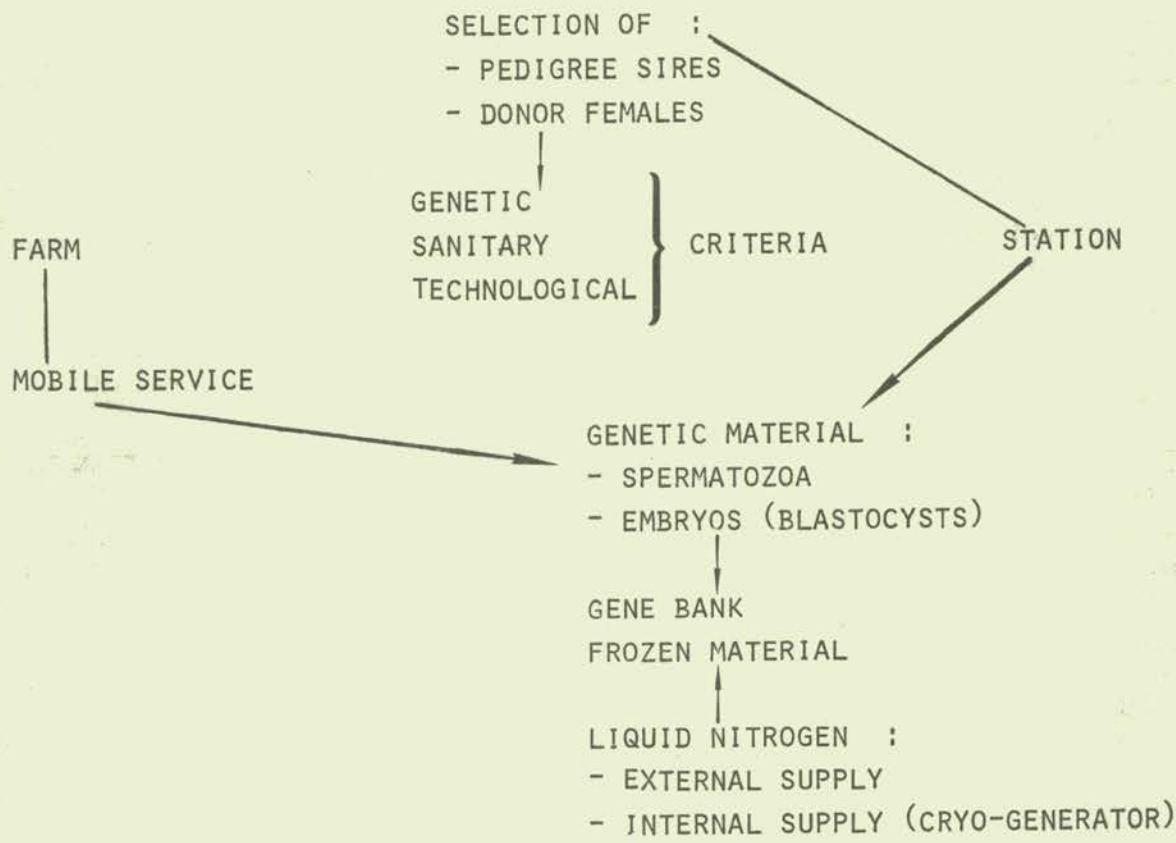
The last 40 years have demonstrated the importance of AI as an agent of genetic improvement. On the other hand, genetic effects of embryo transfer are still under discussion (Jandrain 1977; Smith 1976). Embryo transfer remains, however, a valuable tool for disseminating the genetic merit of outstanding females at the herd level, even though the contribution to genetic progress at the breed or population level is low (Menissier 1983). Obviously, semen will not be stored with the same genetic programme aims as embryos. For instance, if one expects to produce high-yielding individuals as purebreds, then embryos should be kept. On the other hand, if one expects to produce the best crossbred progeny, then spermatozoa should be kept. Purely as a gene bank, embryo storage must be considered more suitable than semen storage (Land 1977). If it is necessary to reconstitute a breed from a gene bank, this can be done in a single generation from embryos, whereas it will take several generations of backcrossing with semen storage.

2. RESOURCES AVAILABLE FOR THE CREATION OF GENE BANKS

In a sense, gene banks are already available in developed countries. AI enterprises keep stores of frozen semen, and frozen embryos are also stored to some extent. Facilities for processing and cryogenic storage of animal genetic material are much less available in developing countries. Tables 2 to 4 list countries (not necessarily all) with at least one centre involved in semen processing and storage. As yet, there are no developing countries involved in the production and storage of frozen embryos.

3. EQUIPMENT REQUIREMENTS FOR A GENE BANK

The diagram hereunder indicates the steps involved in the supply, processing and storage of genetic material.



Should the three functions be carried out at a single place? Is specific equipment required? Should donor animals be brought to a station or should harvesting of the genetic material take place where the donor animals are normally kept? Local conditions will determine the answers to such questions. If part of the genetic material is to be collected on the farm (as is becoming common in developed countries in the case of embryo collection) then adequate mobile facilities are required. Another requirement is a high level of management in herds of donor animals, to permit

Table 2 SEMEN BANKS IN DEVELOPING COUNTRIES - AFRICA

Country	Technical aid granted by	Technique used	Liquid nitrogen supply	Location	Private or state	Number of 1st inseminations per year
Algeria		Mn.S.	yes	Tizi - Ouzou	S	4 000
Egypt		Md.S.	yes	Cairo/Beni - Souif/ Alexandria	S	50 000 to 100 000
Ethiopia	World Bank	0.25 ml straw	yes	Addis Ababa	S	
	SIDA	0.50 ml straw	no	Arrusie	S	7 000
Gabon	France	Mn.S.	yes	Franceville	S	
Ivory Coast		Mn.S.	yes	Bingerville	S	
Kenya	SIDA	Md.S.	yes	Lower-Kabete	S	500 000
Libya		Mn.S.	yes	Tripoli	S	5 000
Malawi		Mn.S.	yes	Blantyre	S	
Morocco		Md.S.	yes	Ain D'Jema/Kenitra	S	30 000
Mozambique		Md.S.	yes	Maduto	S	15 000
Nigeria		0.25 ml straw	yes	Zaria	S	
Somalia	USAID	Amp.	yes	Mogadishu	S	1 500
Tanzania	SIDA	Mn.S.		Arusha	S	30 000
	Cuba	Pel.	yes	Musoma	S	10 000
Tunisia		Mn.S.	yes	Sidi Thabet	S	30 000
Uganda	EEC	Md.S.		Entebbe	S	
Zambia	Netherlands	Mn.S.		Mazabuka	S	15 000
Zimbabwe		Md.S.	yes	Harare	P,S	80 000

SIDA : Swedish International Development Authority
Amp. : Ampoule
Md.S. : Medium straws
Min. : Minitubes

Mn.S. : Ministraws
Pel. : Pellets
P : Private
S : State

Table 3 SEMEN BANKS IN DEVELOPING COUNTRIES - LATIN AMERICA

Country	Technical aid granted by	Technique used	Liquid nitrogen supply	Location	Private or state	Number of 1st inseminations per year
Argentina		Pel/Md.S.	yes	22 AIC	P	2 500 000
Bolivia		Mn.S.	yes	La Paz	S	25 000
		Md.S.	yes	Santa Cruz	P	
Brazil		Mn.S.	yes	6 AIC	P	800 000
Chile		Min.Md.S.	yes	Valdivia	S	95 000
Colombia	Switz. + GTZ	Md.S.	yes	3 AIC	P	250 000
Costa Rica	BID	Md.S.	yes	San Jose	S	35 000
Cuba		Pel.	yes	9 AIC	S	1 700 000
Dominican		Amp.	yes	Gulf Rancy	P	10 000
Republic	BID	Mn.S.	yes	Santo Domingo	S	?
Ecuador		Mn.S.	yes	Quito	S	45 000
Jamaica	Canada	Md.S.	yes	Kingston	S	
Mexico		Mn.S.,Md,S.	yes	1 AIC	S	600 000
				11 AIC	P	
Nicaragua	FAO	Md.S.	yes	Managua	S	
Paraguay	BID	Md.S.	yes	Asuncion	S	15 000
Peru	BID	Mn.S.		La Molina	S	80 000
Salvador		Md.S.	yes	San Salvador	S	
Venezuela		Mn.Md.S.	yes	16 AIC	P	300 000

AIC: AI Centre
GTZ: Technical Aid from German Federal Republic
BID: Bank for International Development

Table 4 SEMEN BANKS IN DEVELOPING COUNTRIES - ASIA

Country	Technical aid granted by	Technique used	Liquid nitrogen supply	Location	Private or state	Number of 1st inseminations per year
Afghanistan	FAO	Mn.S.	yes	Kabul Univ.	S	
Burma		Mn.S.	yes	Rangoon	S	10 000
India	Denmark Australia Switzerland	Md.S.				
	New Zealand UK France World Bank	Mn.S. Min.	yes		S	1 000 000
Indonesia	New Zealand	Mn.S.	yes	3 AIC	S	
Iraq		Mn.S.	yes	1 AIC	S	10 000
Iran		Md.S.Mn.S.	yes	2 AIC	S	50 000
Pakistan	FAO	Mn.S.	yes	Lahore	S	
	World Bank	Mn.S.	yes	Sindh	S	100 000
	GTZ	Mn.S.	yes	Punjab	S	
South Korea		Md.S.	yes	2 AIC	S	
South Yemen	FAO	Md.S.	yes	Aden	S	3 000
Syria		Mn.S. Pel.	yes yes	Damascus	S S	10 000
Thailand		Mn.S.	yes	2 AIC	S	50 000
Turkey	World Bank	Md.S.	yes	5 AIC	S	

GTZ : Technical aid from German Federal Republic

adequate control of the operations. On-farm collection of material reduces investment in laboratories, and provides a larger catchment area for the genetic material. In this case, the gene bank *per se* will be reduced to a storage area. Harvesting of genetic material at established stations provides the best technical control of operations, leading to the best results.

3.1 Semen Production Units

The requirements for a processing and storage centre for frozen bull semen were discussed by Parez (1978). There are two basic considerations:

- A semen production and storage unit can exist only if it is regularly and easily provided with liquid nitrogen.

- If semen exports are planned, it will be necessary to meet the health, biological and technological requirements of the importing countries. The semen production unit should be sited in an area which is satisfactory climatically and free of the major communicable diseases. A nearby animal pathology laboratory could provide valuable assistance.

3.1.1 The station should be completely isolated from any herds

The housing of bulls in individual boxes obviates managemental difficulties. Additional premises should be located within the AI centre.

Technical premises should be made up of three rooms:

- A laboratory, with benches and sinks on two well-lighted walls. The area of the laboratory should be about 20 m^2.
- A room for processing, storage, parcel despatch, and cleaning and sterilizing of equipment. The area of this room should be about 15 m^2.
- An office of about 15 m^2.

Laboratory air-conditioning reduces temperature and relative humidity, leading to trouble-free working of electrical equipment, and limits the liquid nitrogen consumption of cryogenic containers to about the same level as in a temperate climate. A flat area near the laboratory can be used for semen collection. This area should measure 7 x 7 m. In a hot, dry climate, trees will give sufficient shade. In a hot, humid climate, roof cover is necessary.

3.1.2 Semen donor bulls

Bulls selected initially on the basis of genetic merit should meet the following requirements: (i) freedom from diseases transmissible through semen; (ii) satisfactory sexual performance; (iii) good semen freezability. The fulfilment of these requirements may considerably reduce the number of bulls originally selected as semen donors on the basis of their genetic merit. The data below, which illustrate the reduction in the number of bulls due to various reasons, are drawn from a project for semen production from N'Dama bulls. There were 60 bulls initially.

	Rejected	Accepted (Number remaining)
Genetic selection (by inspection)	38	22
Clinical examination of the genital organs and the locomotory system	4	18
Examination of sexual function and health tests (42 days' quarantine)	12	6
Second health test	0	6

The best time for semen collection will have to be chosen in accordance with climatic and geographical conditions. In the tropics, fertility and libido are more satisfactory at the end of the rainy season and during dry periods than during the main part of the rainy season. As an example, in the equatorial Guinea zone, with two rainy seasons per year (May to mid-June and October-November), six N'Dama bulls regularly collected for semen once weekly had an average production in February, May, June and November of 900, 100, 300 and 700 doses per bull per month respectively.

Bulls should not be given internal or external treatments that would be harmful to semen quality. For instance, some organo-chlorine products used to remove external parasites have a detrimental effect on semen.

3.1.3 Semen quality

The quality of semen collected in an artificial vagina should correspond to the following minimum standards:

Motility	4 (on scale 0-5)
Percent live spermatozoa	65
Sperm concentration	Not below 700 000/mm^3

The diluent used should be milk-egg yolk or sodium citrate-egg yolk, either specially prepared or a commercial product. Glycerol (14 percent) should be added to half of the diluted semen so as to obtain a final 7 percent rate. The dilution rate should be calculated so as to get a final concentration of 30×10^6 live spermatozoa per dose, and to provide at least 12×10^6 live spermatozoa after freezing and thawing. Freezing of the semen should be carried out by standard methods. Individual doses should be labelled with the following information:

- name of semen collection centre
- breed of bull
- number of semen donor bull
- date of semen collection and processing.

Frozen buffalo semen may be processed by the technique of Crabo (1977). The diluent used in this technique consists of equal parts of milk-egg yolk and TES-TRIS-glucose buffer. The composition of the latter diluent is:

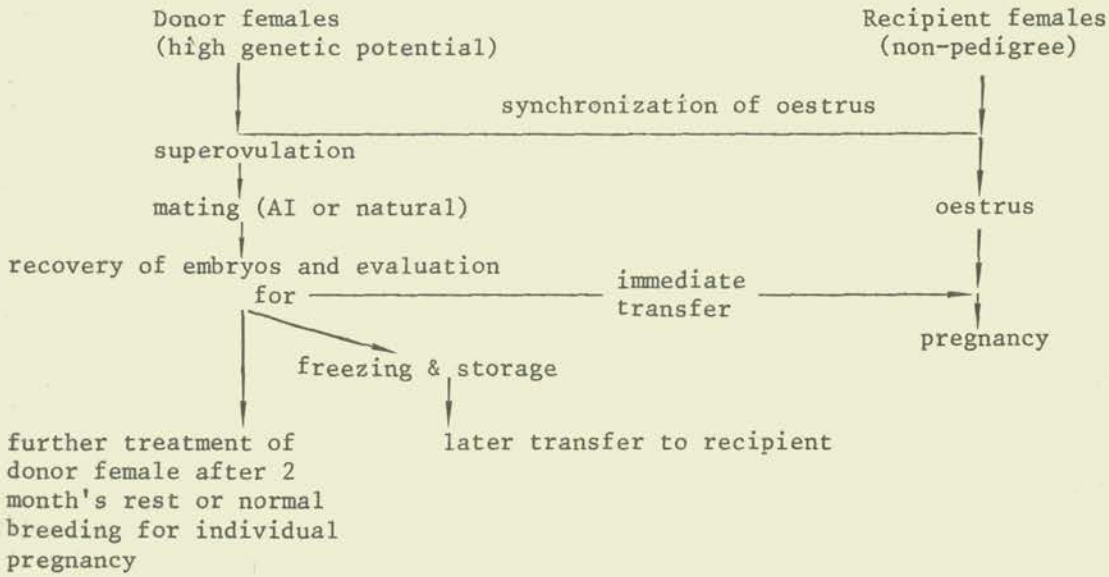
N-(trishydroxymethyl)methyl-2-aminoethane sulphonic acid (TES)	48.3 g
Tris(hydroxymethyl)aminomethane (TRIS)	11.6 g
Glucose	2.0 g
Distilled water	to 1000 ml

Dilution is carried out in four steps: dilution to half the final rate of 37.4°C, cooling to 4° over a one-hour period, further dilution with diluent containing 16 percent glycerol, and equilibration for 3-4 hours.

Before final storage of the frozen semen, a quality test (motility, percent motile spermatozoa) should be carried out 48 hours after freezing. Special care should be taken to maintain the recommended liquid nitrogen level in the storage container.

3.2 Embryo Bank

The main features of the embryo transfer technique may be illustrated as follows (adapted from Hunter 1980):



Embryo collection and processing (as well as semen collection) may be undertaken in three ways:

i. at an embryo transfer station;
ii. on the farm, with embryo processing at a stationary laboratory;
iii. wholly on the farm, with embryos being taken back to the station for final storage.

For the last alternative, the method of working might consist of harvesting and freezing in a given country during one or two months by a mobile team, then final storage in another country at a station, if the latter were not available in the country of collection. The on-farm mode of working is less expensive that the other methods, and is becoming more widely used in developed countries. The breeder or technician is charged with most of the preliminary work (oestrus observation, treatments, etc.). The transfer team is brought in only to harvest embryos, store them and eventually carry out transfers. The main drawback to this pattern is the impossibility of ensuring that each technical step of the process is properly carried out.

3.2.1 Embryo collection station

The premises can be similar to those of a semen collection station. Laboratory space and facilities may be used for both purposes. Donor cows can be kept in boxes, either individually or in groups of 4 or 5 animals. These cows must be handled gently. Stress may cause embryo mortality. A holding pen placed in the shade, close to the examination and embryo collection premises, should be used for handling animals.

3.2.2 Reproductive characteristics of embryo donor females

In order to achieve high reproductive efficiency in embryo transfer, donors should have a good reproductive history (a calving interval of close to 1 year, 1.5-1.8 inseminations per conception, and no dystocia at the last calving), and should have produced at least three calves. They should be at least 90 days *post partum*, have exhibited normal uterine involution, and be regularly cycling. Also, their general condition and health should be good, with no metabolic disorders and metritis. Heifers (if used) should have a record of moderate daily weight gain, and should have reached reproductive age and a corresponding body weight.

Donor cows should be submitted to tests of disease status, as indicated by Wierzbowski (1984).

Oestrus should be detected by an experienced person. A vasectomized bull with a chinball harness may be useful for heat detection in certain circumstances. The appropriate treatment of donor cows for superovulation should be chosen in accordance with their reproductive physiological status. Cycling cows can be treated either with FSH or PMSG. Cows not cycling should be treated either with progestogen-FSH or progesterone-PSMG. Treatment cycles for superovulation of donors are given in Tables 5 and 6.

The response to superovulation varies between individuals. The ovarian stimulus must be administered at the season of highest fertility. In some climatic areas,

Table 5

TREATMENT WITH FSH

Females with unknown heats (but not in an oestrus)

1st day — Implants (2) + progestogen injection*
2nd
3rd
4th
5th
6th
7th
8th day — FSH 08.00 hours (double dose)
FSH 20.00
9th day — FSH 08.00
FSH 20.00
10th day — FSH 08.00 + implant withdrawal
FSH 20.00
Day 0 — 11th day — FSH 08.00
FSH 20.00 + AI
12th day — morning : AI

Day 7 / Day 8 — embryo collection

NB: either 2 AI 12 and 24 hours after heat started, or 2 fixed-time AI 36 and 52 hours after implant withdrawal

* Norgestomet

Females with known heats

1st day — Introduction of progesterone** vaginal device (*in situ* 9-12 days)

11th day — Intramuscular injection of $PGF_{2\alpha}$ analogue

12th day — Device withdrawal 2-3 days later

Day 0 — Oestrus

Day 9 — Rectal exploration for corpus luteum

08.00 hours — FSH 6 mg
20.00 — FSH 6 mg

Day 10 — 08.00 — FSH 5 mg
20.00 — FSH 5 mg

Day 11 — 08.00 — FSH 3 mg — PG (double dose)
20.00 — FSH 3 mg

Day 12 — 08.00 — FSH 2 mg
20.00 — FSH 2 mg

Oestrus

Day 13 — 08.00 — AI
20.00 — AI

Day 0

Day 7 & 8: embryo collection

NB: either 2 AI 12 and 24 hours after heat started, or 2 fixed-time AI 48 and 60 hours after PG (double dose)

** PRID (CEVA)

Table 6

TREATMENT WITH PMSG

Females with unknown heats (but not in an oestrus)

	1st day	Vaginal device introduction (9-12 days) * (progesterone)
	2nd	
	3rd	
	4th	
	5th	
	6th	
	7th	
	8th day	PMSG 08.00 hours (2500 or 2000 IU, double dose)
	9th	
	10th day	Device withdrawal 08.00
Day 0	11th day	morning : oestrus
		20.00 : AI
	12th day	morning : AI
Day 7		embryo collection
Day 8		

NB: either AI 12 and 24 hours after heat started, or 2 fixed-time AI 36 and 52 hours after device withdrawal

* PRID CEVA

Females with known heats

	1st day	Vaginal device introduction (progesterone, 9-12 days)
	11th day	$PGF_{2\alpha}$ **
	12th day	Device withdrawal
		48-72 hours
Day 0		oestrus
Day 9		rectal exploration for corpus luteum
		08.00 PMSG (2500 or 2000 IU)
Day 10		
Day 11		08.00 PG (double dose)
Day 12		
		oestrus
Day 13		08.00 : AI
		20.00 : AI

Day 0 → Day 7 & 8: embryo collection

NB: either 2 AI 12 and 24 hours after heat started, or 2 fixed-time AI 48 and 60 hours after PG (double)

** i.e. 0.5 mg ICI cloprostenol

this depends on the standard of husbandry. Sometimes, there may be a 3-month difference between the time of highest fertility in cows on stations and in village herds. The period of highest fertility can be established from calving results over the two previous years. Response to ovarian stimulation is affected by breed. Some beef animals give a better response than dairy cows, as shown in Table 7.

Table 7 RESPONSE TO SUPEROVULATION

Breed	Number of superovulated animals	Number of embryos collected
Charolais	335	6.8 ± 7.6
Holstein-Friesian	417	5.7 ± 5.5
French Friesian	529	3.2 ± 3.8

French National Programme 1980-81 (UNCEIA 1982)

Heifers give a better response (4.7 ± 4.7) than multiparous cows (2.7 ± 3.5). The average number of embryos collected per treated donor, from my own results based on 1832 observations, was 3.5, with individual variation from 0 to 21. With current techniques, more than 35 percent of females do not respond to ovarian stimulation. The response of 1828 treated cows was as follows (Nibart 1982):

- 1752 cows had at least one corpus luteum; 76 had none.
- 1386 cows had at least one collected embryo; 366 had none.
- 1127 cows had at least one collected, transferable embryo; 259 had none.
  (76 + 366 + 259 = 701; 701/1828 = 0.383 = 38.3 percent)

Thus, response to ovarian stimulation depends on type of treatment used, and on its suitability to the animal's physiological condition. In general, FSH seems more efficient than PMSG for stimulation of ovulation. Values for several reproductive traits are not yet well established for cattle and buffaloes in developing countries, e.g. the duration of superovulation (which determines the proper time for insemination), the stimulation response, and the average number of embryos collectable per female. In practical conditions, the mean efficiency of embryo production can be summarized as follows:

- rate of non-response 35 percent
- mean number of transferable embryos per superovulated cow 4 (0-21)
- pregnancy rate after transfer 45-50 percent

Thus, if 30 donor cows were chosen, at least 10 might not produce normal, transferable embryos. If there is no genetic factor causing a lack of response to ovarian stimulation, statistics show that 7 females from an identical genetic strain must be chosen to have a 95 percent chance of at least one female or male being born from collected embryos. Six embryos must be transferred to have a 95 percent chance of obtaining one animal born. In the course of time, one may expect improvement in these figures, due to new scientific knowledge and increasing technical skills of embryo transfer technicians.

Fertilization of eggs in the donor's genital tract can result from AI or natural

mating. In the case of natural service, the bulls will have to be selected in respect of satisfactory fertilizing ability. One bull per two donor cows will be required, since there is a need for three matings per female per 24 hours (i.e. 6 matings per bull per 24 hours).

3.2.3 Embryo collection

Seven days after oestrus and fertilization, donors are flushed using a three-way catheter (Foley, Hahn or IMV models), successively inserted into each uterine horn. Buffer solution (400-500 ml PBS or Dulbecco's phosphate saline) is injected into each uterine horn, and this enables the collection of 70-75 percent of embryos present. The search for embryos, and their examination, are undertaken by using a microscope at 60 times magnification, after the collected liquid has been decanted. Blastocysts are classified according to quality. The choice of embryos, based on the quality criteria, greatly influences the success of freezing and transfer, as shown in Table 8.

Table 8 EFFECT OF EMBRYO QUALITY ON PREGNANCY RATE

Embryo class	Number of transferred embryos	Pregnancy rate at 90 days
Excellent	275	6.30 (a)
Good	152	58.0 (b)
Mean	42	31.0 (c)
Poor	42	12.0 (d)

(a), (b), (c), (d) : significant different $P < 0.05$

The time required to carry out procedures is as follows:

- team installation and settling in of cows 30 minutes
- embryo collection per donor 60 "
- embryo search and examination 60 "
- freezing 60 "

On the same day, a three-person team, with some help for animal handling, will be able to flush 4-5 donor females.

Freezing techniques are as easy to carry out for embryos as for semen. A French technique (IMV-INRA) and an American one (Leibo 1983), very similar in nature, are being patented. Both techniques enable one-step thawing and on-farm transfer, and are as easy to perform as AI.

The basis of these techniques is freezing of the embryo in PBS solution plus 2.0 M glycerol in a straw which also contains a 1.08 M solution of sucrose, the two fractions being separated by an air bubble. Embryos are automatically frozen in a single, rapid step to -6° to -8°C, then progressively until -35° (0.5° per minute) after induction of crystallization (seeding). Storage of embryos is in liquid nitrogen (-196°).

Thawing is carried out in a water bath at 25°-30°C for one or two minutes. A

mixture of both fractions in the straw is obtained by shaking. The thawed embryo can be used without removing the glycerol. Transfer of the embryo into the uterus of the recipient is done by the non-surgical, utero-cervical route. The embryo is deposited deep in the uterine horn ipsilateral to the ovary bearing the corpus luteum. A special instrument is available for this transfer.

3.2.4 Oestrus synchronization between donor and recipient

In cattle, there is a 24-hour maximum time tolerance in the synchronization of the physiological status of the reproductive tract of donor and recipient (Rowson 1972). To obtain synchrony, the best way is to have available a group of recipient females, in natural oestrus, large enough for the planned transfers on a certain day. For example, 300 closely observed females provide, on average, 10 females in oestrus daily. However, treatment with prostaglandin analogues produces very satisfactory synchronization 48-72 hours after its injection between the 5th and 16th days of the oestrus cycle. Embryo transfer has to be made seven days after oestrus. Surgical transfer initially gave better results than cervical transfer. Recent results with cervical transfer indicate a tendency towards equality of results, therefore the easier, cervical transfer technique is replacing the surgical method.

4. MATERIAL AND EQUIPMENT

Details of material and equipment for semen collection and embryo transfer are listed in Appendixes 1 and 2. For both semen and embryo banks the general requirements are:

- equipment for animals (bulls and cows);
- laboratory equipment and supplies;
- office furniture and supplies;
- vehicles.

4.1 Equipment for Animals

Suitable facilities are required for restraining animals at collection of semen and embryos.

4.2 Laboratory Supplies

Few items are common to semen and embryo banks. Cryogenic containers and glassware are usable for both purposes (see Appendixes 1 and 2).

4.3 Office Furniture

Requirements are limited (table, chair, files, etc.).

4.4 Transport

If embryo transfer is undertaken at stations, there is no need for special transport. If on-farm collection is used, a laboratory van is necessary.

4.5 Liquid Nitrogen

This is a very important item. The following data are relevant:

- one semen freezing operation requires about 7 litres of nitrogen (500-1000 doses);
- one embryo freezing operation (24 embryos) requires about 7 litres of nitrogen;
- a 30 litre container in static storage consumes about 0.2 litres per day;
- a 200 litre container in static storage consumes about 5 litres per day.

5. STAFF

Collection and processing techniques for bull semen are well known worldwide. However, this is not the case for bovine embryo collection and processing. Embryo transfer cannot be improvised. The different techniques so far developed require precise step-by-step enactment of the procedures. Ovarian stimulation, oestrus synchronization, etc., can only be successfully applied by the use of treatment regimes adapted to different physiological conditions of animals. Consequently, successful embryo collection, processing and transfer depend on the skills and qualifications of staff.

A team for semen production and/or embryo production should include as a minimum:

- a team leader in charge of all operations. He/she should have a university degree, and be specialized in reproductive physiology and AI, oestrus control and cryogenic techniques;

- a laboratory assistant to undertake the work on quality evaluation and semen or embryo processing;

- an inseminator with experience of more than 1000 inseminations is required. The cervix is closed at day 7 in the cycle. Consequently, this technician will have to be sufficiently skilled to avoid damaging the cervix when inserting and removing the three-way catheter;

- sufficient staff for animal care and handling.

The technicians must possess particularly high levels of manual dexterity. Sufficient ability may be acquired after about 100 collections and transfers. This training may be obtained by participation in operation teams for at least 3 months. There are now numerous training courses in the USA and Europe for this type of specialist. They are generally intended for experienced AI technicians who want to add training in embryo transfer to their AI training. The duration of the courses is usually 15-30 days.

6. COSTS

These may vary from one project to another. Investment is about the same for the processing of 1000 or 10 000 semen doses, or to process 50-500 embryos.

6.1 Estimated Costs in Developed Countries

6.1.1 Semen bank

The average laboratory cost per dose of frozen semen in France is 0.53± 0.10 francs (US$ 0.66 ± 0.01; hereafter, $ means US$). The average cost of semen storage is 0.25 francs ($ 0.03) per dose per year. However, these figures are derived from units processing and storing more than 200 000 doses per year.

6.1.2 Embryo bank

French studies in 1982 indicated the following costs:

- at a station, about $ 1060 per pregnancy
- on-farm, about $ 725 per pregnancy.

Various types of contract, often complex, have been offered by the US embryo transfer industry. Average prices offered are $ 300 for on-farm collection (exclusive of team travel expenses), $ 300 per transferred embryo, and $ 1000 per pregnant recipient female at a station. For the USA, Seidel and Seidel (1981) gave the cost price per calf born from embryo transfer at $ 2300 to $ 2800. These values are only indicative. Furthermore, they are derived from the operations of very well-trained teams, working in a very favourable environment.

6.2 Estimated Costs in Developing Countries

Very little is known on this topic. The Ivory Coast Ministry of Animal Production provided the following data:

i. Investment for 10 bulls to produce about 10 000 doses of semen per year (in 1979): 15 million CFA francs ($ 37 500), including buildings and equipment.

ii. Annually recurrent expenditure (exclusive of salaries):

1980	$ 17 500
1981	$ 17 875
1982	$ 26 250

Under these conditions, the cost of a semen dose would be $ 5.6, with the investment amortized over 5 years. No costs are available for embryo production in developing countries.

The following is an estimate of investment and recurrent costs for the production and storage of 10 000 semen doses and 1000 embryos per year. Assuming a 20 percent annual depreciation on investment, the total cost would be about $ 69 000, leading to a cost price of $ 137.50 per frozen embryo (excluding the cost of donor females, their feed, and animal and team transportation). For storage, one frozen embryo requires the same space as two semen doses. However, taking into account the need for easy location of individual embryos in the storage container, the space required in practice will be about twelve times as great as for semen. Thus, in a 30 litre container with 6-9 canisters, 6000 straws may be stored, but only about 500

embryos. Embryo storage cost, therefore, will be about $ 1 per year per embryo.

To summarize, the costs for this storage bank would be:

- $ 7.50 to produce a dose of frozen semen;
- $ 0.15 to store a dose of semen for one year;
- $ 140 to produce a frozen embryo;
- $ 1 to store a frozen embryo for one year.

7. USE OF FROZEN SEMEN AND EMBRYOS

This paper does not deal with the problems and prospects for the utilization of semen and embryos stored for conservation of genetic resources. It should, however, be noted that whilst the fertilizing capacity of bull semen stored at -196°C for 20 years is well known, this is not yet the case for bovine embryos.

8. CONCLUSION

Although results achieved so far, particularly with frozen embryos, are nct as good as one would wish, and parameters for some reproductive traits in developing countries have not yet been adequately determined, physiologists and technologists have the necessary knowledge and expertise for gene preservation. Geneticists must now specify what material should be stored, and for what purposes.

REFERENCES

Brackett B.G., Seidel G.E. Jr. and Seidel S.M. (eds). New Technologies in Animal 1981 Breeding. Academic Press, New York.

Cole H.H. and Cupps P.T. Reproduction in Domestic Animals. Third Edition. Academic 1977 Press, New York.

Crabo B.G. Freezing of water buffalo semen for the Egyptian AI programme. FAO 1977 Report No. W/K 6082.

Foote R.H. The artificial insemination industry. In: New Technologies in Animal 1981 Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 13-39.

Gomes W.H. Artificial insemination. In: Reproduction in Domestic Animals. Third 1977 Edition. H.H. Cole and P.T. Cupps (eds). Academic Press, New York. pp. 257-284.

Hafez E.S. Reproduction in Farm Animals. Lea and Febiger, Philadelphia. 1968

Hunter R.H.F. Physiology and Technology of Reproduction in Female Domestic Animals. 1980 Academic Press, New York.

Jandrain M. 1977 Les répercussions génétiques de la transplantation ovulaire. II. Le cas d'une population de bovins laitiers. The genetic effects of egg transfer. II. The case of a dairy cattle population. Ann. Méd. Vét. 121:175-192.

Land R.B. 1977 The genetics of breed improvement. In: Embryo Transfer in Farm Animals. Monograph, Canada Department of Agriculture No. 16.

Leibo S.P. 1983 Embryo transfer method and apparatus. United States Patent. No. 4. 380. 997.

Menissier F. 1982 Aspects génétiques de la transplantation d'embryons chez les bovins. Genetic aspects of egg transfer in cattle. In: La Reproduction des bovins. Compte-rendu de journées UNCEIA.

Nibart M. 1982 Programme national de transplantation d'embryons dans l'espèce bovine. In: La Reproduction des bovins. Compte-rendu des journées UNCEIA.

Parez M. 1978 Study of "Minimum requirements AI service". report, AGA Division, FAO, Rome.

Rowson L.E.A. 1972 Egg transfer in the cow: Synchronisation requirements. J. Reprod. Fertil. 28:427-431.

Seidel G.E. Jr. and Seidel S.M. 1981 The embryo transfer industry. In: New Technologies in Animal Breeding. B.G. Brackett, G.E. Seidel Jr. and S.M. Seidel (eds). Academic Press, New York. pp. 41-80.

Smith C. 1976 Use of stored frozen semen and embryos to measure genetic trends in farm livestock. Z. Tierzücht. Züchtungsbiol. 94:119-127.

UNCEIA. 1983 Programme national pour l'étude et développement de la transplantation embryonnaire en France. National programme for the study and development of egg transfer in France.

Wierzbowski S. 1984 Cryopreservation of genetic material: disease risk and control. In: Cryogenic Storage of Germplasm and Genetic Engineering. FAO Animal Production and Health Paper No. 44/2. Rome.

APPENDIX 1

LIST OF EQUIPMENT AND MATERIALS NEEDED FOR A SEMEN BANK

1. SEMEN COLLECTION

- Artificial vagina (AV)
- Rough liners for AV
- Cones for AV
- Felt cover for AV
- Collection tubes
- Thermometers for AV
- Vaseline

2. SEMEN EXAMINATION AND PROCESSING

- Stainless electric water-bath ($37^{\circ} \pm 1^{\circ}C$)
- Binocular microscope
- Warm stage for microscope
- Thermometer for microscope
- Slide
- Cover slip
- 500 ml graduated test tube
- 50 ml graduated tube
- Erlenmeyer 500 ml flask
- Erlenmeyer 100 ml flask
- Laboratory glassware
- 10 ml pipettes
- Stand for 6 test tubes
- Photocolorimeter
- Tubes for photocolorimeter
- Haemocytometer
- Diluent
- Glycerol
- Plastic straws (0.5 or 0.25 ml)
- Plug powder
- Filling pump
- Filling nozzle (20 nozzles)
- Clip for mini straws
- Manual printing machine
- Block for printing machine
- Special ink for block
- Complete set of letters and numbers
- Electric cold-storage cabinet ($4^{\circ}C$)
- Bubbler dishes
- Bubbler dish support
- Penicillin
- Streptomycin

3. FREEZING AND STORAGE

Ministraws
- Rack for 100 ministraws
- Rack for 175 ministraws
- Thermostat
- Thermocouple for thermostat
- Freezing grill
- Funnel
- Divider
- Pit basket
- Container 200 litre; wide opening with canisters
- Working basket, 3 place for the container
- Plastic lid for container
- Container (40 litre) with canisters
- Container (15-20 litre) with canisters
- Pressurized 120 litre container for liquid nitrogen transportation
- Standard plastic goblets
- Polygonal plastic goblets
- Goblets for 15-20 litre canisters
- Long forceps
- Short forceps
- 90° alcohol
- Electric sterilizer (500 W)

4. TRANSPORTATION

- Semen and liquid nitrogen transporation vehicle

APPENDIX 2

LIST OF EQUIPMENT AND MATERIALS NEEDED FOR AN EMBRYO BANK

1. EQUIPMENT

1. Embryo Harvesting

- Three-way flushing catheter with inflatable cuff
- Cow restraining crate
- Glass flasks
- Thermostatic chamber
- Medical plug in silicone elastomers

2. Examination and Processing of Embryos

- Hood with vertical laminar flux
- Binocular lens
- Water bath
- Analytical balance
- Magnetic mixer
- Filtration-sterilization apparatus (Millipore)
- Incubator (37°C)
- Glassware
- Disposable Petri dishes
- Straws

3. Embryo Freezing

- Programmable freezer
- Cryogenic container (30 litre) for freezer
- Cryogenic container (15-18 litre) for storage
- Cryogenic container for nitrogen transportation
- Straw printing machine

4. Travelling Equipment

- Mobile laboratory
- Small car

II. MATERIALS

- Epidural anaesthetic
- Uterine disinfectant (cream)
- Progestogen implants
- Vaginal progesterone implants
- PMSG
- FSH
- Prostaglandin analogue

- Poly-oxy-methylen for rubber probe sterilization
- PBS buffer solution
- Semen doses
- Disinfectants and antibiotics
- Sucrose solution
- Crystal ministraws "transfer"
- Plastic rods for ministraws

MOLECULAR BIOLOGY AND ITS APPLICATION TO DOMESTIC ANIMALS

R. Frankham and M.R. Gillings[1]

1. INTRODUCTION

Historically, genetic improvement of domestic animals has been accomplished by selection on phenotype. In a few cases, individual genes of large effect have been identified and manipulated. Most selection has been done in closed populations with periodic episodes of between population selection and occasional outcrossing. Such traditional selection schemes have their drawbacks. Desirable alleles are not present initially at all loci within a particular population. Further, favourable alleles are lost due to genetic drift in finite sized populations of domestic animals (Robertson 1960). Consequently, favourable alleles need to be introduced from other populations. At present, this can only be done by crossing, but results in the simultaneous introduction of unfavourable alleles at other loci. This is a particularly serious problem when genetic variation is added to highly improved populations by crossing to unimproved populations. Unfavourable alleles can be removed by backcrossing but this is time consuming and expensive. Using crossing, the sources of new alleles are limited to populations producing fertile hybrids.

Genetic engineering offers the exciting prospect of eliminating these problems. It should:

(a) allow single genes to be transferred from unimproved populations to improved ones without any accompanying undesirable alleles, and

(b) allow gene transfer across species boundaries from other animal species, or even from plants, microorganisms or viruses.

The feasibility of such gene transfer across species boundaries has been dramatically demonstrated by Palmiter *et al.* (1982). They transferred the rat growth hormone gene to mice by microinjection and established that the transferred gene functioned (resulting in gigantism). Further, they showed that the gene was transmitted to second generation progeny. Several other gene transfers have been reported in animal cell lines (see Pellicer *et al.* 1980) and animals. The following genes have been transferred to mice: thymidine kinase from Herpes Simplex Virus (HSV) (Gordon *et al.* 1980; Gordon and Ruddle 1981; Wagner, Stewart and Mintz 1981; Wagner *et al.* 1981), rabbit or human β-globin (Costantini and Lacy 1981; Wagner, Stewart and Mintz 1981; Wagner *et al.* 1981), human interferon (Gordon and Ruddle 1981), chicken transferrin (McKnight *et al.* 1983), Moloney leukaemia virus genome (Harbers, Jahner and Jaenisch 1981) and the mouse metallothionein gene promoter (a control sequence) linked to the HSV thymidine kinase gene (Brinster *et al.* 1981; Palmiter, Chen and Brinster 1982). However, we shall use the study of Palmiter *et al.* (1982) as a focus to discuss molecular biology and its applications to the genetic

---

[1] School of Biological Sciences, Macquarie University, North Ryde, NSW 2113, Australia.

improvement of domestic animals. We shall also allude briefly to other applications of molecular biology to animal production in the broader sense. Various aspects of these subjects have been reviewed recently by Brackett, Seidel and Seidel (1981), Lascelles (1982), Robertson (1982), Schuman and Shoffner (1982), Sleigh (1982), Ward (1982), and Ward *et al.* (1982). Fitzhugh (1983) deals in detail with the implications of germ plasm storage and genetic engineering for breeding programmes.

2. TECHNIQUES

The work by Palmiter *et al*, (1982) involved the identification of fragments of DNA coding for rat growth hormone and for the transcriptional control sequence from a mouse metallothionein gene. These fragments were joined to a prokaryotic DNA vector (plasmid), and subsequently introduced in to the bacterium *Escherichia coli* where milligram quantities of the hybrid DNA molecule were made. The cloned DNA was purified and microinjected into the pronucleii of fertilized mouse eggs. These eggs were implanted into pseudopregnant foster mice and resulted in transgenic progeny. It must be emphasized that this work rests on the combined efforts of many research teams.

Each of the techniques in this sequence will be described along with alternative procedures and some other relevant techniques not actually used by Palmiter *et al.* (1982). The efficient utilization of genetic engineering in animal breeding requires the simultaneous use of a range of non-molecular techniques. Consequently, we mention some important non-molecular techniques that have great potential for animal production, especially when used in conjunction with molecular techniques. We review the relevant fields briefly and refer primarily to recent papers and reviews rather than present a detailed literature review. Cherfas (1982) provides a lucid review of molecular topics and Maniatis *et al.* (1982) cover techniques in their laboratory manual.

Work such as that of Palmiter *et al.* (1982) is made possible through the use of new biochemical tools for modifying and manipulating nucleic acids. These tools include nucleic acid modifying enzymes and small circular DNA molecules called plasmids. We will describe each of these in turn.

2.1 Nucleic Acid Modifying Enzymes

i. Alkaline phosphatase removes the phosphate group from the 5' end of DNA or RNA. This prevents linear molecules from circularizing, and allows the replacement of the terminal phosphate with a P^{32} label (end-labelling). End-labelling is used in DNA sequencing.

ii. Polynucleotide kinase does the reverse of alkaline phosphatase by adding phosphate to the unphosphorylated end of a nucleic acid. This enzyme is used to end-label nucleic acids.

iii. Terminal transferase adds a nucleotide to the 3' end of a DNA molecule. It is also used for end-labelling. Other transferases add nucleotides to the 5' end of DNA molecules. DNA molecules can be joined by creating poly dA extensions on one molecule and poly dT extensions on the other using terminal transferases (Wensink *et al.* 1974). The base complementarity of the A (Adenine) and T

(Thymine) residues allows annealing to take place. G (Guanine) and C (Cytosine) bases can be used in a similar way.

iv. Ligases repair nicks or gaps in the sugar phosphate backbone of DNA or RNA. T^4 ligase can join double stranded DNA molecules that have "blunt ends", i.e. molecules without single stranded extensions. Ligase is an essential tool for making hybrid DNA molecules and for circularizing DNA fragments.

v. DNA Polymerase I (Kornberg enzyme) has three catalytic activities; adding nucleotides to the 3' end of DNA or removing nucleotides from 5' or 3' ends. The enzyme recognizes gaps or nicks in one strand of double stranded DNA. It moves along the DNA from the nick, gradually transferring the gap to the end of the molecule by adding nucleotides at the 3' side and removing them from the 5' side. This process, called nick translation (Rigby *et al.* 1977) can be used to incorporate nucleotides labelled with P^{32}, S^{35}, or I^{125}, thus allowing detection of minute quantities of DNA by autoradiography (Laskey and Mills 1977). In an alternative procedure biotin labelled nucleotides are incorporated, and subsequently detected using immunological methods (Manuelides, Langer-Safer and Ward 1982).

vi. Reverse transcriptase synthesises a complementary single stranded DNA copy of single stranded RNA (Temin 1978). This is recovered as an RNA/DNA hybrid molecule. The RNA is hydrolysed with alkali, and the single stranded DNA incubated with DNA polymerase 1 to produce a double stranded copy (cDNA) of the RNA.

vii. DNase 1 creates nicks in double stranded DNA (with short incubation times), and so is used to generate gaps for recognition by the Kornberg polymerase in the nick-translation procedure.

viii. Other nucleases are available that are specific for double stranded DNA, single stranded DNA, double stranded RNA, single stranded RNA, or combinations of these. They may be used to degrade unwanted components in solutions or to modify existing components.

ix. Restriction endonucleases are perhaps the most powerful of the array of enzymes used by molecular biologists (Arber 1979; Smith 1979; Roberts 1983). Type II restriction enzymes recognize and cleave specific palindromic nucleotide sequences, thus providing a means of generating specific and repeatable fragments from any particular genome. Furthermore, some of the enzymes cleave in a staggered formation leaving short single stranded tails at either end of the restircted DNA. The tails are complementary and so can reanneal (sticky ends). Consequently, DNAs from different organisms, cut with the same restriction enzyme, can anneal to form hybrid DNA molecules, and can then be stabilized by subsequent treatment with ligase. This procedure forms the basis of recombinant DNA technology.

2.2 Plasmids

Plasmids are probably best known as the multiple resistance transfer factors in bacteria, causing simultaneous resistance to several antibiotics. They are small circular non-chromosomal DNA molecules that replicate autonomously. Consequently,

plasmids are ideal vectors for transferring and manipulating DNA in prokaryotes (Bolivar and Backman 1979; Kahn *et al.* 1979). Useful plasmids contain antibiotic resistance genes with internal restriction enzyme sites. Circular hybrid DNA molecules, consisting of foreign DNA spliced to plasmid DNA, can be created by cleaving both foreign DNA and plasmid DNA with the same restriction enzyme and allowing the cleaved DNA to anneal and ligate. The plasmid pBR322 contains two antibiotic resistance genes, one for tetracycline and the other for ampicillin. Depending on the restriction endonuclease used, foreign DNA sequences can be spliced specifically into either of the genes, giving a hybrid plasmid which has lost one or other of its drug resistant properties. The hybrid plasmid can be reinserted into the bacterial host, simply by treating the bacteria with calcium chloride to make them permeable to the plasmids. Once inserted, the plasmid start to replicate and to direct synthesis of plasmid specific polypeptides, including those resulting in drug resistance. Thus, growing the host cells on culture plates containing either ampicillin or tetracycline will allow selection of bacteria containing plasmids, with foreign DNA inserts, evident by resistance to only one of the antibiotics. An additional advantage of some plasmids is their ability to replicate in the presence of chloramphenicol, an antibiotic that shuts down synthesis of cell protein and DNA in the host. Relaxed replicating plasmids occur in thousands of copies per cell after incubation of transformed cells with chloramphenical. This allows isolation of milligram quantities of recombinant DNAs from the host cells with fewer problems of contamination by host cell DNA.

2.3 Cloning Specific Genes

2.3.1 Isolating mRNA

The rat growth hormone mRNA was isolated from rat pituitary tumour cells induced to secrete high levels of growth hormone by the use of glucocorticoids (Harpold *et al.* 1978). The mouse metallothionein mRNA was isolated from mouse livers induced to high levels of metallothionein gene expression by injecting mice with cadmium and zinc (Durnam *et al.* 1980). The mRNA may be purified further by running on gels (Seeburg *et al.* 1977) or by preparative centrifugation. The mRNAs obtained by these procedures contained high levels of the required mRNA (1.4% metallothionein mRNA; 50% rat growth hormone mRNA). The appropriate mRNA in these cases was identified using the rabbit reticulocyte *in vitro* translation system (Durnam *et al.* 1980). Alternatively, a monoclonal antibody may be used to isolate the specific mRNA-polysome complex (Shapiro and Young 1981).

2.3.2 Making a DNA copy of the mRNA

DNA copies of the mRNAs were produced using the enzyme reverse transcriptase, and DNA polymerase I.

2.3.3 Inserting the cDNA into a plasmid

The cDNA was cloned into a plasmid. In the case of the metallothionein gene, synthetic oligonucleotides (linkers) containing restriction sites for the restriction enzymes Eco R1 and Hin dIII were ligated to the cDNA. The cDNA plus linkers was digested with Eco R1 and Hin dIII and ligated to Eco/Hin-digested pBR322 plasmid DNA (Durnam *et al.*1980). The rat growth hormone cDNA was inserted into the Bam H1 site

of pBR322 by the poly dA/dT connector method (Harpold *et al.* 1978; Seeburg *et al.* 1977) to produce a recombinant plasmid. In both cases, the DNA inserts were into the tetracycline resistance gene of the pBR322 plasmid.

2.3.4 Inserting the recombinant plasmid into *E. coli*

E. coli was transformed with these recombinant plasmids. In both the above cases the transformed *E. coli* cells were ampicillin resistant and tetracycline susceptible and so were selected on antibiotic-containing media plates.

2.3.5 Identifying cDNA from the gene of interest

The cDNA-containing colonies were copies of all active genes in the original tissue. Several methods are available to identify individual genes from amongst the thousands of colonies. Most involve isolation of colony DNA and its fixation on nitrocellulose or filter paper as single strands (Taub and Thompson 1982). In the two cases in question, mRNA from the original tissues was incubated with the nitrocellulose discs to anneal (hybridise) complementary mRNA to the bound cDNA. The bound mRNA was eluted (hybrid release) and translated in the rabbit reticulocyte system. Colonies of interest bound the mRNA coding for the protein of interest, so this protein was detected in the translation system. The translated proteins were identified by electrophoresing them besides known samples of the protein of interest.

Alternatively, colonies fixed on nitrocellulose may be probed with the original cDNA and cDNA made from a non-producing tissue. Colonies that hybridise only to the former are tissue specific genes. Colonies of interest may be identified by DNA sequencing if the amino acid sequence of the protein is known or by *in situ* hybridization of labelled cDNA to fixed chromosomes if the chromosomal location is known. The cDNA may be identified using a probe from a related species if one is available.

2.3.6 Constructing a DNA library

Because cDNA is a copy of mRNA sequences, it does not represent a functional gene and lacks control sequences and introns. Consequently it is not suitable for gene transfers. To obviate this problem a so-called DNA library is made and the full gene isolated. One method of doing this is to shear mechanically or partially cleave genomic DNA with restriction enzymes and ligate it to modified DNA from bacteriophage lambda.

The DNA is packaged inside the lambda capsid and the recombinant virus used to infect *E. coli*. This allows large pieces of DNA to be inserted into the bacterium using the phage as a vector. Cloning systems using lambda Charon 4A recover recombinants as plaques, since the phage is still lytic. More recently, lambda arms spliced to the ampicillin resistance gene of pBR322 have been used. This vector (called a cosmid) is non-lytic and transformants are recovered as ampicillin resistant bacterial colonies. Such cloned DNA libraries are available for many species, including mice, chickens, pigs, sheep, rats, rabbits, ducks, goats, *Xenopus*, *Drosophila*, man and many plants.

2.3.7 Probing the DNA library to detect the required gene

Single stranded, nick-translated (labelled) cDNAs from the cloned fragments, previously identified as representing the mRNAs for growth hormone and metallothionein, were hybridized to the single stranded library DNAs bound to nitrocellulose filters, in order to detect the desired genes (Barta *et al*. 1981; Doehmer *et al*. 1982). Cloned DNA fragments giving hybridization contain at least part of the desired genes and are chosen for further analysis.

A variety of other methods is also available for cloning genes. A gene may be cloned in a well-studied species using overlapping DNA sequences to "walk" along the DNA from a nearby known cloned gene (e.g. Bender *et al*. 1983), even if its product has not been defined. In *Drosophila*, the salivary chromosome band containing a gene of interest can be cut out and cloned directly. Transposable elements can be used to induce mutations of interest, and the transposable element probe used to identify the gene, even if the gene is otherwise unknown (Schnieke, Harbers and Jaenisch 1983).

2.3.8 Mapping the gene

A restriction map of the gene is usually made at this stage. Restriction endonucleases are used to cut the library DNA at specific base sequences. The cut DNA is run on agarose or polyacrylamide gels to separate the fragments by size (Southern 1979), the separated DNA is transferred to a nitrocellulose filter or DBM paper (a Southern blot; Southern 1975; Smith and Summers 1980) and probed with the appropriate nick-translated cDNA. The sites of hybridization of the labelled cDNA to the immobilized single stranded DNA on the filter are detected by autoradiography or by using the biotin procedure. Gene fragments hybridizing to the cDNA are represented in the mRNA, while those that do not hybridize represent sequences not coding for amino acids (introns or non-transcribed control sequences) or sequences not part of the gene. In addition the restriction sites within the gene can be aligned to provide a map of the gene.

Heteroduplex mapping may be used to define the presence of non-translated sequences (introns) within the gene. In this procedure, purified mRNA or cDNA is annealed to the cloned gene. Internal non-coding regions appear as single stranded loops when visualized with electron microscopy (Davis *et al*. 1971). The chromosomal location of the gene may be determined by *in situ* hybridization of the labelled gene probe to chromosome spreads (Harper and Saunders 1981), hybridization of the probe to somatic cell hybrid lines (Lai *et al*. 1983), or even to machine sorted chromosomes (Lebo *et al*. 1979). Hybridization of total genomic DNA with the gene probe may be appropriate at this stage to determine the number of copies of the gene present in the genome, and to screen for polymorphisms in gene number or restriction enzyme sites in the gene.

2.3.9 Sequencing the gene

Both the rat growth hormone gene (Barta *et al*. 1981) and the mouse metallothionein-I gene (Glanville *et al*. 1981) were sequenced to define the leading sequences (control regions), the starts and ends of the genes, the non-coding regions within the genes, and to check, using the amino acid sequences of the proteins, that the desired genes had been isolated (rather than related genes or non-functional

pseudogenes). The DNA was sequenced using the methods of Maxam and Gilbert (1980) and Sanger (1977).

2.3.10 Recloning the gene

The desired gene from the DNA library usually requires recloning at this stage to remove unwanted sequences. Consequently it will be cut again using appropriate restriction enzymes and reinserted into a plasmid. A DNA control sequence (promoter) is also inserted into the plasmid in front of the required gene, to allow the gene to be expressed in a new host. This promoter may be the gene's own promoter or an inducible promoter from another gene. In the work of Palmiter *et al.* (1982), a plasmid was constructed with the control sequence (promoter) from the mouse metallothionein-I gene (inducible using metal) linked to the rat growth hormone gene, so the expression of the rat growth hormone gene could be induced using metal supplementation in the diet of transformed mice.

2.3.11 Isolating the desired DNA

This plasmid was inserted into *E. coli* and the required DNA amplified and reisolated. The DNA was cut with the appropriate restriction enzymes, run on a gel, and the appropriate-sized DNA fragment containing the rat growth hormone gene plus metallothionein promoter extracted from the gel (Chen and Thomas 1980).

2.4 Microinjection into Mouse Embryos

This DNA fragment was microinjected into the pronucleus of fertilized mouse eggs. Other techniques have been used to perform the genetic transformation in cultured cells or in other species. DNA has been calcium phosphate-precipitated on to cells to transform them (Pellicer *et al.* 1980); DNA has been inserted into a virus and cells infected to cause transformation (Mulligan and Berg 1980; Doehmer *et al.* 1982); DNA has been cotransformed with a selectable gene (Pellicer *et al.* 1980); transformation in *Drosophila* has been achieved by inserting the required DNA into a mobile genetic element, followed by injection into fertilized eggs (Rubin and Spradling 1982).

2.5 Raising Transformed Progeny

These microinjected eggs were implanted into pseudopregnant mice and allowed to develop. The resulting progeny were checked for growth and assayed for the presence and expression of the rat growth hormone gene. The progeny were shown to contain the rat growth hormone gene by probing isolated samples of their DNA with labelled DNA from the original clone. In this and another case (Costantini and Lacy 1981) there was evidence to indicate that the injected gene had inserted into the chromosomal DNA. Expression of the gene was demonstrated by probing mRNA from the progeny with labelled DNA from the original cloned gene and by radioimunoassay for rat growth hormone. These mice were fed a metal supplement to induce the expression of the rat growth hormone gene by acting on the metal-sensitive metallothionein gene promoter. Several of the progeny contained and expressed the rat growth hormone gene and most of these exhibited accelerated growth and general gigantism. One individual containing the inserted gene (transgenic) was shown to transmit the gene to about

half its progeny as expected with Mendelian inheritance. In other related cases transmission to about one half of progeny has also been demonstrated (Costantini and Lacy 1981; Gordon and Ruddle 1981; Palmiter *et al.* 1982).

2.6 Other Techniques of Potential Use

2.6.1 *In vitro* mutagenesis

Specific mutations can be induced at defined sites in cloned genes using chemical and biochemical methods (Shortle *et al.* 1981). In theory, these techniques will obviate the need for germ plasm resources, as all natural variants could be artifically produced. However, natural variants have been through the sieve of natural selection and so are likely to be more useful than randomly engineered mutants. The efficient use of *in vitro* mutagenesis presupposes a very detailed knowledge of the crucial functional sites of a gene.

2.6.2 Synthesizing genes

Automated systems are now available for chemically synthesizing genes (Alvarado-Urbina *et al.* 1981). From a known amino acid sequence, likely coding sequences can be inferred, and short DNA fragments manufactured. These fragments can be used as DNA probes to screen DNA libraries (e.g. Wallace *et al.* 1979). A chemically synthesized gene was used in making one of the human insulins using genetically engineered bacteria (Cherfas 1982).

2.6.3 Cloning animals

If individual animals can be cloned (as is possible for many plants) both "normal" animal breeding and genetic engineering will be much more productive. Cloning would allow much higher selection intensities, repeat testing of genotypes to increase the effective heritability and much more efficient utilization of specific combining ability. As well, cloned progeny should be relatively uniform, a desirable attribute for processing and marketing.

The advantages of cloning individuals in the context of genetic engineering are perhaps even greater. Selection in cultures containing millions of cells can be carried out for specific mutations, and for the presence, expression, number and site of gene insertion. These procedures would increase the yield of appropriately engineered genes and would reduce the time and expense involved in testing and evaluating individuals containing engineered genes. However, it must be possible to differentiate whole animals from cultured cells (e.g. using nuclear transplantation) or at least to get cultured cells to produce functional germ line cells in chimaeras, for this to be useful. Some success in this latter direction has been reported by Mintz and colleagues using a stable diploid mouse teratocarcinoma line that can be grown in culture, and that undergoes normal development in chimaeric mice. Stewart and Mintz (1981) reported the successful introduction of these teratocarcinoma cells into the germ line of chimaeric mice, and the subsequent transmission of teratocarcinoma genes to successive generations of offspring. Wagner and Mintz (1982) succeeded in introducing foreign genes into these teratocarcinoma cells in culture.

Cloning by splitting embryos has already been done in several species of domestic animals (Polge 1983). However, this seems likely to yield only a small number of clones of each genotype. Large numbers of clones of particular genotypes are more likely to be achieved using nuclear transplantation. This type of transplantation has been done successfully in frogs for the last 30 years. Recently, Illmensee and Hoppe (1981) and Hoppe and Illmensee (1982) reported successful nuclear transplants in mice. Questions about this work have been raised (Marx 1983a, b), but McGrath and Solter (1983) have independently reported success with an improved technique. It seems likely that cloning of mice using nuclear transplantation will shortly be a reality, as McGrath and Solter (1983) have developed all the required techniques. It must be emphasized that cloning of adult animals may not be possible as their cells may be irreversibly differentiated.

2.6.4 Instant inbred lines

It may prove possible to develop inbred lines of domestic animals by means akin to colchicine treatment of anther cultures in plants. Attempts have already been made to fertilize mouse eggs, to remove the male pronucleus, and to duplicate the haploid chromosome complement from the mother.

2.6.5 Somaclonal variation

In clones of cultured plant cells, genetic variation arises, without the use of mutagens, at abnormally high rates (somaclonal variation - Larkin and Scowcroft 1981). This may also occur in animal cells (Scowcroft 1982). Somaclonal variation has been exploited in plants by selecting in cell cultures for desirable mutants.

2.6.6 Choosing the sex of offspring

The ability to choose the sex of offspring would be of enormous value in animal production. Early embryos can already be sexed in a variety of species using molecular probes, HY antigens or karotyping (Beardsley 1983). Separation of X and Y bearing spermatozoa may be a practical reality in the near future, as the efficacy of separation methods can be assessed promptly by karyotyping spermatozoa or by measuring their DNA content.

3. IMPLICATIONS

3.1 Animal Breeding Methods

Genetic engineering is a powerful technique with the potential to yield great benefits in animal breeding. However, formidable technical difficulties must be solved before genetic engineering can contribute to practical animal breeding. It has yet to be demonstrated that the functioning of inserted genes can be adequately regulated by the vertebrate host. Inserted genes must function at the correct time in the correct tissues. At present, the number of gene copies inserted and the sites of insertion are not controllable. Genetic engineering may not be of practical utility in animal breeding in the near future. However, *Drosophila* workers have successfully coped with the above problems (Goldberg, Posakony and Maniatis 1983; Scholnick, Morgan and

Hirsh 1983; Spradling and Rubin 1983), so we are optimistic that these technical barriers will be overcome in domestic animals. Many equally daunting technical barriers have been crossed in the last few years.

There are few candidate genes for genetic engineering. The single genes that have been manipulated in animal breeding have typically been those affecting colour and morphology. They include colour genes in many populations, polled genes in sheep and cattle, the double-muscling gene in cattle, the Boroola high fertility gene in sheep, feather-sexing, colour-sexing, white feather colour, skin coloration and sex-linked dwarfism genes in chickens, the halothane sensitivity-stress susceptibility gene in pigs and rare deleterious recessives in many populations. We simply do not know the genes with important effects on animal performance at this time. Such genes will have to be guessed at from the best available knowledge of physiologists, geneticists, biochemists and molecular biologists, or defined by the joint work of people in these fields. Chimaeric animals will probably be a useful tool in such studies (see Falconer *et al.* 1981). The most likely candidates for genetic engineering are the hormone loci, the major histocompatibility complex and multigene families. The rRNA multigene family has been shown to have an effect on a quantitative character in *Drosophila* (Frankham 1980).

A complete evaluation of the effects of engineered genes will have to be carried out, especially an evaluation of their effects on reproductive fitness. Genetic engineering is likely to be an adjunct to normal selection practices, as only a small proportion of the genome is likely to be identifiable as having sufficient effect to be worth manipulating in this way. The remainder of the genome will continue to be treated as a "black box" by conventional methods of selection based on phenotype. In the context of plant breeding, Borlaug (1983) has warned against a reduction in conventional breeding efforts as they represent the major line of defence on the food front. The same applies to animal breeding where the major problem is not the lack of useful genetic variation and workable method but the limited application of the highly successful procedures that we already possess (note for example the enormous advances achieved in broiler chicken performance in the last 20 years).

3.2 Testing for Deleterious Recessive Genes

Molecular biological methods can be used to test for the presence of deleterious recessive alleles in valuable sires. This has the potential to remove the need for expensive progeny testing. Deleterious alleles have been detected in humans by defining restriction fragment differences between the DNA of the normal and deleterious alleles (Chang and Kan 1982; Orkin *et al.* 1982), or by using a short chemically synthesized DNA fragment as a probe (Kidd *et al.* 1983).

There is considerable interest in the possibilities of gene therapy to alleviate genetic diseases in humans but this does not seem likely to be important in animals. Such defective animals are likely to be culled.

3.3 Storage of Germ Plasm Resources

Nuclear transplantation and genetic engineering, when operational, will allow the storage of genetic material as frozen cell cultures or frozen DNA samples. Frozen semen and frozen embryos can be used to regenerate individuals for those species where these techniques have been perfected.

3.4 Vaccine Production

Genetic engineering has already led to the production of much safer vaccines for virulent pathogens, e.g. Foot and Mouth Disease (Kleid *et al* 1981). This is being done by cloning the gene responsible for the pathogen's main antigenic protein, and using *E. coli* containing the recombinant DNA plasmid to produce the antigenic protein. This protein is then extracted and used for vaccination without the risks attendant on the use of killed pathogen or avirulent strains.

3.5 Producing Valuable Animal Proteins by Biotechnology

In some cases, valuable animal products will be made in bacterial, fungal or yeast cultures transformed with cloned genes. For example, the epidermal growth factor may be produced in transformed *E. coli* and the purified protein used in chemical defleecing of sheep. Alternatively, many copies of such genes may be genetically engineered into laboratory animals and the product collected from serum or urine. Higher concentrations of growth hormone have been obtained from the serum of mice transformed with the rat growth hormone gene than from *E. coli* transformed with a cloned growth hormone gene (Palmiter *et al.* 1982).

3.6 Genetically Engineering Rumen Bacteria

By genetic engineering it may be possible to improve the functioning of rumen bacteria (Ward 1982), or of gut microorganisms in general.

3.7 Molecular Biological Methods as Research Tools

Many fields of biology and agriculture are being revolutionized by the application of molecular biological techniques. For example, our knowledge of the genome has been dramatically advanced in recent years due to the efforts of molecular biologists (Gillings and Frankham 1982). There are immediate prospects of a very productive era in many areas of animal production research using molecular biological techniques. The efficient use of genetic engineering in animal breeding requires a detailed knowledge of the genome of domestic animals. Molecular methods will be extremely useful in mapping animal genomes (Southern 1982), e.g. restriction enzyme fragments can provide many markers for mapping (Skolnick and White 1982; Soller and Beckman 1982).

4. PROSPECTS

Animal breeders are showing a great deal of interest in the potential uses of molecular biology and genetic engineering in animal improvement. A number of practically orientated projects on domestic animals are underway, but all are in the very early stages. For example, Ward *et al.* (1982) are working on the sheep keratin genes as a possible means for improving wool production in Merino sheep. However gene transfers have not yet been reported in any species of commercial livestock. By contrast, genetically engineered plants are reported to have reached the field test stage (Joyce 1983). Rather surprisingly, developments in poultry seem to be lagging behind those in mammals (see Schuman and Shoffner 1982), though Pandey and Patchell

(1982) have reported genetic transformation in chickens by a non-molecular technique, the use of irradiated spermatozoa.

Vaccine production using genetically engineered microbes is already going on (see Lascelles 1982; Sleigh 1982). The use of genetically engineered reagents in animal production is likely to follow soon, while the benefits of genetic engineering in animal breeding are likely to be longer in coming.

It is notable that the work of Palmiter *et al.* (1982) was reported less than 30 years after the elucidation of the structure of DNA by Watson and Crick (1953). At least 11 people have received Nobel Prizes for relevant research in molecular biology in the intervening period. We foresee no slowing of the pace of innovation in the next 30 years.

ACKNOWLEDGEMENTS

We thank Jim Murray, Merilyn Sleigh, Jim Speirs, Malcolm Stewart and Helen Newton Turner for their helpful comments on the manuscript.

REFERENCES

Alvarado-Urbina G., Sathe G.M., Lui W.-C., Gillen M.F., Duck P.D., Bender R. and
1981 Ogilvie K.K. Automated synthesis of gene fragments. Science 214:270-274.

Arber W. Promotion and limitation of genetic exchange. Science 205:361-365.
1979

Barta A., Richards R.I., Baxter J.D. and Shine J. Primary structure and evolution of
1981 rat growth hormone gene. Proc. Nat. Acad. Sci. USA 78:4876-4881.

Beardsley T. Cattle now, people next? Nature 304:301.
1983

Bender W., Spierer P. and Hogness D.S. Chromosomal walking and jumping to isolate
1983 DNA from the *Ace* and *rosy* loci and the bithorax complex in *Drosophila melanogaster*. J. Mol. Biol. 168:17-33.

Bolivar F. and Backman K. Plasmids of *Escherichia coli* as cloning vectors. Meth.
1979 Enzymol. 68:245-267.

Borlaug N.E. Contributions of conventional plant breeding to food production. Science
1983 219:689-693.

Brackett B.G., Seidel G.E. Jr. and Seidel S.M. New Technologies in Animal Breeding.
1981 Academic Press, New York.

Brinster R.L., Chen H.Y., Trumbauer M., Senear A.W., Warren R. and Palmiter R.D.
1981 Somatic expression of Herpes Simplex thymidine kinase in mice following injection of a fusion gene into eggs. Cell 27:223-231.

Chang J.C. and Kan Y.W. A sensitive new prenatal test for sickle-cell anemia. N.
1982 Engl. J. Med. 307:30-32.

Chen C.W. and Thomas C.A. Jr. Recovery of DNA segments from agarose gels. Anal.
1980 Biochem. 101:339-341.

Cherfas J. Man Made Life. Blackwell, Oxford.
1982

Costantini F. and Lacy E. Introduction of a rabbit β-globin gene into the mouse germ
1981 line. Nature 294:92-94.

Davis R.W., Simon M. and Davidson N. Electron microscope heteroduplex methods for
1971 mapping regions of base sequence homology in nucleic acids. Meth. Enzymol. 21:413-428.

Doehmer J., Barinaga M., Vale W., Rosenfeld M.G., Verma I.M. and Evans R.M.
1982 Introduction of rat growth hormone into mouse fibroblast via a retroviral DNA vector: Expression and regulation. Proc. Nat. Acad. Sci. USA 79:2268-2272.

Durnam D.M., Perrin F., Gannon F. and Palmiter R.D. Isolation and characterization
1980 of the mouse metallothionein-I gene. Proc. Nat. Acad. Sci. USA 77: 6511-6515.

Falconer D.S., Gauld I.K., Roberts R.C. and Williams D.A. The control of body size in
1981 mouse chimaeras. Genet. Res. 38:25-46.

Fitzhugh H.A. The impact of germ plasm storage and molecular engineering upon
1984 breeding programmes. Animal Production and Health Paper No. 44/2. FAO, Rome.

Frankham R. Origin of genetic variation in selection lines. In: Selection Experiments
1980 in Laboratory and Domestic Animals. A. Robertson (ed.). Commonwealth Agric. Bur., Slough. pp. 56-68.

Gillings M.R. and Frankham R. Changing views of genomic organization. Proc. 2nd.
1982 World Congr. Genetics Applied to Livestock Production VI. p. 164-181.

Glanville N., Durnam D.M. and Palmiter R.D. Structure of mouse metallothionein-I gene
1981 and its mRNA. Nature 292:267-269.

Goldberg D.A., Posakony J.W. and Maniatis T. Correct developmental expression of a
1983 cloned alcohol dehydrogenase gene transduced into the *Drosophila* germ line. Cell 34:59-73.

Gordon J.W. and Ruddle F.H. Integration and stable germ line transmission of genes
1981 injected into mouse pronuclei. Science 214:1244-1246.

Gordon J.W., Scangos G.A., Plotkin D.J., Barbosa J.A. and Ruddle F.H. Genetic
1980 transformation of mouse embryos by microinjection of purified DNA. Proc. Nat. Acad. Sci. USA 77:7380-7384.

Harbers K., Jahner D. and Jaenisch R. Microinjection of cloned retroviral genomes into
1981 mouse zygotes: integration and expression in the animal. Nature 293:540-542.

Harper M.E. and Saunders G.F. Localization of single copy DNA sequences in G-banded
1981 human chromosomes by *in situ* hybridization. Chromosoma 83:431-439.

Harpold M.M., Dobner P.R., Evans R.M. and Bancroft F.C. Construction and
1978 identification by positive hybridization-translation of a bacterial plasmid containing a rat growth hormone structural gene sequence. Nuc. Acids Res. 5:2039-2052.

Hoppe P.C. and Illmensee K. Full-term development after transplantation of partheno-
1982 genetic embryonic nuclei into fertilized mouse eggs. Proc. Nat. Acad. Sci. USA 79:1912-1916.

Illmensee K. and Hoppe P.C. Nuclear transplantation in *Mus musculus*: Developmental
1981 potential of nuclei from preimplantation embryos. Cell 23:9-18.

Joyce C. Americans let loose new genetic types into environment. New Scient. 98:926.
1983

Kahn M., Kolter R., Thomas C., Figurski D., Meyer R., Remaut E. and Helsinki D.R.
1979 Plasmid coning vehicles derived from plasmids ColE1, F, R6K and RK2. Method. Enzymol. 68:268-280.

Kidd V.J., Wallace R.B., Itakura K. and Woo S.L.C. $\alpha_1$antitrypsin deficiency detection
1983 by direct analysis of the mutation in the gene. Nature 304:230-234.

Kleid D.G., Yansura D., Small B., Dowbenko D., Moore D.M., Grubman M.J., McKercher
1981 P.D., Morgan D.O., Robertson B.H. and Bachrach H.L. Cloned viral protein vaccine for foot-and-mouth disease: Responses in cattle and swine. Science 214:1125-1129.

Lai E.C., Kao F., Law M.L. and Woo S.L.C. Assignment of the α_1-antitrypsin and a
1983 sequence-related gene to human chromosome 14 by molecular hybridization. Am. J. Hum. Genet. 35:385-392.

Larkin P.J. and Scowcroft W.R. Somaclonal variation: A novel source of variability
1981 from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.

Lascelles A.K. Application of R-DNA to animal production. In: Genetic Engineering -
1982 Commercial Opportunities in Australia. Australian Govt. Publishing Service, Canberra. pp. 124-128.

Laskey R.A. and Mills A.D. Enhanced autoradiographic detection of ^{32}P and ^{125}I using
1977 intensifying screens and hypersensitive film. FEBS Letters 82:314-316.

Lebo R.V., Carrano A.V., Burkhart-Schultz K., Dozy A.M., Yu L.-C. and Kan Y.W.
1979 Assignment of human β-, γ-, and α-globin genes to the short arm of chromosome 11 by chromosome sorting and DNA restriction enzyme analysis. Proc. Nat. Acad. Sci. USA 76: 5804-5808.

Maniatis T., Fritsch E.F. and Sambrook J. Molecular Cloning: A Laboratory Manual.
1982 Cold Spring Harbour, New York.

Manuelidis L., Langer-Safer P.R. and Ward D.C. High-resolution mapping of satellite
1982 DNA using biotin-labelled DNA probes. J. Cell Biol. 95:619-625.

Marx J.L. Swiss research questioned. Science 220:1023.
1983a

Marx J.L. Bar Harbour investigation reveals no fraud. Science 220:1254.
1983b

Maxam A.M. and Gilbert W. Sequencing end-labelled DNA with base-specific chemical
1980 cleavages. Methods in Enzymology 65:499-560.

McGrath J. and Solter D. Nuclear transplantation in the mouse embryo by microsurgery
1983 and cell fusion. Science 220:1300-1302.

McKnight G.S., Hammer R.E., Kuenzel E.A. and Brinster R.L. Expression of the
1983 chicken transferrin gene in transgenic mice. Cell 34:335-341.

Mulligan R.C. and Berg P. Expression of a bacterial gene in mammalian cells. Science
1980 209:1422-1427.

Orkin S.H., Little P.F.R., Kazian H.H. Jr. and Boehm C.D. Improved detection of the
1982 sickle mutation by DNA analysis. Application to prenatal diagnosis. N. Engl. J. Med. 307:32-36.

Palmiter R.D., Brinster R.L., Hammer R.E., Trumbauer M.E., Rosenfeld M.G., Birnberg
1982 N.C. and Evans R.M. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature 300:611-615.

Palmiter R.D., Chen H.Y. and Brinster R.L. Differential regulation of metallo-
1982 thionein-thymidine kinase fusion genes in transgenic mice and their offspring. Cell 29:701-710.

Pandey K.K. and Patchell M.R. Genetic transformation in chicken by the use of
1982 irradiated male gametes. Mol. Gen. Genet. 186:305-308.

Pellicer A., Robins D., Wold B., Sweet R., Jackson J., Lowry I., Roberts J.M., Sim
1980 G.K., Silverstein S. and Axel R. Altering genotype and phenotype by DNA-mediated gene transfer. Science 209:1414-1422.

Polge C. Cryogenic storage of mammalian cells. Animal Production and Health Paper
1984 No. 44/2. FAO, Rome.

Rigby P.W.J., Dieckman M., Rhodes C. and Berg P. Labelling Deoxyribonucleic Acid to
1977 high specific activity *in vitro* by nick translation with DNA Polymerase I. J. Mol. Biol. 113:237-251.

Roberts R.J. Restriction and modification enzymes and their recognition sequences.
1983 Nuc. Acids Res. 11, r135-r167.

Robertson A. A theory of limits in artificial selection. Proc. Roy. Soc. Lond.
1960 153B:234-249.

Robertson A. Genetic engineering in animal improvement. In: Proc. 2nd. World Congr.
1982 Genetics Applied to Livestock Production VI. pp. 139-145.

Rubin G.M. and Spradling A.C. Genetic transformation of *Drosophila* with transposable
1982 element vectors. Science 218:348-353.

Sanger F. Determination of nucleotide sequences in DNA. Science 214:1205-1210.
1981

Schnieke A., Harbers K. and Jaenisch R. Embryonic lethal mutation in mice induced by
1983 retrovirus insertion into the α1(I) collagen gene. Nature 304:315-320.

Scholnick S.B., Morgan B.A. and Hirsh J. The cloned dopa decarboxylase gene is
1983 developmentally regulated when reintegrated into the *Drosophila* genome. Cell 34:37-45.

Schuman R. and Shoffner R.N. Potential genetic modification in the chicken,
1982 *Gallus domesticus*. In: Proc. 2nd World Congr. Genetics Applied to Livestock Production VI. pp. 157-163.

Scowcroft W.R. Somatic cell genetics and plant improvement: Discussion. In: Genetic
1982 Engineering Commerical Opportunities in Australia. Australian Govt. Publishing Service, Canberra. p. 115.

Seeburg P.H., Shine J., Martial J.A., Baxter J.D. and Goodman H.M. Nucleotide
1977 sequence and amplification in bacteria of structural gene for rat growth hormone. Nature 270:486-494.

Shapiro S.Z. and Young J.R. An immunochemical method for mRNA purification. J. Biol.
1981 Chem. 256:1495-1498.

Shortle D., Di Maio D. and Nathans D. Directed mutagenesis. Ann. Rev. Genet.
1981 15:265-294.

Skolnick M.H. and White R. Strategies for detecting and characterizing restriction
1982 fragment length polymorphisms (RFLP's). Cytogenet. Cell Genet. 32:58-67.

Sleigh M. Veterinary applications for recombinant DNA technology. In: Genetic
1982 Engineering - Commercial Opportunities in Australia. Australian Govt. Publishing Service, Canberra. pp. 116-123.

Smith G.E. and Summers M.D. The bidirectional transfer of DNA and RNA to
1980 nitrocellulose or diazobenzyloxymethyl-paper. Anal. Biochem. 109:123-129.

Smith H.O. Nucleotide sequence specificity of restriction endonucleases. Science
1979 205:455-462.

Soller M. and Beckman J.S. Restriction fragment length polymorphisms and genetic
1982 improvement. Proc. 2nd World Congr. Genetics Applied to Livestock Production VI. pp. 396-404.

Southern E.M. Detection of specific sequences among DNA fragments separated by gel
1975 electrophoresis. J. Mol. Biol. 98:503-517.

Southern E. Gel electrophoresis of restriction fragments. Meth. Enzymol. 68:152-177. 1979

Southern E.M. Application of DNA analysis to mapping the human genome. Cytogenet. 1982 Cell Genet. 32:52-57.

Spradling A.C. and Rubin G.M. The effect of chromosomal position on the expression of 1983 the *Drosophila* xanthine dehydrogenase gene. Cell 34:47-57.

Stewart T.A. and Mintz B. Successive generations of mice produced from an established 1981 culture line of euploid teratocarcinoma cells. Proc. Nat. Acad. Sci. USA 78:6314-6318.

Taub F. and Thompson E.B. An improved method for preparing large arrays of 1982 bacterial colonies containing plasmids for hybridization: *In situ* purification and stable binding of DNA on paper filters. Anal. Biochem. 126:222-230.

Temin H.M. RNA-directed DNA synthesis. In: Recombinant DNA. D. Freifelder (ed.). 1978 Freeman, San Francisco.

Wagner E.F. and Mintz B. Transfer of nonselectable genes into mouse teratocarcinoma 1982 cells and transcription of the transferred human β-globin gene. Mol. Cell Biol. 2:190-198.

Wagner E.F., Stewart T.A. and Mintz B. The human β-globin gene and a functional 1981 viral thymidine kinase gene in developing mice. Proc. Nat. Acad. Sci. USA 78:5016-5020.

Wagner T.E., Hoppe P.C., Jollick J.D., Scholl D.R., Hodinka R.L. and Gault J.B. 1981 Microinjection of a rabbit -globin gene into zygotes and its subsequent expression in adult mice and their offspring. Proc. Nat. Acad. Sci. USA 78:6376-6380.

Wallace R.B., Shatter J., Murphy R.F., Bonner J., Hirose T. and Itakura K. 1979 Hybridization of synthetic oligodeoxyribonucleotide to ϕX174 DNA: the effect of single base pair mismatch. Nuc. Acids Res. 6:3543-3557.

Ward K.A. Possible contributions of molecular genetics to animal improvement. In: 1982 Future Developments in the Genetic Improvement of Animals. J.S.F. Barker, K. Hammond and A.E. McClintock (eds.). Academic Press, Sydney. pp. 17-39.

Ward K., Sleigh M.J., Powell B.C. and Rogers G.E. The isolation and analysis of the 1982 major wool keratin gene families. In: Proc. 2nd World Congr. Genetics Applied to Livestock Production VI. pp. 146-153.

Watson J.D. and Crick F.H.C. Molecular structure of nucleic acids: A structure for 1953 deoxyribose nucleic acid. Nature 171:737-738.

Wensink P.C., Finnegan D.J., Donelson J.E. and Hogness D.S. A system for mapping 1974 DNA sequences in the chromosomes of *Drosophila melanogaster*. Cell 3:315-325.

RECOMMENDATIONS AND DEFINITIONS

RECOMMENDATIONS*

To FAO/UNEP

1. Since the benefits of conservation and management of domestic animal resources are known to be great relative to costs and since many adapted indigenous populations in developing countries are threatened by loss, the Panel recommended that planned programmes for the conservation of animal genetic resources, especially those adapted to the production conditions of developing countries, should have three mutually supportive strategies for preservation and management.

i. Highest priority should be given to identification and characterization of genetic resources and their adaptation.

ii. Preservation programmes should aim to prevent the loss of those populations containing unusual genetic variations. The preferred techniques will usually be the cryogenic storage of sperm and/or embryos, because most developing countries would not be willing to preserve live animals without utilization, and for most species, satisfactory methods of cryopreservation are available. FAO/UNEP should set up an International Cryogenic Animal Gene Bank, at more than one location using a split sample technique. Particular attention should be paid to ensuring the dependability of the maintenance services.

iii. Resource management programmes with live animals should comprise continued genetic improvement of productivity of local livestock populations, so that wherever possible, they remain economically competitive with imported exotic breeds. To ensure this, development and provision of performance recording systems suited to management needs at the farmer level should be implemented.

2. The Panel recommended that FAO/UNEP should promote the establishment of a list of threatened breeds and populations, indicating their potential genetic value and possible utilization, and proposing priorities for urgent action for conservation.

3. The Panel agreed on the importance of establishing regional data banks on animal genetic resources as soon as possible. It recommended that these should continue to be built up systematically in close cooperation with existing regional bodies such as IBAR/OAU and ILCA for Africa, ALPA for Latin America and SABRAO in Asia and with the leading international information centre on this subject (Commonwealth Agricultural Bureau of Animal Breeding and Genetics) to avoid duplication of effort. The Panel commended FAO/UNEP for initiating its work with a thorough study on the methodology to be applied for the collection, interpretation/summarizing and dissemination of the available information so as to minimize cost, maximize efficiency and to ensure that a globally compatible system should be established.

4. The Panel supported the view that the end point of the methodological study should be (a) a common, worldwide data format for use in the various regions; (b)

---

* These should be read in conjunction with the definitions which follow the recommendations.

the creation of descriptors for each species; and (c) a uniform system of criteria on which information would be judged for inclusion in the data bank. It was therefore recommended that FAO/UNEP continue their work to achieve these objectives without break or delay, and urged them to embark upon the establishment of Regional data bank centres as soon as the methodological studies are complete.

5. The Panel also recommended that FAO/UNEP, when promoting the creation and use of data banks, insist upon the competent scientific screening and compilation of all data entering data banks and that FAO/UNEP ensures that priority be given to this need.

6. The Panel noted that the genetic principles for determining the numbers of parents required to reconstitute a breed are established. It recommended that when cryopreservation of sperm and embryos from a threatened breed is part of its conservation, these principles should serve as guidelines to the amount of material to be stored.

7. The Panel noted that in the long-term storage of sperm and embryos, there should be safeguards for the health of future generations of livestock that will be exposed to contact with animals bred using cryogenically stored cells. It recommended that sperm and embryos stored in gene banks for endangered breeds should have adequate records concerning the health status of the herd and area/country of origin, and of the diseases for which the donors were tested. Future users of these stored cells should have access to these records.

8. The Panel noted that many of the trypanotolerant livestock breeds of West and Central Africa occur as small and scattered populations in a number of countries; that there are inherent difficulties in establishing and maintaining separate conservation and management programmes for these small genetic groups in each of the several locations; and recognized the advantages that may be gained by collaborative programmes of selection, multiplication and conservation which transcend national boundaries; it therefore recommended to FAO/UNEP that they should:

i. continue to foster intercountry cooperation in the development and operation of breeding programmes for trypanotolerant livestock; and

ii. assist in investigations that would help determine the genetic distance between the trypanotolerant breeds in all traits of economic importance, so that rational decisions may be taken to treat populations as discrete or combined for more effective future use.

9. The Panel noted that many domestic animal species in addition to cattle make significant contributions to human welfare and are similarly threatened by loss of important genetic variation, and recommended that greater attention and action be given to the conservation and management of genetic resources in populations of:

i. sheep, goats, buffalo, camelidae, swine and all other domestic species;

ii. breeds kept for draught;

iii. species used only on a small scale but in special environments.

Parallel attention should also be given to ways and means of preserving, for possible future use, populations of wild animals which are related to domestic species.

10. The Panel noted that considerable resources, both intellectual and material have gone into the organization and conduct of the first course on animal genetic resources conservation and management; and that the Hungarian scientists responsible for this course are in a unique position to suggest modifications and improvements that could be incorporated in future courses. It also recognized that there are advantages in organizing future courses at locations in developing countries where the problems exist and using appropriate regional languages for the conduct of the courses. It therefore recommended a two-stage approach to future course locations:

i. Organize the second course in the same location as the first course viz. University of Veterinary Sciences of Hungary, inviting persons who might serve as future Course Directors in developing country locations to participate and benefit from the experience gained by the Hungarian scientists.

ii. Organize subsequent courses on a regional basis giving priority to the needs of developing countries in course organization and selection of teachers and students, and in close contact with the regional associations of animal production, as well as animal breeding and genetics.

11. The Panel recommended that FAO/UNEP encourage educational institutions in the developing countries to include in their animal science curriculum aspects of animal genetic resources conservation (including preservation of threatened genotypes, evaluation, improvement and management). The Manual prepared at the First International Course on Animal Genetic Resources Conservation and Management by FAO/UNEP and the University of Veterinary Sciences, Hungary, should be offered as a source of ideas for inclusion in such curriculum development.

12. The Panel noted that information on genetic distances between breeds of domestic animals would be of value in deciding priorities for action on breed conservation and management, and recommended that research on this topic be encouraged. In particular FAO/UNEP should assist investigations to this end.

13. The Panel recommended that the new techniques of molecular biology should be used to study the functioning of major genes known to be important for animal production, to the discovery of additional major genes, and to the production of gene maps for domestic species, and that FAO/UNEP should promote such studies.

To FAO/UNEP and Governments

1. The Panel agreed that in most developing countries genetic improvement of local breeds would form integral and necessary parts of constructive programmes for their conservation and future use. It was pointed out, however, that such breeding programmes would require more information on production characteristics both under station and field conditions than was usually available. The Panel also noted that recent developments of AI services in developing countries have often been accompanied by massive importations of exotic bovine semen, without concurrent development in semen processing facilities for superior indigenous bulls. The Panel recommended:

i. that Member Governments should give higher priority than hitherto to production recording and breeding programmes adapted to their own conditions so as to ensure the conservation and development of their national livestock resources.

ii. that Member Governments should establish breeding and multiplication centres in countries where breeds are found which are in need of improved utilization;

iii. that FAO/UNEP should render assistance in the establishment of these breeding and multiplication centres and the necessary infrastuctures to support them.

2. The Panel noted that there is only a small number of indigenous cattle breeds suited to milk production under harsh conditions; that these breeds are threatened as a result of a progressive narrowing of their genetic base; that in order to widen their genetic base there must be an exchange of germplasm between countries where the breeds occur; and recommended that:

i. inter-country cooperation in the exchange of germplasm should be encouraged with due regard to quarantine precautions;

ii. FAO should assist in the development of the requisite infrastructure for the exchange of genetic material between countries, for the genetic evaluation and comparison under different harsh conditions and for studies and action on their adaptation in both purebred and crossbred improvement programmes.

DEFINITIONS
pertaining to Animal Genetic Resources

1. CONSERVATION

The management of human use of the biosphere so that it may yield the greatest sustainable benefit to present generations while maintaining its potential to meet the needs and aspirations of future generations. Thus conservation is positive, embracing preservation, maintenance, sustainable utilization, restoration and enhancement of the natural environment.

(This definition of CONSERVATION originates with the World Conservation Strategy, which was prepared by the International Union for the Conservation of Nature and Natural Resources (IUCN), and the following collaborative organizations: United Nations Educational, Scientific and Cultural Organization (Unesco), the Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), and the World Wildlife Fund (WWF).)

2. PRESERVATION

That aspect of CONSERVATION by which a sample of an animal genetic resource population is designated to an isolated process of maintenance, by providing an environment free of the human forces which might bring about genetic change. The process may be in situ, whereby the sample consists of live animals in a natural environment, or it may be ex situ, whereby the sample is placed, for example, in cryogenic storage.

3. CONSERVATION BY MANAGEMENT

That aspect of CONSERVATION by which a sample, or the whole of an animal population is subjected to planned genetic change with the aim of Sustaining, Utilizing, Restoring or Enhancing the quality and/or quantity of the animal genetic resource and its products of food, fibre or draught animal power.

4. THREATENED (Species or breed)

A term used to describe an animal genetic resource population which is subject to some force of change, affecting the likelihood of it continuing indefinitely, either to exist, or to retain sufficient numbers to preserve the genetic characteristics which distinguish it from other populations. THREATENED is a generic term embracing more precise descriptions such as Endangered, or Vulnerable.

(It is also so used in the context of the World Conservation Strategy.)

5. GENE BANK

A physical repository, in one or more locations, where the samples of animal

genetic resource populations which are being preserved are kept. These may include animals, embryos, oocytes, sperm, DNA, etc.

6. DATA BANK

The fund of knowledge comprising the CHARACTERIZATIONS which describe the genetic attributes of animal breeds or species and the various environments in which they occur; these CHARACTERIZATIONS being stored both as numerics and words in a data/word processing system which provides for the addition of further information, for amendment and for analytical use.

7. CHARACTERIZATION

The numeric/word decription of:

i. the genetic attributes of an animal species or breed which has a unique genetic identity; and

ii. the environments to which such species or breed populations are adapted or known to be only partially or not adapted.

The CHARACTERIZATION is a succinct statement, being the distillation of all available knowledge both previously published or unpublished, which contributes to the reliable prediction of genetic performance in defined environments. It is different from the mere accumulation of existing reports or individual findings on genetic performance on specific occasions.

8. DESCRIPTORS (of species or environments)

A series of items with defined meanings for a species and its environments, which are universally used to prepare data bank CHARACTERIZATIONS of:

i. breeds of a given species, covering the phenotypic and genetic parameters of the breed;

ii. environments in which breeds of a given species are found, covering the natural and artificial features relevant to genetic analysis, including such items as climate, topography, endemic disease risk, feed and water supply, and management systems.

The purpose of DESCRIPTORS is to facilitate valid comparison, classification or enumeration of breeds within a species in the context of the environments existing in different countries and regions of the world.

THE FAO TECHNICAL PAPERS

FAO ANIMAL PRODUCTION AND HEALTH PAPERS:

1. Animal breeding: selected articles from World Animal Review, 1977 (C* E* F* S*)
2. Eradication of hog cholera and African swine fever, 1976 (E* F* S*)
3. Insecticides and application equipment for tsetse control, 1977 (E* F*)
4. New feed resources, 1977 (E/F/S*)
5. Bibliography of the criollo cattle of the Americas, 1977 (Bi. E/S*)
6. Mediterranean cattle and sheep in crossbreeding, 1977 (E* F*)
7. Environmental impact of tsetse chemical control, 1977 (E* F*)
7. Rev. Environmental impact of tsetse chemical control, 1980 (E* F*)
8. Declining breeds of Mediterranean sheep, 1978 (E* F*)
9. Slaughterhouse and slaughterslab design and construction, 1978 (E* F* S*)
10. Treating straw for animal feeding, 1978 (C* E* F* S*)
11. Packaging, storage and distribution of processed milk, 1978 (E*)
12. Ruminant nutrition: selected articles from World Animal Review, 1978 (C* E* F* S*)
13. Buffalo reproduction and artificial insemination, 1979 (E**)
14. The African trypanosomiases, 1979 (E* F*)
15. Establishment of dairy training centres, 1979 (E*)
16. Open yard housing for young cattle, 1981 (E* F* S*)
17. Prolific tropical sheep, 1980 (E*)
18. Feed from animal wastes: state of knowledge, 1980 (E*)
19. East Coast fever and related tick-borne diseases, 1980 (E*)
20/1 Trypanotolerant livestock in West and Central Africa, 1980
Vol. 1 - General study (E* F*)
20/2 Trypanotolerant livestock in West and Central Africa, 1980
Vol. 2 - Country studies (E* F*)
21. Guideline for dairy accounting, 1980 (E*)
22. Recursos genéticos animales en América Latina, 1981 (S*)
23. Disease control in semen and embryos (E* F* S*)
24. Animal genetic resources - conservation and management, 1981 (E*)
25. Reproductive efficiency in cattle, 1982 (E*)
26. Camels and camel milk, 1982 (E*)
27. Deer farming, 1982 (E*)
28. Feed from animal wastes: feeding manual, 1982 (E*)
29. Echinococcosis/hydatidosis surveillance, prevention and control: FAO/UNEP/WHO guidelines, 1982 (E*)
30. Sheep and goat breeds of India, 1982 (E*)
31. Hormones in animal production, 1982 (E*)
32. Crop residues and agro-industrial by-products in animal feeding, 1982 (E/F*)
33. Haemorrhagic septicaemia, 1982 (E*)
34. Breeding plans for ruminant livestock in the tropics, 1982 (E* S*)
35. Off-tastes in raw and reconstituted milk, 1983 (E* F* S*)
36. Ticks and tick-borne diseases: selected articles from World Animal Review, 1983 (E* F* S*)
37. African animal trypanosomiasis: selected articles from World Animal Review, 1983 (E* F*)
38. Diagnosis and vaccination for the control of brucellosis in the Near East, 1983 (E*)
39. Solar energy in small-scale milk collection and processing, 1983 (E*)
40. Intensive sheep production in the Near East, 1983 (E*)
41. Integrating crops and livestock in West Africa, 1983 (E*)
42. Animal energy in agriculture in Africa and Asia, 1984 (E/F*)
43. Utilisation des sous-produits de l'olivier en alimentation animale dans le bassin Méditerranéen, 1984 (F*)
44/1 Animal genetic resources conservation by management, data banks and training, 1984 (E*)
44/2 Animal genetic resources: cryogenic storage of germplasm and molecular engineering, 1984 (E*)

FAO PLANT PRODUCTION AND PROTECTION PAPERS: 57 titles published

FAO CONSERVATION GUIDES: 8 titles published

FAO FORESTRY PAPERS: 47 titles published

FAO FOOD AND NUTRITION PAPERS: 30 titles published

FAO AGRICULTURAL SERVICES BULLETINS: 59 titles published

FAO IRRIGATION AND DRAINAGE PAPERS: 41 titles published

FAO SOILS BULLETINS: 52 titles published

Availability: June 1984

C - Chinese
E - English
F - French
S - Spanish

* Available
** Out of print
*** In preparation

The FAO Technical Papers are available through the authorized FAO Sales Agents or directly from Distribution and Sales Section, FAO, Via delle Terme di Caracalla, 00100 Rome, Italy.

M-22

ISBN 92-5-102111-2