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TECHNICAL MEETING ON EMBRYO TRANSFER AND ANIMAL PRODUCTION

Summary Report

Jakarta, Indonesia
February 23-24,1987

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PREFACE

In March 1986, the National Research Council of Indonesia (DRN) and the Board on Science and Technology for International Development (BOSTID) held a joint workshop on biotechnology in agriculture. It was recommended at that workshop that Indonesia examine the potential of developing a Center for Embryo Transfer and Animal Production. A technical meeting to undertake this examination was held in Jakarta, Indonesia, February 23-24, 1987.

The primary objectives of the technical meeting were to assess the present status of knowledge about and experience in embryo technology and animal production technology in Indonesia; identify gaps between the current situation and the actual requirements; determine the economic feasibility of cattle production and management through an embryo transfer program; and set priorities for a program to establish a center for embryo transfer and animal production. The center would provide the advanced technology, animals, embryos, semen, and training needed to produce genetically superior livestock for Indonesia and eventually livestock products for international markets.

As background for the meeting, an ad hoc BOSTID committee met in Washington, D.C. in June 1986 to draft a working paper on the proposed center. Present at the meeting were Drs. Charles C. Muscoplat and Anthony J. Faras, who participated in the March workshop and Dr. Raymond Wright, Jr. professor of animal sciences at Washington State University. A copy of the draft plan that resulted from that meeting is included in Part I of this report.

These activities were one activity in a larger program of cooperation between BOSTID and the Indonesian government. Begun in 1968, this program has featured a series of workshops on food policy, industrial and technological research, natural resources, rural productivity, manpower planning, marine algae biotechnology, biotechnology in agricultural development, and development of a science and technology information system. BOSTID's participation has been supported in the context of a science and technology loan from the U.S. Agency for International Development (USAID) to the government of Indonesia. The current two-year program with BOSTID calls for a number of activities (panel discussions, workshops, follow-up activities, or small advisory groups) to be organized each year.

This meeting was organized by its steering committee under the sponsorship of the Indonesian National Research Council.

Dr. Didin S. Sastrapradja, Assistant (II) Minister of State for Research and Technology and chairman of the National Committee on Biotechnology, opened the meeting on behalf of Dr. B. J. Habibie, Minister of State for Research and Technology and chairman of the DRN. In his keynote address, Dr. Sastrapradja described the long relationship between Indonesia and the U.S. National Research Council (NRC) and its cooperative programs in the field of biotechnology and related subjects (see Appendix A). Dr. Raymond Wright, chairman of the U.S. National Research Council panel, commented on the role that embryo transfer and animal production can play in the development of a dairy and beef industry. Dr. Sediono M. P. Tjondronegoro also voiced his comments about the role of the DRN in hosting the meeting.

After the meeting adjourned, field visits were made to the Lembang area near Bandung where U.S. participants visited an artificial insemination center, a milk cooperative, a government-run dairy, and a private dairy. On the return trip to Jakarta, the group visited the Kariyana feedlot at Cicurug. Comments made by the U.S. participants in response to these visits are also included in Part I of this report.

Part I of this report also contains the conclusions and recommendations that emerged during the two-day plenary session. Papers prepared for this meeting are presented in Part II. The agenda for the meeting and the list of participants are Appendixes B and C, respectively.

This workshop report was prepared by Rose Bannigan of the BOSTID staff using papers written by the Indonesian and NRC participants. The papers have been edited to eliminate duplication, but they accurately reflect the discussions. The final draft was reviewed and approved by the members of the NRC panel and the Indonesian organizing committee. Sabra Bissette Ledent, BOSTID consultant, edited the report.

The participants would like to thank the members of the workshop secretariat for the excellent organization of the workshop.

The U.S. panelists would also like to thank the staff at the various institutions visited at Lembang and Cicurug for taking time to show them the facilities and for their kind hospitality.

CONTENTS

PART I

Conclusions and Recommendations

SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

Participants in this meeting recognized the importance of embryo transfer (ET) in improving the genetic potential and production efficiency of the livestock industry of Indonesia. This advanced technology will lead to increased production and will improve the quality of meat, milk, and other livestock products. It will also contribute significantly to national goals by reducing imports and promoting exports of non-oil commodities. As this advanced technology enters the marketplace, supporting industries will evolve to meet the needs of this growing enterprise.

It is recommended that a Center for Embryo Transfer and Animal Production (CETAP) be established in Indonesia. This center would provide the advanced technology, animals, embryos, semen, and training needed to produce genetically superior livestock for Indonesia and international markets. To ensure rapid implementation of the proposed center's program, resources should be obtained from various private and government agencies.

CETAP should be located at an existing artificial insemination center(s), based on the availability of staff, physical facilities, and land.

To establish a superior genetic pool of animals, CETAP should initially provide or import Holstein, Brahman, and Brahman-infused breed embryos or animals, while using the superior local livestock for embryo production.

CETAP technicians must be trained in embryo transfer procedures and related technologies, since this initial training will ensure the success of the CETAP program and will allow additional technicians to be trained at the center itself.

CETAP should serve as a link among the universities, research institutes, and the private sector to encourage applied research, improve production, and increase efficiency. Private as well as state companies should be encouraged to cooperate with CETAP in increasing production and reducing the reliance on imported animal products.

The intensive use of embryo transfer in livestock should be studied on a commercial basis with the participation of the private sector. The market orientation of this technology should be examined as well. Any research on the technology of embryo transfer should be coordinated

with the efforts of the national artificial insemination and breeding programs for livestock.

Initially, embryo transfer should be carried out by CETAP and farmers with large holdings until the smallholders can participate effectively.

Finally, to enhance animal industries in Indonesia through embryo transfer and related technologies, joint ventures or other relationships with the U.S. government or the U.S. private sector should be encouraged.

COMMENTS BY U.S. PARTICIPANTS

Raymond Wright, Jr. and Jerry J. Reeves
NRC Panelists

GENERAL OBSERVATIONS

Indonesia's universities, private sector, and government were well represented at this two-day meeting in Jakarta, and the government appeared motivated to commence a successful project on dairy or beef cattle. Government representatives have met with the designated ministers, and they will inform the president of Indonesia about the recommendations that emerged from this meeting.

Two different groups in the private sector are interested in some type of beef or dairy embryo transfer program. One group has already imported frozen embryos from the Granada International Corporation (Holstein and White Brahman) and has attained a 35 percent pregnancy rate. They are not happy with the conformation of the White Brahman, however.

The overall impression is that the Indonesians are committed to such a program, but some government support will be needed initially to structure the proposed Center for Embryo Transfer and Animal Production (CETAP). The private sector will support the center by buying embryos and semen and hiring technicians trained at the center. The initial administration chosen to operate CETAP will be critical to its success. The administration should understand the field of embryo transfer and work with the private sector when possible.

SPECIFIC COMMENTS

The artificial insemination center at Lembang is a clean, well-managed facility, but its bulls are of marginal genetic quality. Although bulls of several breeds are used, Holstein and local Brahman predominate. The local Brahmans are the most impressive cattle at the center, but none of the bulls are progeny tested. The use of progeny tested bulls would require that semen be purchased from the United States, England, or Canada.

The Indonesians are currently unable to progeny test their own young bulls because of the almost complete absence of recordkeeping (milk production, etc.) at both the small and large dairies. There has been some discussion of Japanese assistance in developing a computer system to introduce a progeny testing program, but until records are kept this is not possible.

Embryo transfer could be conducted at the existing artificial insemination center if a good supply of recipients is found. Having small farmers supply their animals as recipients is probably not practical, as temperature and nutrition are not well controlled. Association with a large dairy for the provision of recipients is a good possibility. A trade for services such as embryo transfer or artificial insemination could be made.

The concept of establishing a beef feedlot on a now rural island close to Singapore or at an appropriate location on Java is an interesting one. This concept may be unsound, however, because of the high costs of production in a remote area with little or no water. Any project of this type should be examined closely, with particular attention paid to the supply of animals, feed, and water, and transportation costs.

POTENTIAL EMBRYO TRANSFER-ANIMAL PRODUCTION PROJECT

The U.S. participants in this meeting felt that Indonesia could best develop an industry based on embryo transfer and other biotechnological techniques, with immediate benefits, by forming a joint venture with a company from the United States or elsewhere. The overall objective of such a joint venture would be the introduction of state-of-the-art animal biotechnologies in Indonesia. This would be accomplished by:

- Introducing superior frozen Holstein embryos from the United States for transfer into recipient Indonesian cattle. With the proper management and nutrition the resultant calves will mature into high-producing milkers.

- Providing on-site embryo collection and training for local personnel and farmers using Indonesian cattle in a commercial dairy setting to demonstrate embryo transfer. Such a demonstration will not only show Indonesians the feasibility of embryo transfer techniques, it will also show dairy cooperative members and others that an improvement in the genetic quality of their livestock is possible and can be accomplished in a short time. In addition, the superior dairy cattle born as a result of this project will become the property of the host facility.

- Training one or more Indonesian field scientists and one or more laboratory scientists in embryo transfer at a U.S. university, such as Washington State, with follow-up training at a large-scale commercial embryo facility such as AnemTech's embryo technology facility in Eureka, Montana.

- Identifying and, if possible, establishing several embryo transfer research, development, and demonstration efforts aimed at improving overall reproductive efficiency in Indonesia, with a marketable application to other tropical livestock systems.

- Recommending and evaluating with the appropriate Indonesian counterparts and officials the development of a type of dairy and beef cattle that will yield maximum milk and meat production under the agricultural conditions found in Indonesia.

The overall benefit to be gained by Indonesia is the potential to improve milk and meat production over current levels. The immediate impacts and potential long-range payoffs are enormous. Given its current success rate, embryo transfer could lead to an increased rate of genetic improvement of from 10 to nearly 100 percent, depending principally on the current intensity of sire selection and on the trait(s) in question. The possible effects of this alternative technology on the rate of genetic improvement in milk yield in dairy cattle using artificial insemination has been estimated at 100 kg per cow per year, and using embryo transfer, 158 kg per cow per year.

Embryo transfer also has important economic applications in multiplying rare breeds (Bali cattle), strains, or individuals, and, in conjunction with the use of frozen embryos, in avoiding the high costs of shipping and quarantining livestock breeds for new environments.

By using embryos frozen at liquid nitrogen temperatures and imported from the United States, this project will nicely demonstrate how such storage greatly increases flexibility because recipients need not be synchronized precisely with embryo donors in time and space. This leads to tremendous savings in manpower and feed costs.

Proposed Work Plan

A work plan could be devised in conjunction with a U.S. university and a small biotechnology company. It would include the purchase of 100 superior Holstein embryos from the United States, frozen for shipment to Indonesia. Consultants provided by the U.S. company would subsequently visit Indonesia for approximately three weeks to transfer these embryos into recipient Indonesian cattle to attain 40-60 pregnancies.

The Indonesian government would determine the site of the proposed project. Training sessions on embryo recovery and transfer would be held on-site by the consultants responsible for providing detailed instruction to all participants. All interested farmers and personnel from the appropriate Indonesian institutes would be invited without charge. The use of two to three local cows in this training session should demonstrate clearly to the Indonesians that their livestock can be improved genetically in a very short time.

During the nine-month gestation period of the recipient cattle, their maintenance and feeding would be the responsibility of the farm personnel. Several highly qualified veterinarians should reside at the facility site in the event that prophylaxis or treatments are necessary for pregnant cattle. Minor modifications of holding stanchions may be necessary to maximize the comfort of the pregnant cows.

Mother and calving data should be collected by the U.S. consultants and synthesized with firsthand information gathered from their initial visits to the various artificial insemination centers; their discussions with farmers, industry, and government officials; their assessments of dairy herd improvement practices; and so forth. Subsequently, a draft of a final report would be submitted to the appropriate Indonesian government officials for their input and comments. Based upon this in-country review, a final report would be issued evaluating the empirical results and future prospects for embryo transfer in Indonesia. In addition, implementation of a long-term comprehensive program for research, development, and commercial production would be recommended.

Training. To improve and strengthen Indonesia's capabilities in biotechnology, two Indonesian scientists would undergo five months of training in the United States. The nominated scientists must be highly qualified, since they will be the forerunners of research and development in embryo transfer in Indonesia.

Under the direction of the designated head of the U.S. project, training would be conducted at a U.S. university such as Washington State. It is proposed that a laboratory scientist receive training in the general laboratory methods for embryo transfer, including quality control, embryo identification and morphology, and techniques for embryo freezing and storage. In addition, a field scientist would learn techniques for embryo freezing and storage as well as the techniques for nonsurgical embryo collection and transfer.

The advantage of having both a laboratory and field scientist is that together they can use their existing expertise, as well as newly developed skills, to have an immediate impact, resulting in a sense of accomplishment and teamwork. Follow-up after university training would be desirable so that both scientists can observe and study at a large-scale commercial embryo facility, such as AnemTech's Embryo Center in Montana.

Timetable. This project should be designed for a 12-month period. Costs are estimated as follows (based on costs for a Washington State University-AnemTech cooperative program):

Salary and benefits charges for consultants:	
Embryo transfer specialist (one man-month)	$ 9,750
Embryo transfer technician (one man-month)	8,250
Travel (four round-trips from Seattle to Indonesia, $2,500 each)	10,000

Per diem for consultants (60 days @ $85/day)	5,100
Training of two scientists:	
Travel from Indonesia to Seattle, $2,500 each	5,000
Travel within United States, $500 each	1,000
Training and living costs:	
Washington State University, three months	10,000
AnemTech facility in Montana, two months	30,000
Drugs, culture media, and minor equipment	20,000
Farm modifications	5,000
Home office project coordination & support services	10,000
Purchase and shipment of 100 embryos from superior U.S. Holstein cows, $300 each	30,000
Contingency	5,000
TOTAL	$174,000

PROPOSED CENTER FOR EMBRYO TRANSFER AND ANIMAL PRODUCTION

INTRODUCTION

One of the recommendations that emerged from the workshop on biotechnology in agriculture, held in Jakarta in March 1986, was the establishment of a Center for Embryo Transfer and Animal Production (CETAP) in Indonesia. This center would provide the advanced technology, animals, embryos, semen, and training needed to produce genetically superior livestock for Indonesia and international markets. The center would therefore undertake the following activities:

- To meet the increasing demand in Indonesia, establish a supply house to market the supplies, equipment, drugs, and other services needed for embryo transfer in cattle.
- Use embryo transfer in species other than cattle as the opportunity arises.
- Serve as an embryo transfer and animal biotechnology training and research center for scientists and veterinarians.
- Serve as a center for the collection and, in conjunction with the artificial insemination centers, for the distribution of semen produced from the genetically superior bulls.

The center should be located in an area adjacent to the artificial insemination centers and major agricultural research institutes, including universities, to be accessible to the scientists involved in this research. It should also be accessible to dairies or farming areas.

THE BUSINESS OF EMBRYO TRANSFER

General

Recent trends in the economics of livestock production have made it mandatory to increase the productivity of animals for continued

NOTE: This working paper was drafted at an ad hoc meeting held in Washington, D.C. in June 1986.

profitability. This increase can be brought about by improving the productive environment; by better feeding, management, and health; and by breeding better animals. New technologies--such as artificial insemination, multiple ovulation, embryo transfer, embryo splitting, and genetic engineering--can be applied to animal breeding to speed the rate of genetic improvement, to multiply superior animals, and to store or transport them as embryos.

Genetic improvement has been relatively slow, but the gains are cumulative, and they can be passed on to later generations. The genetic gains from superior animals may therefore be marketed. Eventually, the proposed Center for Embryo Transfer and Animal Production, through both its research team, specializing in animal embryo technology and other artificial breeding techniques, and methodologies assembled from both local institutions and leading institutions overseas, will be ideally placed to further this form of livestock science and to exploit this technology commercially in the agricultural marketplace. Thus, the center will be engaged in the business of developing, producing, and eventually marketing and distributing high-quality cow embryos and livestock. The market for semen and for embryos is highly competitive and will accept only animals of proven superiority from recognized breeding programs and from environments that are free of disease.

Given the procedures to be used by the center for the production and genetic development of quality embryos, several years will pass between commencement of the center's proposed plan of operations and the sale of embryos and livestock. Consequently, no sales of embryos or livestock (except for domestic sales) will probably occur during the initial years of the center's operation.

It is increasingly important that farmers achieve maximum milk production per cow at the most economical cost. A balanced feed ration, proper milking procedures, sound herd health, and a carefully selected breeding program are all essential. The quality, volume, and cost-efficiency of milk production per cow--not the number of cows milked--will result in a successful operation. A farmer must integrate into his herd more genetically superior cows capable of producing higher quality milk in greater volumes, thereby raising the average productivity of his entire herd. The center should be able to offer dairy farmers high-quality, low-cost embryos, which will allow farmers to acquire high-quality dairy cows at a relatively low cost, and will enable them to upgrade their herds in a much shorter time than that required for traditional breeding methods. Once integrated into the farmer's operation, these cows will boost the farmer's average herd milk production, regardless of the size of the herd. The center could also offer living yearling cows or younger calves to the farmer.

As an adjunct to its primary business, the center could produce and sell quality semen to artificial insemination stations throughout the country. The center could also serve as an outreach to the farming community by providing information on animal health, proper nutrition, and treating or testing for animal diseases.

Both the embryos and livestock sold by the center will be the product of the center's ongoing process of genetic refinement of favorable traits, and other research and development to be conducted by the center.

Recent applications of mathematical theory using powerful computers have considerably increased the accuracy of estimating the breeding value of animals by allowing production and pedigree information to be combined in an optimal way. With these accurate estimates of genetic superiority, the way is now clear to use the techniques of multiple ovulation and embryo transfer in a similar manner to artificial insemination and to sell genetic gain in national and international markets.

The techniques of multiple ovulation and embryo transfer are now well established in cattle, pigs, sheep, and goats. They can be used not only to multiply the progeny of superior parents, but also to allow progeny to be reared in recipient cows from parents who are themselves too young for normal breeding.

Splitting embryos to obtain identical twins is now feasible, and current research into multiple cloning is expected to bear fruit in the near future. Similarly, methods of determining the sex of embryos are the subject of promising research.

The center could adopt an integrated R&D program for novel applications of established biological techniques, as well as new biotechnologies with the potential to produce premium animals of agricultural importance. Ultimately, by applying the approaches and technologies developed in the center's research program, other or even improved premium animals might be produced that exhibit increased resistance to specific diseases or other desirable traits that are genetically determined. The program of the center could be based on the extensive knowledge of scientific consultants or technologists acquired by joint ventures or from the Indonesian scientific research staff members trained in the fields of molecular and cellular biology and physiology.

Products

The principal products of the center would be animals, embryos, semen, training for technicians, and embryo supplies, including drugs and equipment. The center could produce annually a minimum of 50 frozen embryos and 160 fresh embryos. Some of the embryos and animals could eventually be sold to international markets.

Embryos. The center's primary business would be the mass production and marketing of high-quality cow embryos for research and for sale at comparatively low prices. The genetic research to be undertaken by the center could produce, by creating genetically superior embryos, cows that are more resistant to disease and produce milk more efficiently than the average dairy cows currently used by the Indonesian dairy

industry. Through the application of superovulation and embryo transfer technology, the center could utilize gentically superior donor cows to produce embryos for two uses, the external embryo market and the internal operations of the center. Embryos used internally by the center could be transferred to average recipient cows which will carry the calves through the normal nine-month gestation cycle. Some of the offspring of such embryo transfers could be utilized in the production of more genetically refined embryos. Embryos produced and sold on the market by the center would be sold to buyers who will transplant the embryos into an average recipient cow to produce a high-quality milk cow to add to their producing herd.

The technology associated with embryo transplantation is fairly well established and widely practiced, but training of technicians is necessary.

Livestock. The center would sell high-quality livestock (both cows and bulls) produced by the center's embryo production efforts. In addition, the center would sell the donor cows not necessary for its operations and which have proven milk production records. At the outset of the center's operation, some of the male calves (which are usually 50 percent of all calves born) would be sold for beef purposes. However, initially the calves would be retained for semen production. As techniques for sex determination are developed, the number of male calves born would probably decrease significantly as the center would utilize for its own operations only those embryos likely to produce female offspring. Consequently, the availability of livestock to be sold by the center would be subject to greater control.

EMBRYO TRANSFER

Transfer of cow embryos involves the following procedures:

- Superovulation of the donor cow (induced by the application of certain hormones)
- Artificial insemination of the superovulated cow
- Collection of embryos from the cow
- Examination of the embryos
- Transfer of the normal embryos to recipient cows or freezing the embryos for later transfer.

The basic technologies to be used by the center are well known and generally not subject to patent protection.

Cows normally produce one egg at a time. Consequently, pregnancies generally result in only one calf. Because gestation takes approximately nine months, a cow rarely produces more than one calf each year. Embryo transfer allows a genetically superior cow to produce an increased number of biological offspring in a shorter period of time,

thereby allowing the genetic characteristics of a herd to be changed within a relatively short time span.

In the standard nonsurgical procedure for collection of embryos, the cow's uterus is flushed with a liquid solution used to dislodge the embryos and discharge them out of the uterus into a collecting flask. Once the flushing medium containing the embryos has been removed from the cow, it is searched carefully using a stereomicroscope. The six embryos obtained on average from a superovulated donor cow are then evaluated to select those that are viable. Selected embryos may then be transferred into recipient cows shortly after collection, placed in another medium for freezing and storage, or, if of questionable viability, cultured in a special medium and their development observed for approximately 24 hours to determine whether further use of the embryo is indicated.

Transfer of the embryos to recipient cows can be accomplished surgically or nonsurgically. In a surgical transfer an incision is made in the flank of the recipient cow, and a single embryo is injected from a syringe into the uterus. In a nonsurgical procedure the embryo is placed in the uterus using a technique similar to artificial insemination. Success rates for the nonsurgical transfer of bovine embryos, according to industry sources, range from 40-65 percent, while success rates of 60-80 percent are recorded for surgical transfers. Currently, more than 80 percent of all embryo transfers are being performed nonsurgically because of the convenience and the speed of transfer, and because similar pregnancy rates can be obtained. Healthy donor cows can be successfully superovulated and the embryos collected nonsurgically six times per year. Thus, an average of 21 calves per year can be produced from a single donor cow.

Much of the center's embryo transplant research will be devoted to developing a cost-effective and reliable means of determining an embryo's sex prior to implantation ("sexing" of embryos). If the center can achieve a means of accurate embryo sexing, it could provide this service to other breeders and utilize selective implantation to eliminate its own production of bull calves, whose cost of production nearly always exceeds their market value at birth. The impact of this technnology on the international marketing of frozen embryos would be considerable.

Embryos that have been frozen and stored for up to several months after recovery from a donor cow can be thawed and implanted in recipient animals, but with only about one-half the rate of successful pregnancies of embryos that have not been frozen. If substantial advances can be achieved in the nonharmful freezing of embryos, the market for embryos would increase significantly. Nonharmful frozen storage of embryos would greatly facilitate the shipment of embryos over long distances.

Another area for future research at the center is twinning of embryos (implanting two embryos in one recipient animal). Development of reliable twinning procedures would allow the center to reduce the size of its recipient herd, thereby reducing costs. Such procedures would probably not be fully accepted, however, until the sexing of

embryos can be achieved, thus assuring that each twin will be female and avoiding the possibility that freemartins will be produced.

Research into sexing, storing, and twinning of embryos is being conducted by U.S. breeding organizations and universities. If current research efforts are successful in any one of these areas during the next few years, the economic effect on the purebred cattle breeding industry will be significant. It is hoped that the proposed center would be able to use any new developments in its business.

Embryo Production

The proposed center's embryo production process (whereby offspring born of embryos produced by the center are themselves utilized for embryo production) would become fully operation in approximately two to four years. Once a decision is made to establish the center, an appropriate plan of action will have to be devised.

Research and Development

The proposed center should hire full-time scientific personnel to begin the development and implementation of the center's research and development procedures and supervise initial embryo production. The center would also establish working relationships with local universities and institutes and enter into consulting and service agreements with research scientists and other organizations within the industry, either foreign or local. Through the combined efforts of the center's in-house professionals and its relationships with organizations such as universities, the center could then continue the advancement of embryo production, transplantation, and preservation technologies. This would help both the center and the dairy industry in the development of better methods for producing more efficient and genetically superior cows.

Embryo Freezing. The center would conduct research aimed at increasing the survival rates of frozen-thawed embryos. Current technology in the survival of deep-frozen bovine embryos yields pregnancy rates of approximately 20-38 percent. Most embryos are frozen in glass ampules requiring the presence of a highly trained embryologist to manipulate, evaluate, and package the embryos after thawing.

Recent and promising technology incorporates freezing and thawing of embryos in a 0.5-cc French insemination straw. Embryos can therefore be thawed and transferred nonsurgically without the need for a trained embryologist. Research should be undertaken immediately to enhance the survival rates of bovine embryos frozen and thawed in French straws. (Preliminary field testing conducted by others has yielded 40 percent pregnancy rates with bovine embryos frozen and thawed in such straws.) Specifically, research should aim to develop freezing procedures that

result in an embryo survival rate equivalent to that of nonfrozen embryos, or 60 percent.

Embryo Splitting. This procedure divides a single embryo into two, three, or four parts, resulting in the birth of identical twins, triplets, or quadruplets. Not only are more calves produced per embryo with this method, but those from each embryo are genetically identical. These techniques were recently applied to cattle, sheep, horses, and pigs on an experimental and a limited commercial basis.

Embryos have been successfully divided in half (they are then known as demi-embryos), producing two identical individuals and doubling reproductive efficiency. Embryos are at the "morula" stage when they are 4-5 days old and contain some 30-50 individual cells. A morula and an unfertilized egg, distinguished by having a single cell, can be used in the splitting process. The success rates of splitting embryos are very encouraging.

Embryo Sexing. Numerous attempts have been made with almost all farm species to determine the sex of the embryo before transfer. This is usually attempted by extracting an embryo cell using micromanipulation and fluorescent staining the extracted cells for chromosome analyses. Male and female chromosomes for most farm species can be differentiated under a high-powered microscope. Another method--often called HY antigen staining--involves coupling a male-specific antigen with male cells. Only male cells bind to the male-distinguishing antigen. A female embryo does not take up the fluorescent stain, but the male embryo will glow bright green. Research in this area is continuing.

MARKETING AND DISTRIBUTION

The marketing and distribution plan developed by the center would depend on the rate of production. The first marketable products would likely be embryos, livestock, and semen, which would at first be marketed locally.

The center would market and distribute its products primarily through organizations that are already well established within the dairy industry or government, such as farmer cooperatives and artificial insemination centers.

PART II

Presentations

NATIONAL STRATEGY
IN ANIMAL HUSBAN

Dan
Director-Gene

NATIONAL STRATEGY AND ROLE OF EMBRYO TRANSFER IN ANIMAL HUSBANDRY DEVELOPMENT IN INDONESIA

Daman Danuwidjaja
Director-General of Livestock Services

INTRODUCTION

Indonesia is composed of more than 13,000 islands spanning roughly 5,000 km from east to west. It has a population of over 165 million and an estimated population growth rate of 2.3 percent per annum.

Agriculture continues to be the most important segment of the Indonesian economy, accounting for 30 percent of the gross domestic product, 60 percent of total employment, and 70 percent of non-oil exports. The government has placed high priority on agricultural development through its five-year development plan (Repelita). The objectives of the plan are social justice for all, high economic growth, and national stability.

The overall objectives for agricultural development are the following:

- Meet the need for food and the need for raw materials by domestic industries
- Increase export earnings
- Expand employment opportunities
- Increase farmers' incomes
- Provide production opportunities to entrepreneurs
- Support balanced regional and rural development
- Increase transmigration activities.

Livestock is an intregal part of the rural economy of Indonesia. Farm animals serve as a source of power for cultivation and transport, as a source of food, and as a convenient means of family savings. The livestock sector provides almost all of the domestic requirements for meat and eggs, and part of the requirement for milk. Moreover, it fulfills a considerable part of the requirements of agriculture for fertilizer.

This paper was presented at the meeting by Soemarno P.

STRATEGY FOR DEVELOPING ANIMAL HUSBANDRY

The majority of Indonesia's rural population are farmers whose land holdings are often small, ranging from 0.5 to 3.0 hectares. These farmers depend on livestock draft power (cattle and buffaloes) for 70 percent of their cultivation and on livestock products for consumption. In 1985, cattle and buffaloes raised by small farmers contributed 276,000 tons of the total meat production of 808,000 tons, or about 34 percent. The poultry industry contributed 318,000 tons, or 39 percent of total meat production in the same year. Milk production for 1985 was estimated at 190,000 tons of which 95 percent was produced in Java. This production only fulfilled 25 percent of national consumption, however. Goat is the most important small ruminant in Indonesia and the most important sideline of the smallholder family.

The government is well aware of the importance of the livestock sector as a source of renewable animal protein for human consumption, as a raw material for industry, and as a provider of draft power and manure for crops. The livestock sector also creates jobs and contributes to the productive utilization of land resources.

In Repelita IV, the main objectives of livestock development are:

- To increase farm incomes and employment opportunities through enhanced production and productivity of livestock.
- To increase the livestock population and production to meet domestic demands.
- To increase export earnings and reduce the import of livestock products.
- To meet the demand for draft animals and the manure needed for crop production.
- To conserve indigenous breeds, such as Bali cattle.
- To improve the grassland through establishment of better-quality forage, thus improving both natural resources and the environment.

To meet the national objectives of livestock development, the following strategy is being implemented:

- Maintain and protect the existing and future livestock against disease and disorders.
- Improve feed and feed management.
- Improve the genetic potential of livestock by importing high-value breeds and implementing artificial insemination (AI) and other appropriate breeding programs.
- Improve skills and know-how through extension activities.
- Improve the infrastructure and facilities for processing and marketing livestock and livestock products.

BREEDING AND THE ROLE OF EMBRYO TRANSFER IN LIVESTOCK DEVELOPMENT

The government has placed high priority on the development of dairy and beef cattle. The large ruminants--cattle, buffaloes, and dairy cows--play an important role in the rural economy. Besides providing meat and milk (often a substitute for imported milk powder needed by some milk processing plants) for domestic consumption, they provide draft power for agriculture and transport, sustain a domestic leather industry, and provide hides and skins for export. They are also a means of accumulating capital.

The general strategy for developing the population and improving the genetic potential of large ruminants is:

- Prevent the slaughter of productive female animals by using law enforcement.
- Select good bulls for distribution to a group of farmers.
- Use the best bulls for the artificial insemination program being conducted in several provinces.
- Develop the genetic quality of local breeds--such as Sumba Ongole (SO), Peranakan Ongole (PO), Madura, and Bali cattle--through village breeding programs as well as modern breeding programs controlled by the implementation unit of the government project.
- Move some cattle and buffaloes from the densely populated areas to the vastly unpopulated areas through transmigration of livestock.
- Import breeding stock with a high genetic value and the potential to cope with the lack of domestic breeding stock as well as to improve the genetic quality of the overall livestock population.
- Import proven bulls and progeny-tested frozen semen to meet the demand for high-quality semen for the AI program.
- Implement an embryo transfer program by using frozen embryos from the United States and England and undertaking an embryo transfer pilot project by using Bali cattle as a donor and a recipient.
- Implement a program for progeny testing dairy cattle with the support of the Japanese government through the presidential aid program (BANPRES).

Breeding Large Ruminants

Cattle. Three major breeds of indigenous cattle are found in Indonesia: Ongole, Madura, and Bali, as well as a fourth that is less well known, Aceh. In addition, cattle have been imported from Australia and New Zealand over the past 10 years. These imports include crosses between Bos indicus and Bos taurus cattle, as well as some purebred Bos indicus, often called Brahman bulls, used for mating purposes.

The Ongole breed consists of two major types: Sumba Ongole and Peranakan Ongole. Sumba Ongole (Bos indicus) were imported from India to the island of Sumba in 1914; they have remained purebred. Peranakan Ongole, a cross of Bos indicus cattle with a native breed, are found mainly in Java. These two breeds have been used for improving the quality of draft cattle throughout Java and Sumatra, through the "Ongolisasi" process. The number of Sumba Ongole and Peranakan Ongole that can be distributed to the large pool of Indonesian cattle are limited, however. Thus, this effort has not resulted in a change in the genetic base.

Madura cattle are a cross between Bali or indigenous Madura cattle and Singhala cattle from Sri Lanka. They provide both draft power and meat.

Bali cattle probably resulted from the domestication of Banteng (Bos sundaicus) over a long time, possibly beginning in prehistoric times. The udders and teats of Bali cattle are small and poorly developed, making them poor milkers. This breed is raised for draft power and beef.

Aceh cattle are found in the district of Aceh in North Sumatra. They are very small animals, as small or smaller than the Madura cattle, but they appear to be quite resistant to high temperatures and local parasites. These cattle must be used in pairs to provide draft power.

The Brahman cross from Australia, developed there for commercial purposes, was obtained by crossing a purebred Brahman bull (Bos indicus) with (usually) a crossbred female with equal Hereford and Shorthorn genes. This resulted in an animal that is one-half Brahman, one-fourth Shorthorn, and one-fourth Hereford. This cross exhibits hybrid vigor for growth and resistance to several parasites and diseases, and these animals, which are larger than the local breeds, have performed well in Indonesia. With good conditions, nutrition, and management, the Brahman crosses make excellent mothers because they are good milkers. Moreover, their ability to reproduce is excellent under certain conditions.

Crossbred Friesian cattle, which have from 75 percent to almost 100 percent Friesian genes, are now in demand (especially the young bulls) by the newly developing feedlot industry in some regions.

Finally, there appears to be an increased demand for higher quality beef by the 4,000 hotel and restaurant industries in Indonesia, as well as by the upper middle-class population in the larger cities.

Buffalo. The swamp buffalo is found in the swampy areas of Indonesia, particularly in the rice production areas where the land is heavy and difficult to plow. This animal provides draft power in these areas and appears to be more resistant to disease than cattle.

Dairy Cows. The dairy cow population is made up almost exclusively of purebred and grade Holstein-Friesians. Today, Indonesian farmers accept only Holstein-Friesians as milk cows; no other breeds are considered.

Breeding of dairy cows and beef cattle in Indonesia is accomplished by artificial insemination. Semen is provided by the government. Breeding services are provided either by the government or by the KUD (Koperasi Unit Desa) to which the farmer belongs. In the past a great deal of Holstein-Friesian semen was imported, but since 1972 the government of Indonesia has established bull studs for producing semen, initially at Lembang, West Java, and later at Singosari, East Java. Lembang has the Holstein-Friesian bulls, while Singosari produces frozen semen from the Bali, Ongole, and Brahman breeds. Most dairy cows are bred artificially using semen produced at the AI center in Lembang.

The small herd size of typical smallholders leaves little room for selection on the female side. All heifers must generally be raised as replacements. Interviews with farmers reveal that culling for low productivity is secondary to culling for loss of production from mastitis and reproductive disorders. Very rarely are female calves sold. Finally, a record system for milk production is not yet in place.

Artificial Insemination and Needs of Embryo Transfer

Artificial Insemination. The artificial insemination program was started in 1972 following the establishment of bull studs at Lembang. About 500,000 doses of frozen semen are currently being produced by the AI centers at Lembang and Singosari and distributed to more than 20 provinces throughout Indonesia. About 200,000 head of cattle and buffalo are inseminated each year with a conception rate of 40-70 percent.

Among the alternative strategies mentioned above, artificial insemination is the most important tool in efforts to improve the genetic potential of large ruminants. Using AI, a genetically superior bull can be used to impregnate more than 3,000 cows per year instead of only 70 using natural mating.

This year the Indonesian government intends to increase the quality of frozen semen produced by instituting a progeny testing program. This activity will be organized by the AI Center at Singosari with Japanese assistance. The use of imported Holstein-Friesian bulls or progeny-tested bulls for producing semen will have a positive effect on milk and beef production by F_1 and higher-grade daughters because the bulls belong to populations with average milk yields for dairy cows and rapid growth and high daily gain for beef cattle.

Embryo Transfer. Artificial insemination techniques use the genetic superiority of the male animal only. It may then be desirable to introduce a new technique--embryo transfer--that makes use of superior females as well. With the embryo transfer technique, a superior cow can produce more than 30 offspring per year instead of only one when mated naturally or through artificial insemination.

Embryo transfer may be an effective and efficient method of obtaining a group of elite cows to produce candidate bulls for

artificial insemination, but it still faces many problems: lack of knowledge and skills, especially for farmers and technicians, and its high price. Introduction of embryo transfer directly into the field is an expensive operation, considered a luxury by small farmers. Another problem is the difficulty faced in organizing implementation of embryo transfer in the field given the widely sporatic distribution of cattle and the small herd size (one to three cows) of farmers. Nevertheless, in the future this technique should be implemented along with the AI program.

In a pilot embryo transfer project established at Cicurug, Java, frozen embryos were imported in liquid nitrogen containers from the United States. Embryo transfer using the surgical method was carried out on three occasions seven days after estrus. Calves born weighing 50 kg are now growing into adult cows and bulls. At the age of about 24 months, the bulls now weigh more than 700 kg. These animals have the genetic potential of their parents and produce an average of more than 9,000 kg of milk per annum compared to only 3,000 kg per annum for local dairy cattle. Thus, they represent a genetic pool of superior dairy cattle that should be fully utilized for improving the quality of dairy cattle in indonesia. Maximum utilization of these animals can only be accomplished by using AI and embryo transfer techniques.

Another embryo transfer pilot project was carried out at Pulukan, Denpasar, Bali, in August and September 1985, using Bali cattle. The nonsurgical method was used to collect embryos from selected Bali cows and to transfer them directly to recipients of the same breed after evaluation of the embryos. Unfortunately, not all the data on this project are available. It is known, however, that the Bali cows produced 5-13 embryos at each collection, and that the pregnancy rate was about 50 percent.

The purpose of this pilot project was to provide in-service training for young researchers and field workers, as well as a means of transfering embryo transfer technology from the foreign experts to Indonesian technicians. It is hoped that the 16 workers trained in the embryo transfer process can later apply their knowledge.

PRESENT STATUS OF AND PROSPECTS FOR EMBRYO TRANSFER IN ANIMAL PRODUCTION IN INDONESIA

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INTRODUCTION

Lack of animal protein is the major problem faced by the human population of developing countries, including Indonesia. Animal production, the main source of the animal protein, has long been neglected or less appreciated in these countries. Thus, the production of cereals is steadily increasing, while the production of animal protein is lagging far behind.

The Basic Outline of the State Policy (GBHN) of the Republic of Indonesia states that overall national development should be directed toward improving the standard of living and well-being of all Indonesian people. It also states that this goal should be pursued by fully utilizing science and technology.

The fourth five-year national development plan (Repelita IV) emphasizes development of the agricultural and industrial sectors, leading to self-sufficiency in food supplies. In the middle of Repelita IV (late 1985), Indonesia was proclaimed self-sufficient in rice production by President Soeharto at a meeting of the Food and Agriculture Organization in Rome. Thus from a nutritional point of view, the current supply of carbohydrates as the main component of nutrition is more than enough to feed the Indonesian people. Protein, however, an essential component of nutrition, is still lacking.

As stated in Repelita IV, agricultural development includes the development of fisheries and livestock production to provide animal protein. The consumption of animal protein in Indonesia is still very low, averaging less than 3 g per person per day, or about half of the minimum daily requirement of 5 g. Growth of the population of Indonesia, which is currently more than 160 million and increasing at a rate of 2.34 percent per year, has far surpassed that of animal production, the main source of animal protein aside from aquatic resources. The cattle population remains fairly static at about 6.7 million head, while the population of water buffaloes decreases each year (Table 1).

The Directorate-General of Livestock Services, Department of Agriculture is making a major effort to cope with Indonesia's livestock problems by intervening in the livestock population, supporting farm cooperatives, and facilitating the market flow.

TABLE 1 Population (Thousands) of Farm Animals (Except Horses and Poultry) in Indonesia, 1969-1984

Year	Beef Cattle	Dairy Cattle	Buffalo	Goat	Sheep	Swine
1969	6,447	52	2,976	7,544	2,998	2,878
1970	6,130	59	2,876	6,336	3,362	3,169
1971	6,245	66	2,976	6,943	3,146	3,382
1972	6,286	68	2,822	7,189	2,996	3,350
1973	6,637	78	2,489	6,793	3,457	2,768
1974	6,380	86	2,415	6,517	3,403	2,906
1975	6,242	90	2,432	6,315	3,374	2,707
1976	6,237	87	2,284	6,906	3,603	2,947
1977	6,217	91	2,292	7,292	3,804	2,979
1978	6,330	93	2,312	8,051	3,611	2,902
1979	6,362	94	2,432	7,659	4,071	3,183
1980	6,440	103	2,457	7,691	4,124	3,155
1981	6,516	113	2,488	7,790	4,177	3,364
1982	6,594	140	2,513	7,231	4,231	3,587
1983	6,660	162	2,538	8,049	4,316	3,677
1984	6,751	169	2,533	8,098	4,343	4,079

SOURCE: Directorate-General of Livestock Services, Ministry of Agriculture, 1985.

Intervention in the farm animal population includes manipulation of quantity and quality, distribution, supporting environmental conditions, and product orientation of the animals. To increase the quantity and quality of farm animals, the government has imported cattle and water buffaloes, reinforced the law to prohibit the slaughter of productive female animals, focused on prevention of infectious diseases, and applied the AI technique to reproduce large farm animals. Animals imported from New Zealand, Australia, and recently from the United States, as well as those from densely populated regions in Indonesia, are being distributed to other less populated regions of the county. Special attention is being given to the geographic, geopopulation, and geoculture of these regions. Efforts are also being made to improve supporting environmental conditions, particularly management and feeding. Preferences for particular products of farm animals are being considered as well, with an emphasis on increases in their quantity and quality.

In the case of large farm animals, sophisticated reproductive techniques--artificial insemination (AI) and embryo transfer (ET)--are required. Artifical insemination using frozen semen has been applied to cattle and buffaloes in Indonesia since 1972 and 1981, respectively.

Indonesia's two AI centers, located at Lembang, West Java, and Singosari near Malang, East Java, are concerned mainly with the production and distribution of frozen semen stored in liquid nitrogen

containers. This semen is distributed to more than 20 provinces throughout Indonesia, each of which has its own network of AIactivities under the Office of Livestock Services. About 300,000 head of cattle and buffalo are inseminated each year with a conception rate of 40-70 percent. Infertility problems with farm animals and the inexperience and lack of attention of farmers are still hampering the progress of the AI program in Indonesia.

INTRODUCTION OF EMBRYO TRANSFER IN DAIRY CATTLE IN INDONESIA

Under the initiative of the minister of cooperatives, a pilot project was established at the PT Berdikari United Livestock feedlot and dairy farm at Cicurug, West Java, to introduce the use of embryo transfer in dairy cattle in Indonesia. This pilot project was carried out with the cooperation of Indonesia's Department of Cooperatives and the Granada International Corporation (Texas).

In this project, frozen Holstein-Friesian embryos, collected from registered, genetically superior cows at the Granada International Corporation farm in Texas, were imported in liquid nitrogen containers.

Embryo transfer was carried out on three occasions. In March 1984, 207 cows were selected and brought into the PT Berdikari United Livestock farm at Cicurug for treatment to meet the necessary requirements for embryo transfer (Gunn and Old, 1981; Anonymous, 1984). The 207 cows were then divided into four groups of about 50 animals each, and the estrus of each was synchronized in such a way that within five days embryo transfer could be carried out in not more than 40 cows per day.

Estrus was synchronized using intramuscular injections of 25 mg of Lutalyse (prostaglandin F_{2alpha}) per animal and repeated 11 days later. Only 113 head (54.6 percent) of these cows were in heat, however, after the second injection. According to G. D. Mahon (personal communication, 1981), after administration of prostaglandin estrus should appear in 80-85 percent of the treated cows. The low response to prostaglandin can be caused by many factors, including management and nutritional status. Only 77 head (68 percent) were, in the end, used as recipients.

Before transfer, the glass ampules containing the frozen embryos were taken out of the liquid nitrogen container and thawed in tap water at 30°C. The embryos were then put into phosphate buffer saline (PBS) solution and downgraded concentrations of glycerol of from 10, 8.7, 5, 3.3, to 1.7 percent for as long as 10 minutes each. At the end of the process the embryos were put into pure PBS solution for 20-30 minutes. Meanwhile, the embryos were evaluated and divided into four categories (A, B, C, and D), according to their quality. Before transfer, the recipient animals were rectally palpated to determine on which side the corpus luteum was located.

The embryos were transferred seven days after estrus using the surgical method. After local anesthesia with lidocaine 2 percent was

administered, an incision of 20 cm was made at the caudodorsal end of the flank on the same side as the corpus luteum. The uterine horn was gently pulled out, and an embryo was deposited near the end. The uterine horn was then pushed back into its previous position, and the wound was closed with several stitches.

Rectal palpation carried out two months later revealed that 26 (35.14 percent) of the 77 recipients were pregnant. This result was higher than that obtained in South Korea (20 percent) by the same workers (G. D. Mahon, personal communication, 1984). This figure was lower, however, than that mentioned by Jillela (1982)--40-70 percent.

Most (92.3 percent) of the pregnant animals had received A-quality embryos, while the rest of the pregnant animals (7.7 percent) received B-quality embryos. Those receiving C-quality embryos were not pregnant. Most (84.6 percent) of the pregnant animals had a first-grade corpus luteum, and the rest (15.4 percent) had second-grade. Those having a third-grade corpus luteum were not pregnant. In summary, most (76.9 percent) of the pregnant recipients had a first-grade corpus luteum and received an A-quality embryo.

The results of this embryo transfer project were thought to be good, since it was the first time ET had been introduced and applied in Indonesia with the existing constraints.

The second embryo transfer activity took place in July 1984 at Cicurug, West Java, At that time, 228 healthy and nonpregnant adult cows were selected and synchronized. Of these, 175 cows went into heat, and 145 were used as recipients. The embryos were then transferred using the surgical method and 62 (42.76 percent) cows became pregnant.

The birth weights of the calves ranged from 30 to 50 kg. At the age of about 24 months, the bulls have reached more than 700 kg body weight. In January 1987, semen samples from 17 bulls, aged less than 24 months, were collected. They showed an average volume of 5 ml per ejaculate, a sperm concentration of about 1,000 million cells per milliliter, and an average motility of 75 percent. All the female animals have been mated and are currently pregnant. More than 80 Holstein-Friesian cows and bulls of superior quality are now available at Cicurug.

In another project, a small number of frozen Brahman embryos were brought together with frozen Friesian embryos and transplanted surgically into beef cattle of the graded Ongole and Brahman crossbreeds at the Bila River Ranch in South Sulawesi. The pregnancy rate was over that of the dairy cattle.

In July 1986, the Directorate-General of Livestock Services imported from England 200 frozen Holstein-Friesian embryos which were stored in ampules in a liquid nitrogen container. About half of the embryos were used in an embryo transfer activity in East and West Java.

Selected Holstein-Friesian recipients underwent estrous synchronization by means of intramuscular injections of the prostaglandin F_{2alpha} 11 days apart. Embryo transfer was carried out 7-8 days into the estrous cycle after the last prostaglandin injection.

The frozen embryos were thawed by immersing the ampules in a water bath at 35°C. The embryos were then removed and placed into a series of small petri dishes containing PBS and downgraded concentrations ofglycerol from 10 to 0 percent. Finally, each embryo was placed in a transparent plastic ministraw and transferred to the recipient using the nonsurgical method.

No data are available on the results of this activity. The conception rate was low, however.

RESEARCH ON EMBRYO TRANSFER IN LARGE FARM ANIMALS

Water Buffalo

In November 1985, an experiment using embryo transfer was carried out in Padang Lawas, Southern Tapanuli, North Sumatra. It was aimed at upgrading the local swamp water buffalo toward dairy buffalo.

Twelve female swamp buffaloes were selected as donors. Estrus was synchronized by an intramuscular injection of 20 mg Enzaprost (PGF_{2alpha}), and superovulation was induced by an intramuscular injection of 2,500 IU PMSG (Pregnant Mare Serum Gonadotropin). This drug has been used by other investigators (Betteridge, 1977; Jillela, 1982; Parnpai et al., 1985). These animals were then inseminated with frozen semen of the Murrah dairy buffalo breed.

Embryoes were collected seven days after insemination using the nonsurgical method. A microscopic examination showed that no embryos were produced in this trial. Instead, many luteal and follicular cysts were formed in the ovaries.

Apparently, the swamp buffalo does not respond to this treatment. Further intensive research on the kind, dosage, and combination of hormones used in established embryo transfer in this particular animal should be carried out.

Based on the experience described above, a study of embryo transfer in the swamp buffalo is being undertaken by Tuty L. Yusuf, a postgraduate (S-3) student and staff member of the Department of Reproduction and Obstetrics, Faculty of Veterinary Medicine, Bogor Agricultural University. This trial is intended to compare the effect of pure Follicle Stimulating Hormone (FSH) and Pregnant Mare Serum Gonadotropin on superovulation and embryo production in the swamp buffalo. According to Heath (1981) and Brand and Hoogenkamp (1982), a dose of 30-50 mg pure FSH injected in the fragment twice a day for five consecutive days could induce superovulation.

Two groups of three buffaloes each are undergoing treatment as donors with injections of 30 mg FSH and 2,500 IU PMSG, respectively. Each animal in both groups is also injected intravenously with Human Chorionic Gonadotropin (HCG) to enhance ovulation. All animals are artificially inseminated three times at estrus. Embryos are then retrieved using the nonsurgical method.

The results obtained thus far indicate that animals injected with pure FSH produce more corpora lutea, as a physiological proof of superovulation, than those injected with PMSG. So far no embryos have been obtained from these animals.

Dairy Cattle

After succeeding with embryo transfer using frozen embryos, the dairy farm of PT Berdikari United Livestock in Cicurug, now called PT Kariyana Gita Utama, will attempt to produce its own embryos from the ET progenies. Before ET is applied in these superior animals, however, this technique is being tested in the local inferior dairy animals. Sabdi Hasan Aliambar, an S-2 graduate student and staff member of the Department of Veterinary Clinics, Faculty of Veterinary Medicine, Bogor Agricultural University, is currently undertaking research in this area at the dairy farm in Cicurug.

Two groups of four donors each are being treated alternatively with pure FSH and pure FSH combined with HCG, respectively, for superovulation. Embryos are then collected using the nonsurgical method.

The preliminary results show that the combination of FSH with HCG produced more corpora lutea (about 10 per donor) than FSH alone (about five per donor).

On January 23, 1987, eight embryos were obtained successfully from one donor that was treated with FSH and HCG. The first embryos of large farm animals ever produced in Indonesia, they were transplanted into five recipients. No estrus has been detected over two weeks after transfer or three weeks after estrus, which may indicate pregnancy in at least some of the recipients.

CONCLUSIONS

The following conclusions were reached after field and laboratory studies of embryo transfer in cattle and swamp buffaloes were carried out in Indonesia:

- The embryo transfer technique could be applied in improving the quality of cattle in Indonesia, but intensive studies should be carried out before this technique is applied to water buffaloes.
- The nonsurgical method seems to be more practical than the surgical method, and the conception rate obtained from the nonsurgical method does not differ from that obtained using the surgical method.
- The donor as well as the recipient animals should be in optimum reproductive condition before they are subjected to ET activities.
- Prostaglandin in the form of Lutelyze and Enzaprost can be used effectively to synchronize estrus in cattle as well as in buffaloes.
- The transfer of embryos in dairy cows at day seven would bring maximum results.
- The quality of the embryos and of the corpora lutea determine the success rate of the embryo transfer technique.

- An intramuscular injection of 2,500 IU PMSG does not successfully induce superovulation in the swamp buffalo.
- An injection of pure FSH in combination with HCG produces more corpora lutea in cattle and swamp buffaloes than an injection of PMSG.
- Successful application of the nonsurgical method and conduct of the laboratory work related to embyro transfer require well-trained and highly skilled technicians and veterinarians.

RECOMMENDATIONS

Based on past experience with embryo transfer in the field and laboratory using the surgical as well as the nonsurgical method in large farm animals in Indonesia, the following steps are recommended:

- The national policy, strategy, and program for research on and the application of embryo transfer to farm animals should seek to improve livestock production in Indonesia.
- The priority of field applications of embryo transfer should be the improvement of dairy cattle production, in line with the national livestock development policy.
- Given the lack of selected genetically superior female animals to be used as donors (other than the available ET progenies at Cicurug), more embryos should be imported to establish a national stock of genetically superior Holstein-Friesian cattle.
- Intensive studies should be carried out on the domestic dairy and beef cows to be used as recipients under the standard compared to the traditional methods of feeding and management.
- Skilled technicians and experienced staff should be trained to undertake the embryo transfer technique.
- Easy access to the hormones needed for estrous synchronization and superovulation, the materials for suspension and preservation of embryos, and the equipment needed for embryo collection, freezing, and transportation (at reasonable prices) should be approved.
- Further research should be carried out on the development and application of embryo transfer techniques to beef cattle and swamp buffaloes.

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PRESENT STATUS OF AND PROSPECTS FOR EMBRYO TRANSFER IN THE UNITED STATES

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HISTORICAL PERSPECTIVE

Walter Heape performed the first embryo transfers at the end of the last century, and had a profound influence on other studies of reproduction as well, including uterine environment, sperm-egg interaction, and anatomy of the reproductive tract. Heape's first transfer in 1890 stimulated an interest in embryo transfer in both Europe and the United States; for a review, see Betteridge (1981).

Gregory Pincus and his colleague M. C. Chang are probably best known for their work in developing the oral contraceptive while at the Worcester Foundation in Shrewsbury, Massachusetts. Before that, however, Pincus had successfully transferred rabbit embryos to recipient does in 1929 while serving as a visiting National Research Council Fellow from Harvard University.

This work by Pincus stimulated interest in how this new science might be applied to the genetic improvement of domestic livestock. For example, the Foundation of Applied Research in San Antonio, Texas, founded in the 1910s, collected over 750 donor cattle during an eight-year period. The results were disappointing, however. The effort achieved only four pregnancies, all of which aborted before eight months. Nevertheless, what was learned about embryo collection and transfer, superovulation, and embryo handling played a role in a cooperative effort undertaken by the U.S. Department of Agriculture and the University of Wisconsin in 1951 to achieve the first live birth in cattle from embryo transfer (see Table 1).

Although synchronization and superovulation were relatively routine procedures in the 1950s, the success rates of embryo transfer remained low in domestic animals. Much interest was centered around the embryo collection and transfer procedures patterned after earlier work in laboratory animals. Superior results were reported in the 1960s by L. E. A. Rowson who used surgical methods in sheep, goats, and cattle, with exploitation of this technology for the commercial embryo transfer marketplace. The complications of adhesions, surgical trauma, and the high cost of embryo transfer limited its application, however, to donors of superior genetic or monetary value. In the 1970s, laboratories in Japan, the United States, and Europe developed the

TABLE 1 First Records of Successful Embryo Transfers

Date	Species
1891	Rabbit
1933	Rat
1934	Sheep
1934	Goat[a]
1942	Mouse
1949	Cow[b]
1949	Goat
1951	Cow[c]
1951	Pig
1964	Cow (cervical)
1968	Ferret
1974	Horse
1976	Baboon
1978	Man[c]
1978	Cat
1979	Dog
1981	Gaur[d]
1984	Marmoset
1984	Zebra[d]
1984	Przewalskis horse[d]

[a]Reinsertion.
[b]Aborted.
[c]In vitro fertilization and reinsertion.
[d]Interspecies recipient.

nonsurgical embryo collection techniques for cattle used today. This technological advance allowed the collection and transfer of embryos outside the surgical arena, and a new "on-the-farm" embryo transfer industry evolved (Figure 1).

The embryo transfer industry is now fully established in over 14 countries. As of 1979, an estimated 13,000 cattle embryos had been transferred in the United States and over 4,000 in Canada (Seidel and Seidel, 1981). Although the collection and transfer of embryos is now routine, the related technologies of embryo preservation and micromanipulation for the production of identical twins, determination of sex, and control of genetic makeup of the embryo will result in reduced costs and wider application of embryo transfer technology.

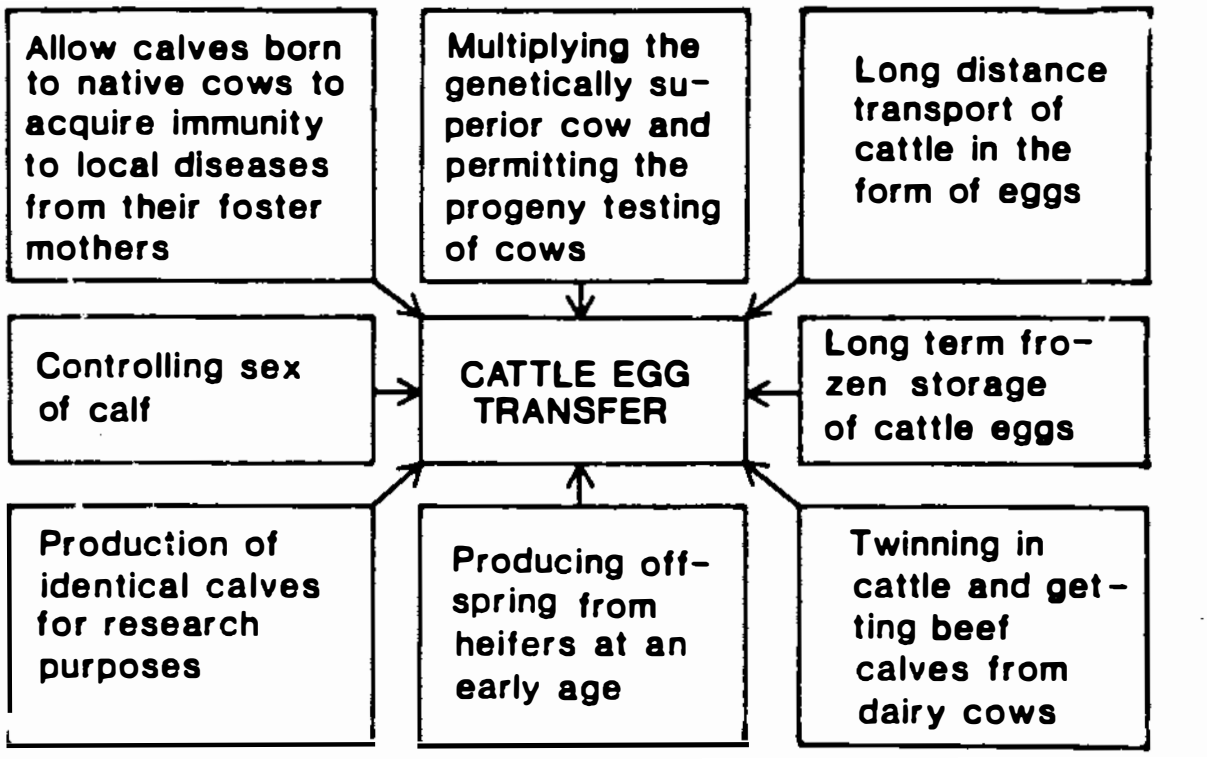

FIGURE 1 Potential uses and application of embryo transfer in cattle.

TECHNOLOGY OF EMBRYO TRANSFER

Donor and Recipient Selection

Selection of an animal to be an embryo transfer donor is based on many criteria. In cattle the decision may be based on economics rather than true genetic superiority. The following selection criteria will insure genetic superiority as well as a good probability of producing embryos of high quality:

- Whenever possible, a donor with a previous success record in embryo transfer should be selected. Recent information suggests that certainly in sheep and perhaps in cattle successful superovulation is related to the total number of oocytes in the ovary before treatment. The number of oocytes present in the ovary depends on the breed used.
- Donors should be between the ages of 1 and 4 years in sheep and 3 and 10 years in cattle.
- Donors should be free of genetic and other disease and conformational abnormalities. This is particularly critical for meeting the health requirements for the exportation of embryos.

- o Donors should exhibit regular estrous cycles and have had at least two regular cycles following a seasonal or lactational anestrus.
- o Donors should include animals with superior production traits of economic importance and above-average production of offspring from previous matings of the same dam and sire.
- o Donors should have a sound reproductive record, including no more than two inseminations per conception or three calves born within two calendar years.
- o Donors should be part of a routine herd health program that includes genetic consultation and evaluation of the production performance of offspring. Practitioners should educate clients about the expectations for success and the difficulty of obtaining offspring from problem donors.

Recipient management is critical to the success of an embryo transfer program, but it is commonly overlooked. Generally, recipients should be selected and culled according to the same criteria used by superior commercial or purebred cattle operations. Purchase of recipients from the sale barn and use of infertile recipients or animals previously treated with growth stimulants should be avoided at all costs. Recipients that are too thin or too fat should also be excluded from an embryo transfer program. Cows, when well managed, can serve as well as heifers as recipients because of the lower occurrence of perinatal mortality and good conception rates associated with them.

New recipients should have been vaccinated as calves. Upon arrival, they should be isolated and tested for tuberculosis and brucellosis, treated for internal and ectoparasites, given preventive virus and leptospirosis vaccinations, and identified with both metal and plastic ear tags. Recipients can be grazed on pasture, but they should receive about 20 lb of high-energy grain ration daily while lactating. Recipients in a growing phase of nutrition and with a dystocia-free history can be used as early as 60-90 days after calving.

Cattle

Estrous Synchronization. A high degree of estrous synchronization between donor and recipient is essential for high conception rates. An equally important factor for high conception rates is embryo quality. A high-quality embryo can adjust better to a greater degree of asynchrony than an embryo of poor quality.

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or its analogues are the most common luteolytic agent used to synchronize estrus in cattle (see Table 2). While there are differences in the structure and biological activities of prostaglandin products, there is no evidence to suggest that one particular product is superior to another.

Estrus occurs approximately 48 hours following administration of a luteolytic dose of $PGF_{2\alpha}$. Ideally, recipients should be in estrus 12-18 hours before or on the same day as the donor. Poor pregnancy

TABLE 2 Prostaglandin and Prostaglandin Analogues

Agent	Trade Name	Manufacturer	Dose[a]
Dinoprost	Lutalyse	Upjohn	25 mg
Cloprostenol[b]	Estrumate	ICI Pharma	500 g
Alfaprostol[b]	Alfave	Hoffman-LaRoche	5 mg
Fenprostalene[b]	Bovilene	Syntex	1 mg

[a]Dose recommended by the manufacturer.
[b]Analogue.

rates may result when recipients exhibit estrus 60 hours or longer following the $PGF_{2\alpha}$ injection.

Superovulation. Donor cows will respond more predictably when the superovulatory hormone is given 8-14 days following estrus. The drugs Pregnant Mare Serum Gonadotrophin (PMSG) and Follicle Stimulating Hormone (FSH) are most commonly used to induce superovulation. When compared to PMSG, however, FSH has been shown to result in the recovery of more embryos which are of superior quality. PMSG may be used for a donor suspected of being refractory to FSH because of repeated stimulation or for other problem donors.

FSH is generally administered twice daily, although several studies have shown no decrease in response with once-a-day injections. Certain donors may require the addition of luteinizing hormone (LH) to FSH at a 1:5 ratio. It is not routinely added, however, as most commercial preparations of FSH contain some LH. Several superovulation treatment schedules are shown in Table 3. Schedule A is used for a donor that does not respond well to FSH treatment. Schedule B is most often used for superovulation of heifers or donors not previously superovulated where hyperstimulation could exist. Donors previously stimulated with FSH and obese or older cows may respond better to Schedule C.

Practitioners should recognize that superovulation is a highly variable event, and that hyperstimulation is always possible (Figure 2). Strict attention to detail is necessary for a successful embryo transfer program, including donor and recipient selection, health and nutrition, timing of injection, and estrous detection.

Breeding. Little difference in conception rates are observed between fresh semen, natural breeding, and artificial insemination when timed correctly with ovulation. Donors may ovulate over 24 hours, with the interval from estrus to the first ovulation remaining unchanged. Multiple breedings at 12-hour intervals are generally used (Table 3), with some breeders using two units of frozen semen at each breeding.

TABLE 3 Superovulation Treatment Schedules for Cattle

Day[a]	Time	Schedule A	Schedule B	Schedule C
10	AM	2,500-5,000 IU PMSG	5 mg FSH	5 mg FSH
	PM		5 mg FSH	5 mg FSH
11	AM		4 mg FSH	5 mg FSH
	PM	Recipient, $PGF_{2\alpha}$	4 mg FSH[b]	5 mg FSH[b]
12	AM	Donor, $PGF_{2\alpha}$	3 mg FSH[c]	5 mg FSH[c]
	PM		3 mg FSH	5 mg FSH
13	AM		2 mg FSH	5 mg FSH
	PM		2 mg FSH	5 mg FSH
14	AM			
	PM	Breed	Breed	Breed
15	AM	Breed	Breed	Breed
	PM	Breed	Breed	Breed

[a]Day _ of onset of estrus.
[b]Recipient, $PGF_{2\alpha}$
[c]Donor, $PGF_{2\alpha}$

FIGURE 2 Large cystic follicles formed following superovulation with PMSG in sheep.

Semen should be routinely examined before insemination, and particular care should be given to cleanliness and good insemination techniques. Bull fertility has been shown to be highly correlated with the number of fertilized embryos recovered, and it may affect the viability of embryos transferred.

Sheep and Goats

Estrous Synchronization. Recipients can be synchronized with $PGF_{2\alpha}$, progesterone, or intravaginal progestins (Table 4). $PGF_{2\alpha}$ given during the luteal phase of the cycle will produce estrus within one to three days. A double injection of $PGF_{2\alpha}$ given 8-10 days apart in sheep and 12-14 days apart in goats is useful when the date of the last estrus is unknown. Progestin pessaries can be used for estrous synchronization in cycling recipients or for the induction of estrus in anestrous animals (Table 4). Adminstration of PMSG (350-500 IU) or 1,000 units of LH (IV) at the time of pessary removal can be used to aid ovulation for animals treated during anestrus.

Superovulation. The poor ovulation rate and increased number of unovulated follicles associated with the use of PMSG in sheep and goats has made FSH the drug of choice. FSH treatment is initiated two to three days before $PGF_{2\alpha}$ pessary removal (Table 5). Teaser males are useful in the detection of estrus at 12-hour intervals starting 12 hours after $PGF_{2\alpha}$ injection or pessary removal.

Pessary removal or $PGF_{2\alpha}$ injection for recipients should occur 24 hours before that for superovulated donors to insure estrous synchronization. Like cattle, the superovulation response in sheep and goats is highly variable, depending on the age and nutritional status of the donor and time of breeding season. Breeds of high fecundity (Finnish Landrace) generally respond better than breeds of low fecundity (Suffock).

TABLE 4 Synchronization Treatment in Sheep and Goats

Agent	Dose	Comments
Progesterone	10-15 mg (IM)	Duration 12-14 days, sheep; 14-18 days, goat
Progestin (Cronolone)	30-45 mg (pessary)	Duration 12-14 days, sheep; 14-18 days, goat
$PGF_{2\alpha}$	8-15 mg (IM)	Luteal phase of cycle
Cloprostenol	150-250g (IM)	Luteal phase of cycle

TABLE 5 Superovulation Schedule

Sheep			Goat		
Day[a]	Time	Treatment	Day[a]	Time	Treatment
10	AM	5 mg	14	AM	5 mg
	PM	5 mg		PM	5 mg
11	AM	4 mg	15	AM	4 mg
	PM	4 mg		PM	4 mg
12	AM	3 mg + $PGF_{2\alpha}$	16	AM	3 mg
	PM	3 mg		PM	3 mg
13	AM	2 mg[b]	17	AM	2 mg + $PGF_{2\alpha}$
	PM	2 mg		PM	2 mg
			18	AM	2 mg[b]
				PM	2 mg

[a]Day _ of onset of estrus.
[b]Pessary removal.

Breeding. Superovulated ewes consistently show a lower fertilization rate compared to does, possibly as a result of poor sperm transport through the cervix, endogenous progesterone, or the residual affect of the progestin pessary. Ewes and does should be inseminated or hand-mated at 12-hour intervals until the cessation of estrus. Fertilization rates are generally higher with natural mating compared to artificial insemination for both ewes and does.

Swine

Embryo transfer is performed in swine to prevent and control disease, not to produce genetically superior offspring. A survey conducted by P. A. Martin in 1981 showed that 43 percent of commercial embryo transfers were performed to establish new herds from herds with pseudorabies, 26 percent to make additions to specific pathogen-free herds, 23 percent to obtain boars for closed commercial herds, and only 7 percent to obtain offspring from genetically superior donors.

Estrous Synchronization. Synchronization can be achieved by weaning a group of sows for which estrus will occur in 4-10 days. A high proportion of sows will be in estrus within 4-5 days when 500-800 IU of PMSG are given at the time of weaning. An alternative method is to breed sows and abort them between the sixteenth and forty-fifth days of pregnancy with two injections of $PGF_{2\alpha}$ 12 hours apart. A tighter synchrony can be achieved with an injection of 500-800 IU of PMSG at the time of the second $PGF_{2\alpha}$ injection.

Superovulation. In females from which embryos are collected for more than two consecutive estrous cycles, the time of estrus is usually not controlled and superovulation is not used. Sows can be superovulated with a single injection of 1,200-1,500 IU of PMSG at weaning or at the first $PGF_{2\alpha}$ injection administered between the sixteenth and forty-fifth days of pregnancy. The superovulation response ranges from 0 to 45 ovulations and is quite variable among individual females and breeds.

Breeding. Optimum conception is achieved with natural mating or artificial insemination every 12 hours from the onset of estrus. Approximately 75 ml of semen should be used for artificial insemination, and it should contain at least 5 billion live spermatozoa.

EMBRYO COLLECTION AND TRANSFER

Before 1975, most embryos were collected using surgical methods. Nonsurgical approaches are now commonly used for embryo collections in cattle and horses. Surgical procedures are still being used in the other farm species, primarily because of the difficulty encountered in passing a catheter through the cervix. Nonsurgical techniques avoid potential damage to the reproductive tract by adhesions, they are repeatable, they do not require elaborate facilities, and they can be performed on the farm. Nonsurgical techniques can only be applied, however, when the embryos have reached the uterus and in animals where the cervix can be penetrated with a catheter.

Cattle

Collection. One- to eight-cell embryos (one to five days following estrus) can be collected by flushing media either from the fimbriae toward the uterus or from the uterotubal junction toward the fimbriae following insertion of a glass or Teflon cannula into the infundibulum. This surgical approach has limited commercial application as it requires general anesthesia and surgical invasion of the abdominal cavity.

Embryos are routinely collected by nonsurgical methods six to eight days after the onset of estrus. Prior to day five the embryos may be in the oviduct and after day eight may be hatched (escaped from the zona pellucida), difficult to visualize, and fragile.

The donor is placed in a restraining chute, and the rectum is cleared of feces. The front end of the donor should be elevated a minimum of 14 inches to create a positive pressure in the abdomen. The perineal region and vulvar lips are thoroughly washed with water with particular attention given to removing all soap from the region. The

tail is tied out of the way and an epidural anesthetic is administered. The practitioner must not introduce air into the rectum; this problem is frequently encountered in older dairy cows. The ovaries can be palpated to approximate the number of corpora lutea and large, unovulated follicles.

Two types of catheters are commonly used for nonsurgical collection in cattle. The Foley catheter is inexpensive, is readily available, and can be reused following gas sterilization (ethylene oxide). The length of the Foley catheter may be limiting, however, in collecting embryos from large dairy cows, and the rubber is very flexible which can cause difficulty in threading the uterus. In addition, a stilette is not provided with this catheter and must be manufactured.

The Rausch catheter is 67 cm long with an 18-gauge outside diameter. A stilette with a Luer-Lik fitting is also supplied. This catheter is somewhat stiffer than the Foley catheter which makes threading the uterus simpler. The catheter tip to the cuff is 5.5 cm long (longer than the Foley) and has four holes to inject and retrieve fluid. The Rausch catheter is expensive and not generally available from medical supply houses, but it can be reused following gas sterilization.

Two systems are commonly used for nonsurgical collection in cattle: the continuous-flow, closed-circuit system and the interrupted-syringe method. Practitioners may use a combination of these two systems, and both are equally effective when done properly. The closed system offers the advantage of sterility and fluid control, but it requires additional tubing and connections. The syringe method is faster and allows embryo searching before the flush is complete (Figure 3).

The sterile catheter with the stilette locked into position is coated with a sterile lubricant (it is advisable to use a plastic sheath over the catheter to prevent contamination of the uterus). Extreme care should be taken to remove all fecal contamination from the lips of the vulva which are parted wide for catheter insertion. The catheter is passed through the cervix aided by manipulation through the rectal wall and into the right uterine horn while withdrawing the stilette slowly. The catheter is positioned so that the cuff is about halfway between the uterine body and the top of the uterine horn. The cuff is then slowly inflated with saline solution or air until it completely fills the uterine lumen. Care should be taken to avoid overinflation of the cuff because this can result in endometrial damage and loss of flushing medium into the broad ligament. The stilette is then removed, and a clamp is placed on the exteriorized end of the catheter.

A conservative approach to filling the horn with fluid should be used to avoid overdistention and possible rupture of the endometrium. Generally, 25-35 ml of fluid (about the size of a 35-day pregnancy) is sufficient to distend the horn, and this amount is repeated about eight times. Eighty-five percent of the embryos collected will be found in the first 100-150 ml of fluid. The uterus is gently agitated and lifted to insure that the fluid reaches all of the endometrium.

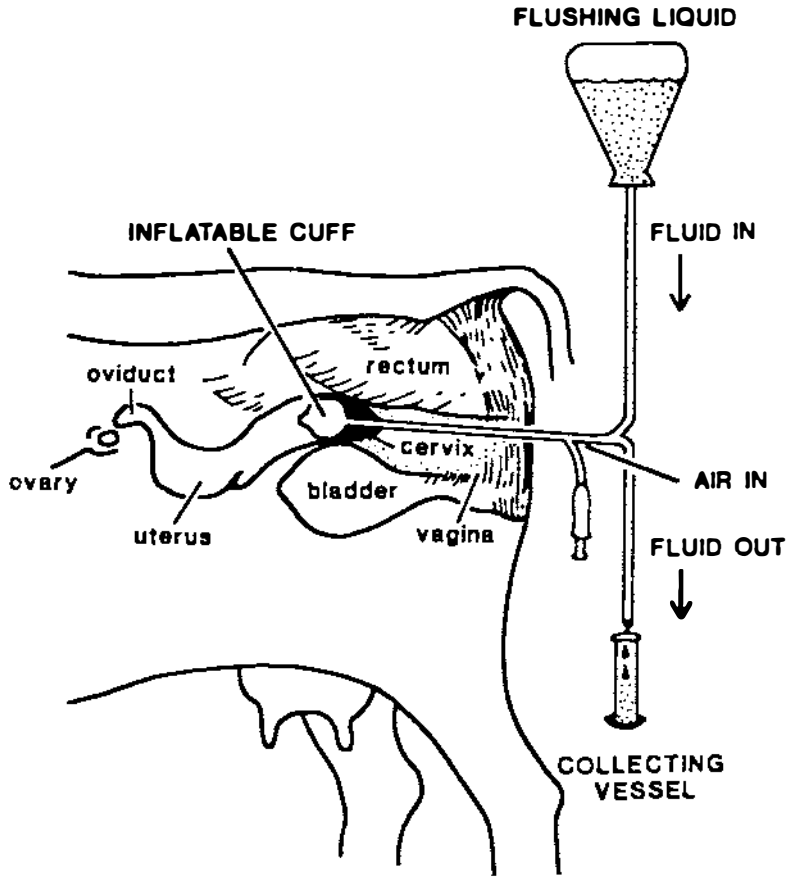

FIGURE 3 Continuous-flow method for the nonsurgical collection of embryos in cattle.

When the collection of the right uterine horn is completed, the stilette may be reinserted or the catheter removed completely from the reproductive tract before reinserting the stilette. The latter procedure is advisable for inexperienced practitioners to insure proper locking of the stilette in the catheter. The flushing procedure is repeated for the left uterine horn.

Following completion of the flush each uterine horn is infused with 30-50 ml of an antibiotic solution using a uterine infusion catheter. $PGF_{2\alpha}$ can be given at this time or within a week of the flush, but the return to estrus can vary from a few days to several weeks.

Transfer. Most practitioners utilize a flank approach to transfer embryos surgically. The recipient is palpated to determine the side on which the corpus luteum is located, which will be the same side as the flank incision. An epidural and local (L-block) anesthesia (Lidocaine) is administered, and the surgical site is shaved and cleaned. A 15-20 cm vertical incision is made in the paralumbar fossa, and the muscle layers and peritoneum are bluntly dissected. Existence of the

corpus luteum is verified, and the top of the uterine horn is gently grasped and exteriorized. A small puncture is then made into the uterine lumen with the blunt end of a cutting suture needle. The embryo is loaded into a transfer pipette in less than 0.5 ml of fluid which is positioned between two air spaces. The loaded pipette is then passed through the puncture into the uterine lumen and the embryo deposited.

Practitioners use a variety of instruments to transfer embryos nonsurgically, including telescoping rods and transvaginal surgical techniques, but the most commonly used is the Cassou AI gun with a French straw. The recipient is restrained and palpated to determine the presence and location of the corpus luteum. The vulvar area is cleaned and wiped dry with careful attention paid to removing all fecal contamination. A double sheath is used to reduce contamination from the vagina, and the embryo Cassou gun is threaded through the cervix to a position cranial to the external uterine bifurcation. The site of embryo deposition is secondary to an atraumatic placement for good results. The embryo should be deposited in the uterine horn ipsilateral to the corpus luteum using slow but even pressure from the Cassou gun.

Sheep and Goats

Embryos are collected and transferred surgically from sheep and goats because of the difficulty in passing a catheter into the uterus through the cervix. One- to eight-cell embryos can be collected from the oviduct with later stages flushed from the uterus. Surgical collection is performed under general or local anesthesia with the animal in dorsal recumbency. A 3-5 cm midline incision is made as close to the udder as possible using blunt dissection to reach the peritoneum. The uterus and ovaries are exteriorized onto a sterile field and the corpora lutea counted.

The oviduct can be cannulated with a glass or Teflon tube (2 mm outside diameter) held in place by plastic clips, and a blunt needle is inserted into the uterine lumen a few centimeters from the tip of the horn. The caudal segment is pinched off with thumb and forefinger or intestinal forceps, and flushing media is gently forced in a retrograde manner through the uterus and oviduct. Caution must be taken to limit the pressure as it can rupture the endometrium and force fluid into the broad ligament. The practitioner must also manipulate the oviduct to remove twists that can occlude fluid flow. This procedure is generally used to collect embryos composed of from one to eight cells. The technique has the disadvantage of manipulating the delicate fimbriae of the oviduct which often leads to surgical adhesions.

An alternative method that avoids manipulation of the delicate fimbriae is to place a small pediatric Foley catheter in the uterine lumen at the uterine bifurcation and to expand the cuff gently to secure its position. A blunt needle with syringe is then inserted at the top of the uterine horn, and fluid is forced slowly into the uterus

with the practitioner gently massaging the uterus to insure fluid expansion of the endometrial folds. A simple suture is used to close the site of catheter insertion, and the uterus is cleansed with a high-molecular weight dextran solution to minimize surgical adhesions. The abdomen is then closed routinely, and the animal is given an injection of antibiotics.

Swine

Collection. Embryos are collected and transferred surgically in swine. Anesthesia is induced by an injection of barbiturate into the marginal ear vein and maintained with halothane gas. The midventral area is shaved and washed, and a 1-3 cm incision is made. The adjacent ovary, oviduct, and 30 cm of uterus is exteriorized onto a sterile field. A small incision is made for cannula insertion in the antimesometrial side of the uterus, about 20 cm from the uterotubal junction and away from vascular regions. The cannula, usually made of glass with a length of 12 cm and a diameter of 10 mm, is inserted a few centimeters into the horn and held in place by a clamp. A blunt needle is then inserted into the oviduct, and 49-60 ml of fluid is forced through the oviduct, into the uterus, and out the cannula. The uterus is gently milked with the thumb and forefinger to remove all the fluid. The cannula is then removed, and the uterus is washed with a dextran solution to minimize surgical adhesions. Finally, the abdomen is closed, and the entire procedure is repeated for the second uterine horn.

Transfer. Optimal results are achieved when recipients are in estrus one or two days after the donor and receive at least 12 good-quality embryos. Transfers are performed surgically following injection of a barbiturate anesthesia into the marginal ear vein. A midventral incision is then made, and the ovaries are evaluated for corpora lutea and the general condition of the reproductive tract. Embryos can be transferred by depositing them into the lumen of the uterus some 5 cm away from the puncture wound, or by threading a catheter into the oviduct and washing the embryos into the uterus with a minimum amount of fluid. All embryos transferred can be placed in one uterine horn because swine embryos will become equally distributed in both uterine horns.

EMBRYO EVALUATION

Embryo evaluation is an important determinant of the success of embryo transfer procedures. A gross morphological evaluation of embryos has been shown to be useful in predicting pregnancy rates for groups of embryos, but it is of limited value in determining survival on an individual embryo basis. Dye exclusion tests, measures of enzyme activity, glucose uptake tests, and live-dead stains seem to correlate

well with morphology and embryo survival following transfer. Several of these methods require complex equipment and (or) a lengthy in vitro culture period. Thus, they are of little value for on-the-farm embryo transfer conditions.

A morphological evaluation has been used widely to delineate embryo quality. Several schemes for evaluating embryos have been described, and all appear equally reliable when based on actual pregnancy rates. Parameters commonly used to evaluate embryo quality include shape, color, number and compactness of cells, size of the perivitelline space, number of extuded and degenerated cells, and number and size of vesicles. Categorization of embryos from the most ideal to the poorest varies among practitioners. Some utilize simple two-way classifications (good and poor), while others use a more complex system. Systems that classify embryos as good, fair, and poor appear to be the simplest and most reliable. The most extensively used criterion to evaluate bovine embryos is whether an embryo has attained an expected stage of development based upon day of flush post-estrus. Embryos retarded two days or more appear less successful than those at an expected stage of development. To date, embryo evaluation remains one of the most subjective and qualitative aspects of embryo transfer.

Gross Morphology of Bovine Embryos

The gross morphological characteristics of the bovine embryo have been described by several authors. The overall diameter of the bovine embryo is estimated to be 150-190 m, including a zona pellucida thickness of approximately 12-15 m. The overall diameter of the embryo remains virtually unchanged from the one-cell stage to blastocyst expansion. Early cleavage-stage embryos are commonly known by the number of cells present (such as one-cell, two-cell, and so forth), up to the 16-cell stage. Because only an estimate can be made of the number of cells present for early morulae and beyond, other morphological criteria must be used. The various developmental stages commonly recovered nonsurgically from superovulated donors are described briefly below:

- Morula (four days of age). This is commonly referred to as a ball of cells. Individual blastomeres are difficult to discern. The cellular mass of the embryo occupies most of the perivitelline space.

- Compact morula (five days of age). Individual blastomeres have coalesced, forming a compact mass. The embryo mass occupies 60-70 percent of the perivitelline space.

- Early blastocyst (six days of age). The embryo has formed a fluid-filled cavity or blastocoele and has the general appearance of a signet ring. It occupies 70-80 percent of the

perivitelline space. Visual differentiation between the trophoblast and the inner cell mass may be possible at this stage of development.

- o Blastocyst (seven days of age). Pronounced differentiation of the outer trophoblast layer and the darker, more compact inner cell mass is evident. The blastocoele is highly prominent, with the embryo occupying most of the perivitelline space.

- o Expanded blastocyst (eight days of age). Overall diameter of the embryo dramatically increases (1.2 to 1.5 times), with a concurrent thinning of the zona pellucida to approximately one-third of its original thickness. Embryos recovered at the expanded blastocyst stage frequently appear collapsed, characterized by complete or partial loss of the blastocoele. The zona pellucida rarely regains its original thickness.

- o Hatched blastocyst (nine days of age). Embryos recovered at this developmental stage may be in the process of hatching or may have completely shed the zona pellucida. Hatched blastocysts can be spherical with a well-defined or collapsed blastocoele. Identification of embryos at this stage can be difficult for the inexperienced practitioner.

The quality of individual embryos can be determined using the following criteria:

- o Excellent. An ideal embryo--spherical and symmetrical with cells of uniform size, color, and texture.
- o Good. Trivial imperfections such as a few extruded blastomeres, an irregular shape, and few vesicles.
- o Fair. Definite but no severe problems such as the presence of extruded blastomeres, vesiculation, and few degenerated cells.
- o Poor. Severe problems such as numerous extruded blastomeres, degenerated cells, cells of varying sizes, and large numerous vesicles but a viable appearing embryo mass.

Summary

A great deal of variability exists in morphological development and embryo quality within and among donors. Embryo recovery in a superovulated cow commonly results in a range of embryonic cell stages that differ in estimated developmental ages of from 24 to 48 hours. Embryo evaluation involves identification of the embryonic cell stage of development and assessment of quality based on morphological characteristics. Whether an embryo is of excellent or good quality appears to make little difference in pregnancy rates. Thus, a system that classifies embryos as good, fair, and poor appears to be the simplest and as reliable as more complex systems. Further assessment of the developmental capabilities of poor-quality embryos can increase

the efficiency of embryo transfer. Depending on the available facilities, embryos of poor quality can be cultured for 24 hours to assess their viability. Post-culture embryo evaluation and transfer results in pregnancy rates equal to those of noncultured embryos of similar quality.

Results indicate that recipient-donor estrous cycle asynchrony of two days in either direction does not drastically alter pregnancy rates. Synchronization of embryonic development with the estrous cycle of the recipient may have more stringent requirements, however. It has been shown that bovine blastocysts can be refrigerated ($4^{o}C$) for up to two days without significant losses in viability. This technique can be used to store blastocysts in a dormant state, while recipients that exhibit estrus after the donor progress to a more synchronous stage of their cycle.

As evidenced by actual pregnancy rates, microscopic evaluation of embryos, even at high magnifications (200X), remains very subjective. Development of a reliable, rapid, and practical method of assessing embryo viability would greatly benefit the embryo transfer industry.

CULTURE MEDIA AND SHORT-TERM STORAGE OF EMBRYOS

Media Components

Culture systems for embryos of major farm species were designed to meet one of two objectives. The first objective was a long-term culture system (days) in which various media, gaseous atmospheres, and embryo-handling methods could be studied. The second objective was a short-term culture system (hours), in which embryos could be held briefly before transfer to recipient females.

Embryos from farm animals have been cultured in a wide variety of defined and undefined media. Defined media, rather than undefined media (in which the composition is unknown and the components can vary considerably), are the media of choice when the objective is to study embryo development. When the objective is to provide a system that supports in vitro embryo survival, however, the appropriate medium is the one that is effective.

The ions required for successful embryo development include K^{+}, CA^{+2}, NA^{+}, Mg^{+2}, CL^{-}, PO_4^{-3}, and HCO_3^{-}. Optimum levels of these ions in media are similar to serum values except for K^{+}, which is sometimes found in higher concentration in synthetic oviduct fluid (SOF) and Menezo's medium. The role of individual ions in embryonic development is not well understood; however, CA^{+2} is known to be important in membrane stability and permeability and also at the time of morula compaction. It is generally agreed that sodium chloride primarily acts as the osmotic balance of the medium. The osmolarity of commonly used media is in the range of 250-300 millosmoles (mOsm).

It is often difficult when working under field conditions to maintain embryos in a controlled gaseous atmosphere. This has led to the use of phosphate-buffered medium, which eliminates the need for CO_2 incubators or alternative gassing systems. It has been found, however, that mouse embryo development decreases following incubation in phosphate- (not bicarbonate-) buffered medium when 5 percent CO_2 is not maintained in the air atmosphere.

The most commonly used gas atmosphere for embryo culture is five percent CO_2 in air. Some investigators have found that reducing the oxygen concentration from 20 to 5 percent (a total gas phase of five percent CO_2, 5 percent O_2, and 90 percent N_2) was beneficial for embryo development. No development occurs in the absence of O_2, and the lower O_2 tensions are probably closer to physiological states. No role for N_2 in this mixture has been found, and it is generally considered to be inert.

The bicarbonate ion in the medium controls the pH, but it also functions in equilibrium with CO_2 as a source of carbon during embryo development. For this reason, long-term storage of embryos in phosphate- (instead of bicarbonate-) buffered medium is not recommended.

Pyruvate and lactate are the preferred energy sources for early preimplantation embryos, while glucose is incorporated into the embryo at all stages of development in much greater amounts than either pyruvate or lactate. There seems to be little benefit in including pyruvate or lactate in media for the culture of embryos of eight cells or greater in development. Glucose should be a component of phosphate- as well as bicarbonate-buffered medium at a concentration of 0.5-1.0 g/l.

Little benefit is gained by using a concentration of serum greater than 10 percent in complete culture medium. When using a phosphate-buffered medium, however, serum concentrations of 20 percent are recommended for holding embryos for several hours. Considerably less serum, from 1 to 10 percent, can be used for flushing. All serum should be heat treated (56°C for 30 min) to remove complement activity and should be sterilized by filtration before being used in any medium. Recent results suggest that newborn calf serum and steer serum can be used in place of the more expensive and difficult to obtain fetal calf serum for embryo flushing and transfer. Bovine serum albumin (BSA) can be an effective media supplement for long-term culture, but care should be taken to adjust the pH of the medium, particularly when concentrations of BSA are greater than 2 percent. As a matter of convenience, serum is used more frequently than BSA, which is generally supplied in a powdered form.

Methods for Short-term Embryo Culture in the Field

Embryos should be stored in the same medium that was used for flushing. It is not desirable to move embryos, for example, from a

phosphate- to a bicarbonate-buffered medium because of the possible changes in osmolarity, pH, and energy substrates. When a bicarbonate-buffered medium is used, precautions should be taken to prevent changes in the pH caused by CO_2 escaping into the atmosphere. This is done by using capped tubes that were previously gassed, or by placing embryo vessels in an incubator in which the atmosphere can be controlled. No precautions are necessary with phosphate-buffered medium, and it is recommended that a 20 percent serum concentration be used if less than this amount was employed for the flush.

Flushing and holding medium should contain antibiotics to prevent bacterial contamination. Moreover, embryo vessels should be sterilized before use and kept in a dust-free environment. No particular precautions are needed to maintain embryos at 37°C, but extremes in temperature should be avoided.

The viability of bovine, cattle, sheep, goat, and swine embryos begins to decline following 12 hours of storage in phosphate-buffered medium supplemented with serum. Thus, except for swine embryos which do not survive cold storage, embryos that must be stored longer than 24 hours before transfer should be frozen. If freezing is not possible, cattle, sheep, goat, and swine embryos should be placed in a bicarbonate-buffered medium with 1 g/l glucose and 10 percent heat-treated serum of 1.5 percent w/v BSA and held at 37°C in a gaseous atmosphere of 5 percent CO_2 in air.

Recent evidence indicates that cattle embryos will survive for two to three days at 4°C. When embryos in culture medium were placed in a stoppered tube in a water bath in a refrigerator, survival was very good up to 48 hours. This provides an alternative for transporting embryos or for retarding the development of embryos while recipients are allowed to "catch up."

Attempts to freeze porcine embryos or to even cool them to temperatures below 5°C have proven unsuccessful. Long-term storage systems for porcine embryos should include a bicarbonate-buffered medium supplemented with glucose and BSA at a concentration of 1.5 percent w/v. Porcine embryos should be held at 37°C in a gaseous atmosphere of 5 percent CO_2 in air for optimal results.

Unfrozen embryos must be shipped in a container in which a temperature of 37°C can be maintained for a long time. The Trans-Temp Container maintains a temperature of 37°C for 48 hours and still offers enough space for a embryo container. In addition, this system is well padded and secure enough for shipment under most conditions. Embryos can be shipped in tightly capped plastic tubes that were previously gassed with a 5 percent CO_2 in air mixture. Because the gas will leak from the tube and the atmosphere will not be maintained indefinitely, plastic tubes can be placed in a stainless steel anaerobic chamber, which can be gassed directly by a 5 percent CO_2 in air source. The anaerobic chamber is then placed in the Trans-Temp Container. This system controls both the temperature and gas atmosphere and places the embryos in a secure environment.

Live animals--including their fluids, secretions, excretions, and gametes--are potential transmitters of disease. This is of great concern when animals, semen (34 pathogens have been recorded in semen and 17 have transmitted diseases), and embryos are exported to regions that are disease free. Historically, embryos have been accepted for import if the dam and sire of the embryos satisfy the health requirements of the importing country.

For an embryo to transmit an infectious disease to a recipient animal it must have been infected with the disease in utero (environmental infection) or carry the infectious agent on its surface (gametic infection). For an environmental infection to occur, the disease organism must penetrate the zona pellucida. Viral infectious agents, because of their small size, are of particular concern compared to bacterial, protozoal, chlamydial, or rickettsial infectious agents.

Cattle diseases related to embryo infectious agents have received the most attention; only scant research has been devoted to other farm species. Table 6 shows the results of disease transmission from cattle embryos transferred from infected donors.

As previously discussed in this section, 93 percent of commercial swine embryo transfers are performed to control the spread of disease or produce disease-free offspring. Table 7 lists the diseases that

TABLE 6 Disease Transmission by Cattle Embryos Transferred from Diseased Donors

Agent	Zona Penetration	Results
Foot-and-mouth disease virus	Negative	A,B
Brucella abortus	Negative	B
Bovine parvovirus	Not determined	C
Bovine viral diarrhea	Only one of many studies showed zona penetration	A,B
Blue tongue virus	Negative	A,B
Bovine leukemia virus	Negative	A,B
Infectious bovine rhinotracheitis	Not determined	A,B
Akabane virus	Negative	B
Mycobacterium paratuberculosis	Negative	B

A Recipients/calves seronegative.
B Embryos seronegative.
C Embryos developed normally in culture following exposure to the agent.
D Results not conclusive.

TABLE 7 Disease Transmission by Swine Embryos Transferred from Diseased Donors

Agent	Zona Penetration	Results
Porcine parvovirus	Binds to zona pellucida	Recipients seropositive Embryos positive
Pseudorabies virus	Binds to zona pellucida	Recipients seropositive/ seronegative Embryos positive
African swine fever virus	Binds to zona pellucida	Not determined
Swine vesicular disease virus	Binds to zona pellucida	Not determined
Foot-and-mouth disease virus	Binds to zona pellucida	Not determined
Enteroviruses (ECOP-3 and 6)	Binds to zona pellucida	Not determined

have been studied. In contrast to cattle, several infectious swine agents are known to bind or penetrate the zona pellucida. The large number of sperm attached and embedded into the swine zona pellucida and the sperm furrows found may be involved (Figure 4).

Procedures for Disease Control

A tremendous advantage would be gained if embryo transfer could be used to move disease-free genetic material across continents or from diseased parents into disease-free environments.

Extreme care should be taken to use sterile procedures (sterile equipment and solutions) and to clean the rectum and the vulva meticulously before collection. All serum used in the flushing medium and semen used for breeding must be free of mycoplasma and viruses.

Embryos should be washed through 10 changes of medium containing antibiotic to reduce the potential of disease transmission from the uterine flushing. It may be possible to effectively dilute out the concentration of the virus some 10^7-fold if the agent does not bind to the zona pellucida.

Trypsin, pronase, and other enzymes and antisera have been used to remove bound infectious agents from the zona pellucida. It is unlikely that these techniques will be approved for the international exchange of embryos, however.

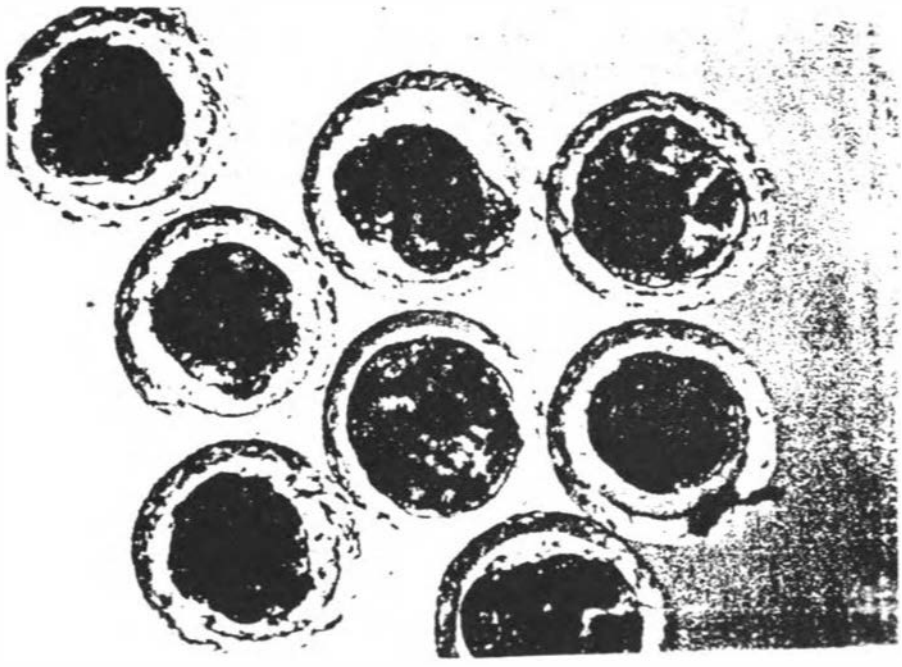

FIGURE 4 Excellent-quality swine morulae and early blastocysts. Observe the large number of spermatozoa embedded in the zona pellucida. This is characteristic of swine embryos.

Embryos frozen for international shipment offer a greater potential for disease transmission than fresh embryos because the zona pellucida may fracture during freezing. The zona pellucida cannot then serve effectively as a barrier to infectious agents entering the embryo. Thus, extreme care in sterile procedures, health testing of dams and sires before collection, and thorough washing of the embryo is imperative for the international exchange of embryos.

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NEW BIOTECHNOLOGY FOR CATTLE PRODUCTION

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Four additional breakthroughs in biotechnology will be important to both the dairy and beef cattle industries in the future. They are: recombinant growth hormone, growth hormone-releasing hormone, adrenergic blockers, and vaccines against hormones. These breakthroughs will increase production by as much as 30 percent (by a simple injection), an increase never before realized in animal production.

RECOMBINANT GROWTH HORMONE

At least four major U.S. companies are making and testing recombinant derived growth hormone (GH) for use in agriculture. The major uses in cattle will be for increased milk production by 15-35 percent and increased body weight gain by 10 percent.

Exogenous GH acts on the liver to increase the release of insulin growth factor-1 (IGF-1) which in turn is responsible for increased protein synthesis, leading to increased milk production, and for cartilage formation, leading to increased bone growth. Present trials in the United States require daily injection of this material which is a troublesome management problem. A longer-acting delivery system would aid this technology. There is little doubt that injections of GH will increase production to a magnitude never seen before in agriculture.

GROWTH HORMONE-RELEASING HORMONE

Two U.S. companies are developing a growth hormone-releasing hormone (GHRH) analog for use in increasing growth and milk production. GHRH is naturally produced in the hypothalamus (part of the brain), and in turn induces release of GH from the pituitary which increases growth and milk production as described above.

Chemists have found that only 29 of the original 44 amino acids are necessary for biological activity. Dr. A. V. Schally (Nobel Laureate) has been developing changes in the 29-amino acid GHRH-A, and he has produced a series of analogs that have a greater biological potency than the natural hormone.

The releasing hormone can be prepared by peptide synthesis for testing and marketing. One advantage of this material is that its synthesis is less expensive than that for recombinant GH. Another advantage is that, unlike GH, it can be placed in a microcapsule for slow release. These microcapsules, which are made from the same material as the soluble suture, can release peptides for one to six months. Thus, this technology could make GH injections outdated even before the technology comes on the market.

ADRENERGIC BLOCKERS

At least one U.S. company and probably more are working on adrenergic blockers. These drugs repartition the nutrients of ruminants from fat synthesis to protein synthesis. Reports show a decrease in pelvic and kidney fat of 46 percent and an increase in loin and rump muscles of 25 percent.

These repartitioning agents work best in ruminant animals, and they have been a dream of animal scientists for years. It is not known how they will work on milk production and what interactions they might have with various feedstuffs. Cimaterol, an adrenergic blocking drug, is being tested by the American Cyanamid Company. The future of these drugs in combination with GH and GHRH analogs will be exciting.

VACCINES AGAINST HORMONES

Vaccination against an animal's own hormones can be used to either increase or decrease reproduction. Immunization against the steroid hormones appears to limit the negative feedback of these steroids at the site of the hypothalamus-pituitary, resulting in the increased production of luteinizing hormone and Follicle Stimulating Hormone which increases the ovulation rate.

An Australian product on the market, Fecundin^{R}, is androsteinedione conjugated to a protein. This drug produces an active immunization and increases a lamb crop by 0.2 lambs per ewe. Washington State University used a passive immunization in goats and increased the number of kids born per doe by 70 percent.

The future use of vaccines to increase reproduction and growth is realistic, and it has the interest of the large pharmaceutical companies.

APPROACHES FOR USING EMBRYO TRANSFER TECHNOLOGY IN THE CATTLE INDUSTRY IN INDONESIA

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INTRODUCTION

Indonesia's development strategy calls for transformation of its economy from one based on agriculture to one based on industry. Some characteristics of a typical industry are:

- Commercial orientation
- Standardization and quality control
- Continuous production
- Mass production technology
- Initiation of new processes to increase the added value of products.

The current objectives of agricultural development emphasize:

- Maintaining self-sufficiency in food production and providing proper nutrition for the Indonesian people
- Increasing the farmer's income
- Creating employment opportunities
- Increasing exports and reducing imports
- Encouraging and supporting transmigration
- Achieving balanced regional development
- Supporting industries.

In fitting together the characteristics of industrialization with the objectives of agricultural development, tremendous problems are encountered for which there is no panacea. One must try to tackle the specific problems while always keeping in mind the total package.

This paper addresses one narrow area, embryo transfer in cattle, as it pertains to both industrialization and agricultural development.

CATTLE PRODUCTION IN INDONESIA

To increase both the quantity and quality of cattle species in Indonesia, three approaches are being applied:

1. Upgrading of the local strains of cattle
2. Importing fully grown cattle
3, Artificial insemination.

The first step is the natural approach. Even if the best techniques of cattle husbandry are applied, however, decades of effort are required for substantive results and supplies would still fall short of the demand generated by population growth in Indonesia.

The second approach would upgrade the cattle population quickly, but transport problems often make this approach impractical. After a long journey at sea, full-grown cattle are in bad condition, as was the case in West Sumatra. Moreover, there is always the possibility of importing new diseases.

The third approach is successful only where herds can be continuously checked. Otherwise, weak links from local cows will be passed on randomly to their progeny, making it difficult or impossible to fulfill the original development plan. Mortality caused by weak links can greatly disrupt any development plan. Finally, progress from use of this approach is relatively slow.

All three approaches are, however, being carried out simultaneously in Indonesia, so that the overall results are still better than the traditional approach of natural selective breeding.

A new but well-established technology, embryo transfer, is now being used to rapidly improve and increase the number of cattle for industrial purposes. This technology has the following advantages over the existing technologies:

- It provides instant upgrading of all progeny to pedigree standard for either meat or milk production.
- A calf is born locally with natural maternal immunity to local diseases.
- The new calf has a 100 percent pedigree and is totally unaffected by the genetics of a surrogate mother (as a recipient).
- The quantity and quality of the offspring are controllable.

APPROACHES TO INDUSTRIAL BEEF CATTLE PRODUCTION

Cattle breeding is essential to the meat, dairy, leather and shoe, glue, and feed industries. Each industry has its offshoots. For example, the meat industry needs slaughter houses, refrigerated container fleets, wholesale distributors, and retail butcher chains. The milk industry needs dairy farms and refrigerated milk distribution systems or milk powder factories. In the same way, the leather, glue, and feed industries create new businesses.

Only the beef cattle industry is considered in this paper. Development of this industry can increase foreign exchange earnings through the export of high-quality meat and reduce the import of meat consumed by large hotels and restaurants.

The value of the more than 1.3 million kg of meat imported by Indonesia is US $4.6 million. Thus, local entrepreneurs should take advantage of international meat prices and first export to an affluent market abroad. An emphasis on exports will require the selection of high-value cattle breeds for meat and controlled production because local consumers will not be able to afford the prices obtained in the international market for meat of this quality. This is not, therefore, an agricultural activity designed to directly improve the protein diet of the population.

Beef cattle production is a business proposition, and, its development should be approached as that of any other industry. Because this economic activity is aimed at the export market, a competitive price is a must. Maximum labor productivity, efficiency, and increased capital costs are also necessary. (This type of industry will not, however, produce a large number of jobs.) Through such commercial development, Indonesia could gain a high financial return through taxes. Finally, protection against imported meat should be considered for a certain period of time.

Private and cooperative investment could be welcomed, but it pays to be aware of private investments in which managers try to avoid taking risks and, in case of failure, leave the lending or guaranteeing government institution holding the bag. On the other hand, based on past experience in Indonesia government-conducted business operations are seldom successful. In this pioneering endeavor, the employment of essential specialists, such as expatriates with a long history of successful experience, is imperative. Joint ventures with foreign companies are therefore highly recommended.

In managing a cattle industry using embryo transfer technology, other production components must not be overlooked. These include animal feed and forage production, animal health and hygiene, animal product processing, and marketing and distribution. All four components of animal production should be integrated into one unit. A simplified scheme of an integrated beef cattle industry is shown in Figure 1.

Animal health and forage production are often stumbling blocks in good cattle production. Herds of cattle of more than 500 head require specialists to manage the feed supply and to keep the animals healthy. Cooler locations, 300-500 ft above sea level, are best for animal production. A dryer climate (in eastern Indonesia, for example) than that found in much of Indonesia is probably also better, provided that water availability is guaranteed. Where land is scarce (Batam island or locations near the cities), growing forage through the use of hydroponics might be viable. The economics is, of course, the determining factor.

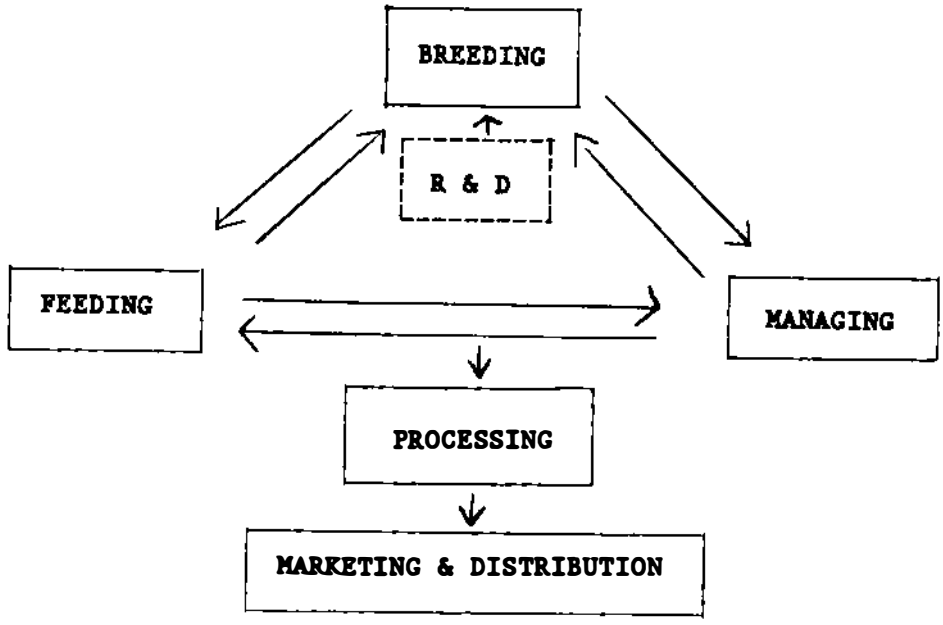

FIGURE 1 Integration of the four components of animal production in the beef cattle industry.

The size of the recipient of the embryo can be a problem, since the high-quality imported beef cattle are large when compared to the local recipients. Any problem with delivery of the calf could be overcome by using dairy cows as recipients--most are pedigreed and relatively large. A flow chart for a beef cattle industry using embryo transfer technology is shown in Figure 2.

A pilot project could be carried out on 1,000 ha of land, using 1,000-1,500 dairy cows as recipients, 1,000 imported/local high-quality beef cattle embryos, and 100,000 hybrid coconuts. It might then produce 300-400 calves (about 50 calves and 250-300 beef cattle) after the third year, 405,000 liters of milk per year after the second year, and 120,000 coconuts per year after the fourth year.

Since this idea has not yet been implemented, it is strongly suggested that a feasibility study be first carried out. Such a study would cost approximately $15,000.

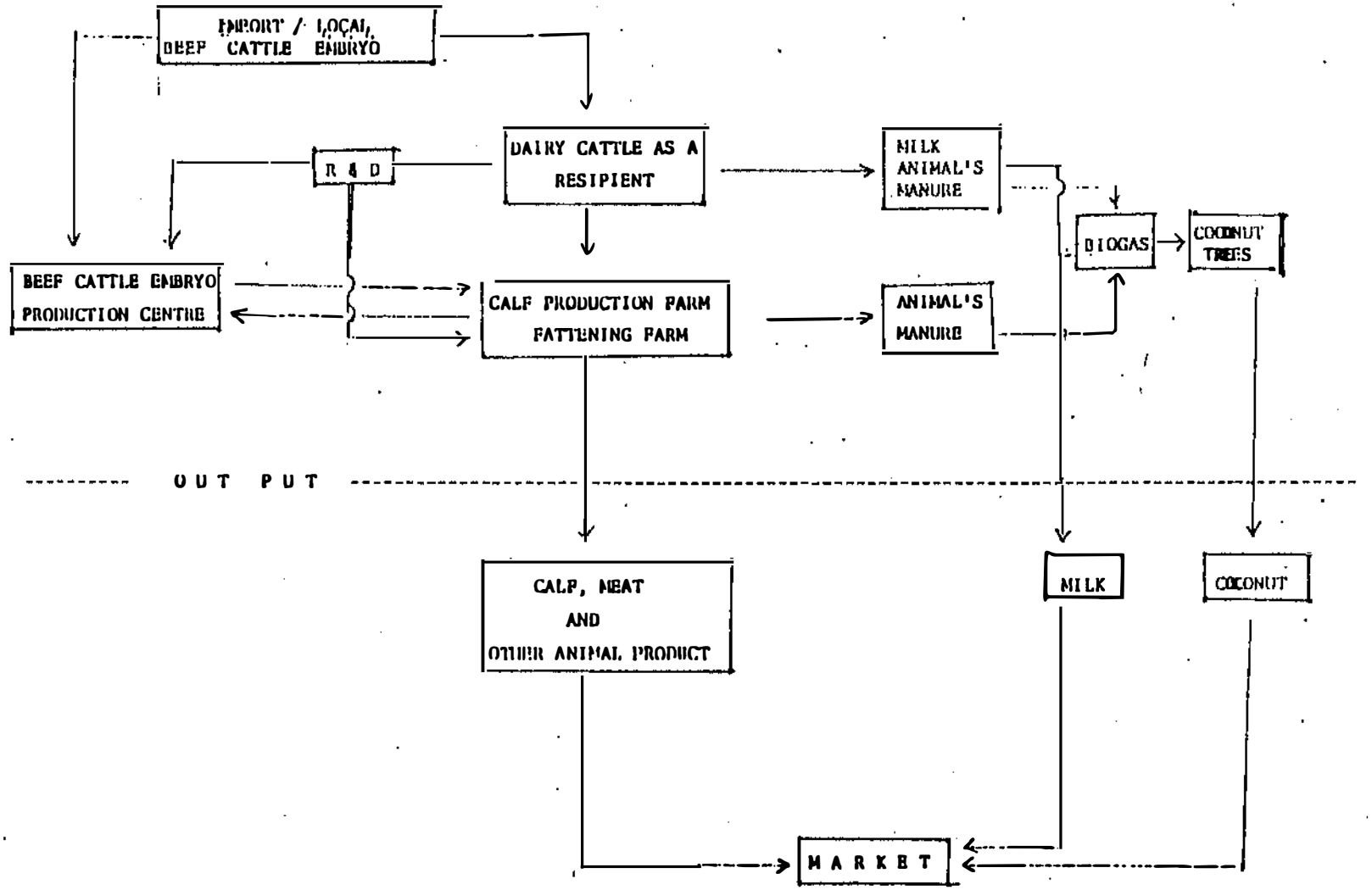

FIGURE 2 Flow chart of a beef cattle industry using embryo transfer technology.

POTENTIAL FOR DEVELOPMENT OF CATTLE IN INDONESIA

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Development of a cattle industry depends heavily on developing a nation's economy and its agricultural sector. It appears that in practically every economy development of the livestock industry has generally lagged behind that of the rest of agriculture and the general economy, in science, technology, management, and marketing.

This is not to say that there has not been great progress; it is just that the nature of the industry has made progress more difficult. Some developing countries have made little progress except in the health area. Africa and Asia have clearly lagged behind in productivity per animal. In 1977, Africa produced an average of 15.7 kg of beef per year for each head of cattle inventory, while the corresponding average for Asia was 13.1 kg. The world average was 40 kg, and Australia produced 60.7 kg, Europe 76.8 kg, and the United States 94.3 kg (Table 1).

An Indonesian Ministry of Agriculture (1981) publication has described the livestock situation in 1981:

> Most of the cattle, sheep, goats, buffalo, chickens and ducks are managed by smallholder farmers using traditional husbandry systems. In most cases, they maintain their animals under scavenging or minimal cost conditions and keep their large animals primarily for power, for manure for their crops, and as a savings account.
>
> With the exception of eggs, the production of livestock products has not kept up with demand and imports have been rather substantial. An inadequate meat supply is due primarily to several factors, including a diminishing livestock population (now about 6.3 million cattle and 2.3 million buffalo), the low genetic potential of existing livestock, the relatively high mortality due to animal disease outbreaks and the shortage of high quality feedstuffs (p. 71).

Clearly, development of Indonesia's potential in cattle and beef production requires the adoption of improved technology and management. This includes improving and expanding good-quality breeding cattle using

TABLE 1 Beef Production (Kilograms) per Head of Cattle Inventory

Region	Year			Percent Change,
	1960	1970	1977	1960-1977
Asia	5.3	14.1	13.1	147
Africa	15.8	16.2	15.7	-1
South America	27.3	30.5	31.2	14
North and Central America (except USA)	31.9	33.8	38.3	20
Australia	37.8	47.2	60.6	60
USSR	-	56.6	60.7	7[a]
Europe	55.9	71.7	76.8	37
United States	74.8	89.3	94.3	26
Total world	30.4	35.8	40.0	32

[a]Change from 1970 to 1977.

SOURCE: Compiled from FAO Production Yearbook, various issues.

a variety of means: imports of cattle, artificial insemination, and embryo transfer.

COMPARATIVE ADVANTAGE

Tropical countries usually have a comparative disadvantage in producing dairy and beef cattle, primarily because they have a comparative disadvantage in producing high-quality pasture and feedstuffs. Where there is no good alternative use for land, however, cattle can sometimes be produced at very low cost even though they may take four years to reach slaughter weight. This situation is typical of many Central and South American countries where canned or frozen boneless beef is produced for export. Productivity is low, but producers have low-cost (and often low-quality) grazing available year-round. In addition, most areas and most farms have land that is not suitable for crops where some livestock can be produced at relatively low cost. Livestock become more expensive when they start competing for cropland or when expensive pasture improvement is necessary.

Typically, developing the cattle industry in the tropics is a greater challenge than in a temperate climate because of lower productivity, lower nutrition, lower calving rates, higher calf mortality rates, higher

incidence of disease and insects, and higher marketing costs for live cattle. Many of the developing countries are in the tropics. These countries have about 65 percent of the world's supply of cattle, but they produce only 33 percent of the world's supply of beef. The developed countries, on the other hand, have 35 percent of the cattle and produce 67 percent of the beef.

Cattle in developing countries are often multipurpose: they provide meat and power, and they serve as a store of capital. Generally, any comparative advantage for cattle in the tropics is related to non-grainfed beef, or producing a joint product with a dairy, or to an unusual source of by-product feed. The dairy industry in Indonesia could be expected to produce a substantial portion of Indonesia's beef supply.

A strong factor in comparative advantage is the level of demand. Beef can be produced nearly anywhere if the price is high enough, and Japan is a prime example of this (Farris, 1984). Per capita meat and poultry consumption in Indonesia is reportedly among the lowest in the world (Table 2). Indonesia's large population could certainly provide the market for expanded production, and dairy and beef development can contribute substantially to improving the nutrition of the Indonesian people. Creating and maintaining an economic environment to foster livestock development are, however, essential (Farris, 1986).

Considering its rapidly growing population, Indonesia will likely continue to be a net importer of meat and dairy products. Thus, it is important to recognize its comparative disadvantage in these areas and not to establish import barriers. To avoid such a step, any subsidies to develop the domestic livestock industry should be direct rather than import restrictions.

Given the concentration of population in Java, some islands could export beef to Java and Singapore, while Indonesia itself would continue to act as a net importer of meat and dairy products.

DEMAND

Demand for meat is related to per capita income except where there are specific religious taboos such as the Islamic and Jewish rules against eating pork. Per capita consumption of meat is higher in the developed countries. Consumption is lowest in South Asia and low in East Asia relative to income. Fish consumption is undoubtedly the most important source of animal protein in Indonesia, where the per capita consumption of meat and poultry is estimated at 3.4 kg per year. Eggs and milk consumption in Indonesia is also estimated to be among the world's lowest (Table 2).

Generally, people like variety and will consume a wide range of protein sources if given the opportunity. Demand for meat within an income group is very responsive to the price level. A study of the international demand for beef shows that, once adjusted for differences in income, demand is closely related to the price level. It appears that the demand curve for beef (corrected for income) is not significantly different for many developed countries (Figure 1).

TABLE 2 Per Capita Meat and Egg Consumption Projections (Kilograms) for 1985, by Region

Region	All Meat	Beef	Pork	Sheep & Goats	Poultry	Eggs	Milk[a]
United States	120.19	62.93	27.34	0.99	28.93	15.99	240.17
Canada	114.69	62.25	25.40	1.24	25.80	12.57	272.73
EC-6	86.92	30.64	37.94	1.83	16.51	14.31	362.79
EC-3	77.94	27.36	28.28	7.90	14.40	13.72	365.09
Other Western Europe	74.38	22.85	29.62	4.54	17.37	14.18	265.77
Japan	37.35	6.71	16.99	3.17	10.48	19.62	88.69
Oceania	129.30	71.44	13.70	27.56	16.60	12.57	220.74
South Africa	43.95	22.85	3.23	7.56	10.31	6.30	113.19
Eastern Europe	80.00	21.76	40.65	2.65	14.94	15.10	357.84
Soviet Union	62.06	32.07	16.76	4.69	8.54	15.75	423.98
China	23.38	2.32	15.31	0.89	4.86	4.45	6.03
Middle America	30.92	15.73	6.43	1.68	7.08	7.71	130.76
Argentina	119.49	95.82	9.91	3.93	9.83	8.15	203.99
Brazil	44.39	27.47	9.11	0.58	7.23	4.12	121.03
Venezuela	47.03	25.99	5.87	0.39	14.78	8.51	99.32
Other South America	34.05	21.45	4.88	2.73	4.99	5.20	102.13
High Income North Africa and Middle East	29.92	5.16	0.34	11.40	13.02	6.90	64.43
Low Income North Africa and Middle East	19.29	7.31	0.04	8.45	3.49	4.71	25.55
East Africa	15.49	10.66	0.78	1.51	2.54	1.84	35.42
Central Africa	9.58	4.87	0.79	2.52	1.40	1.60	12.67
India	1.29	0.33	0.11	0.66	0.19	0.11	14.79
Other South Asia	4.59	2.01	0.03	1.96	0.59	0.73	12.22
Thailand	13.29	4.47	5.23	0.00	3.59	4.72	13.72
Other South East Asia	10.40	1.67	6.86	0.06	1.81	2.72	5.41
Indonesia	3.41	1.40	0.86	0.36	0.79	0.64	4.62
High Income East Asia	25.50	4.90	12.37	0.33	7.90	11.31	27.04
Low Income East Asia	15.10	3.34	7.77	0.19	3.80	5.05	36.32
Rest of world	21.62	10.06	4.66	3.71	3.19	6.11	39.37
World	32.48	12.73	10.95	2.25	6.55	6.45	98.80

[a]Food and Agriculture Organization of the United Nations.

SOURCE: Winrock International.

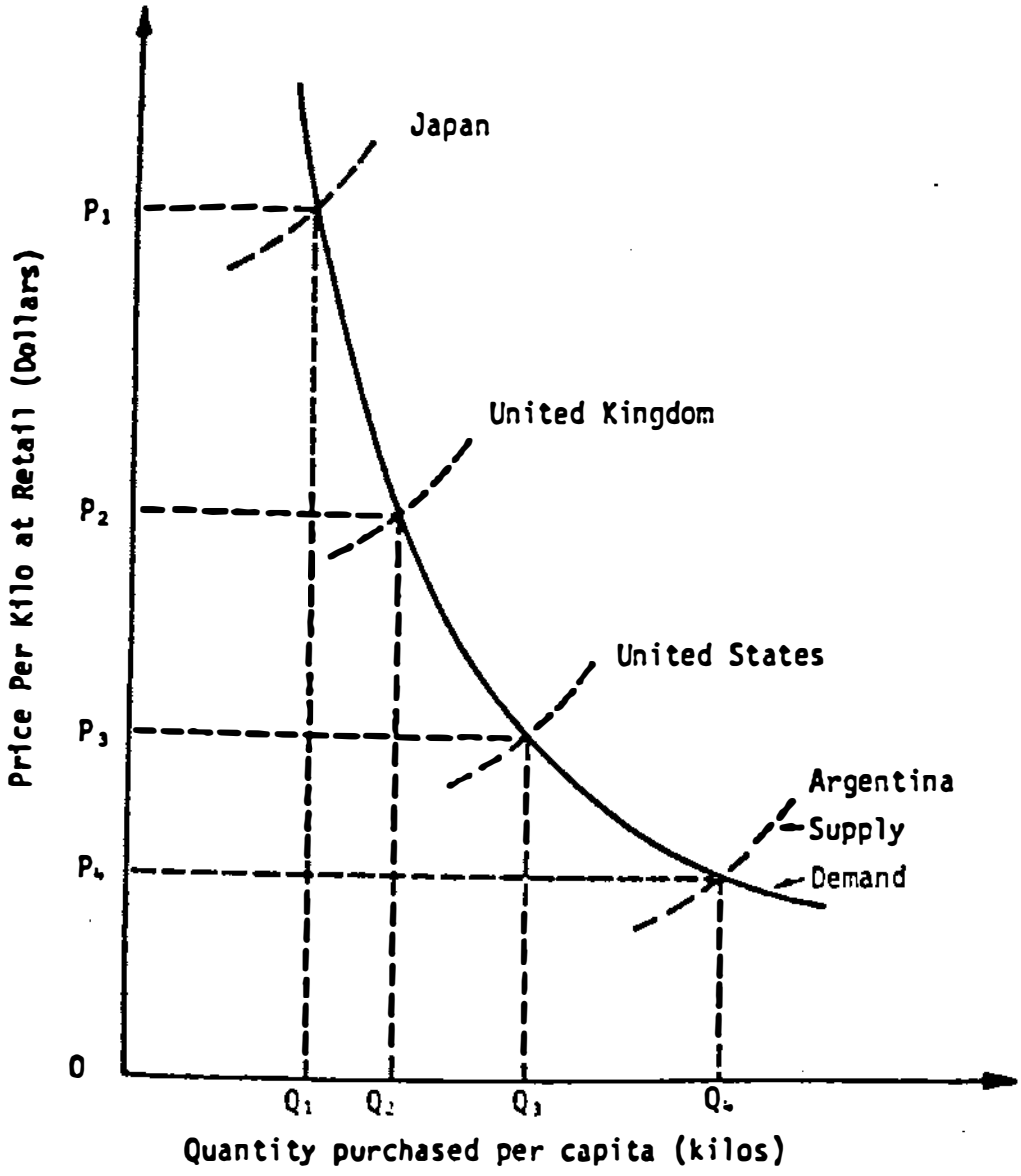

FIGURE 1 Illustration of demand and equilibrium price for steak, assuming a constant income level.

Thus, it is important to proceed in Indonesia with all aspects of beef and dairy development, including the expanded use of embryo transfer. Imported beef and dairy products will be needed into the foreseeable future, but selective expansion of the domestic industry

would have a substantial direct impact on economic development, and would contribute as well toward import substitution.

WORLD TRADE

In considering livestock potential, one must also consider the Australia and New Zealand international market. Indonesia is situated within easy access of a major low-cost source of beef and lamb, and Oceania, also nearby, is the largest exporter of beef and lamb. To compete with these sources it will be necessary to improve overall productivity, and this begins with developing improved breeding herds, followed by use of the other appropriate production and marketing practices.

Apparently at one time Indonesia exported cattle to Singapore, but this was discontinued in 1975. One feedlot in Indonesia has been importing Australian steers for feedlot finishing, and this lot has been finishing local dairy and Zebu-type bulls as well.

Now there is an interest in developing beef exports from Indonesia, especially to Singapore. Without analyses of specific projects, this does not appear to be economically feasible without subsidies. As beef development progresses, however, there may be some specific products for which Singapore or other markets would offer the best price. If relatively free trade is allowed, individual firms should be able to serve the best markets with the appropriate products.

The specific market appears to be the least of Indonesia's development problems, however. Efforts should concentrate on improving livestock productivity and the domestic marketing system. When Indonesia can compete price-wise on the international market, it will be time to look for export opportunities.

CONCLUSIONS

Recent data indicate a serious need for an increased level and variety of animal protein for Indonesia's already large and growing population. Although the tropics generally have a comparative disadvantage in dairy and beef production, there are areas and operations where this kind of production is the best alternative available, especially in the highest elevations. The economic activity multiplier of livestock enterprises in Texas is about 3.4 times the livestock's slaughter value. The multiplier for Indonesia would probably be higher. For an efficient livestock industry, improved technology, management, and marketing are clearly needed. In fact, improvements should be made throughout the system. Improvements in the genetic base are very important for increasing productivity, and this can be done by importing breeding stock, artificial insemination, and embryo transfer. Improved animal health, nutrition, and market practices are also needed.

A pilot program that results in an improved production package seems to be one approach to speeding up development. Such a program could focus on improving the genetic base by coordinating the use of artificial

insemination and embryo transfer, followed by direct support programs, education, and research directed toward increasing productivity per animal and lowering costs--not increasing them.

Marketing should not be neglected. Programs that make it easier to move cattle and widen the market for small operators are important to expediting development. Large potential markets already exist in Indonesia. As per capita incomes rise, the demand for animal products will grow rapidly.

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APPENDIXES

APPENDIX A

Keynote Address

Didin S. Sastrapradja
Assistant (II) Minister of State for
Research and Technology

In March 1986, the Indonesian National Research Council and the U.S. National Research Council held a workshop on biotechnology in agriculture for Indonesia. One of the group discussions focused on embryo transfer and animal production (ETAP). Participants at that workshop felt a need to develop more adequately a capability in embryo transfer technology in Indonesia and recommended the establishment of a Center for Embryo Transfer and Animal Production (CETAP). This national center would facilitate the promotion of Indonesian groups currently working on ETAP activities.

As a follow-up to that meeting, several of these groups have conducted scientific meetings. At one such meeting in July 1986, it was recognized that to master embryo transfer technology under Indonesian conditions, studies had to be undertaken on embryo production techniques, monitoring, and evaluation of embryo development from the time of transfer to the adult stage, as well as economic analyses of the process. A decision on which commodity should receive the highest priority was not made, however, although several choices were offered.

Exactly one week ago the Interuniversity Center for Biology at Bogor Agricultural University held a symposium on the role of embryo transfer and genetic engineering in the improvement of animal quality and production. This meeting recognized the importance of embryo transfer as a means of improving animal quality and production in Indonesia. A program on this technique--which is quite sophisticated--should be carried out using interdisciplinary and integrated approaches. It was also recommended that the related technical departments further develop the details of such a program.

The question to be answered about the practice of embryo transfer in Indonesia is: Where do we go from here? The activities in embryo transfer in Indonesia are limited thus far on how to master the transfer. Embryo and animal production technology need more scientific backup. Therefore, I ask that the participants in this meeting assess and identify the necessary knowledge and science that must be mastered by Indonesian scientists and technicians to develop these technologies under Indonesian conditions.

The success of the development of these technologies will depend upon the manpower available. Therefore, manpower development is very

important. I shall also expect the discussions to identify in detail the quantity and qualifications of the manpower needed in each discipline for the development of these technologies. It is important to identify the supporting disciplines as well.

Manpower and the supporting sciences development should be directed toward long-term development of embryo technology for the improvement of animal production, including embryo production, storage, transport, etc. Thus, the facilities and equipment needed for the supporting sciences must also be specified.

Finally, I expect the formulation of cooperation between the Indonesian and the U.S. national research councils on many aspects of embryo transfer technology.

I wish you all success in your deliberations and discussions.

APPENDIX B

Meeting Agenda

Monday, February 23

Morning

Opening Ceremony

Keynote Address
Didin S. Sastrapradja
Assistant (II) Minister of State
for Research and Technology
Chairman, National Committee on Biotechnology

Remarks
Raymond Wright
Chairman, NRC Panel
President, AnemTech Company
Professor, Department of Animal Sciences,
Washington State University

Address
Sediono M. P. Tjondronegoro
Secretary, Indonesian National Research Council

Coffee

Presentations:
Daman Danuwidjaja
National Strategy and Role of Embryo Transfer in Animal Husbandary Development in Indonesia
Daman Danuwidjaja
Director-General of Livestock Services

Present Status of Prospects for Embryo Transfer in Animal Production in Indonesia
Mozes R. Toelihere
Department of Reproduction and Obstetrics,
Faculty of Veterinary Medicine,
Bogor Agricultural University

Present Status of and Prospects for Embryo Transfer in the United States
Raymond Wright, Jr.

	New Biotechnology for Cattle Production **Jerry J. Reeves** **Department of Animal Sciences,** **Washington State University**
	Approaches for Using Embryo Transfer Technology in the Cattle industry in Indonesia **Ida K. and A. M. Satari** **Agency for the Assessment and Application of Technology**
Lunch	
Afternoon	**Plenary Session**
Coffee	
	Plenary Session

Tuesday, February 24

Morning	**Presentation** **Potential for Development of Cattle in Indonesia** **Donald E. Farris** **Department of Agricultural Economics,** **Texas A&M University**
	Plenary Session
Coffee	
	Plenary Session
Lunch	
	Break for Formulation of Conclusions and Recommendations by Steering Committee
Afternoon	**Summary of Recommendations and Conclusions** **Didin S. Sastrapradja**
	Closing Remarks

APPENDIX C

List of Participants

STEERING COMMITTEE

Didin S. Sastrapradja, Chairman
Haryanto Dhanutirto, Secretary
A. M. Satari
Sediono M. P. Tjondronegoro
Susono Saono
Raymond Wright
Rose Bannigan

ORGANIZING COMMITTEE

Haryanto Dhanutirto, Chairman
Ida Kusumah, Secretary
Jana Anggadiredja, Vice-Secretary
Rachmaniar Rachmat
Saraswati
Dadang A. Permadi
Muhamad Said Did
Roi A. Sparingga

U.S. NATIONAL RESEARCH COUNCIL PANEL

Raymond Wright, Chairman
Donald E. Farris
Jerry J. Reeves
Jack Rutledge
Lorreine Leibfried-Rutledge
Rose Bannigan, Staff Officer

PARTICIPANTS

Mozes R. Toelihere
Yuhara Sukra
Abd. Rachman
Suhadji
Soemarno P.

Lien Sutawiria
I. Komang Wyarsa Sardjana
Ida Kusumah
Sunartono Adisumarto
Rini dharsana
Harimurti Martojo
Wanda G. Piliang
Sabdono Surohadikusumo
Suyadi
H. Azwarli
Poerwanto Soediono
Djaya Gunawan
Herdi Soemeru
T. Widharetna

SECRETARIAT

Betty Sribudiwaty
Ratna Wulan Karang
Indang Wahyurini
Asti Suryani
Rangguh Adven Parmoto
Tina Tambunan