5. Practical Applications of Embryo Banks

Embryos to be stored in embryo banks can be obtained by pursuing two principally different strategies:

One strategy involves resident embryo transfer stations, and the other mobile transfer teams which visit individual breeders to obtain embryos from donor animals.

5.1 Stationary Embryo Transfer (ET) Stations

Such stations require trained personnel and financial resources to cover the costs of establishing and running the station. Recovery of embryos from endangered breeds of cattle will rarely suffice as a solid foundation for such an establishment. Since the organization of laboratory facilities (cleaning of equipment, disinfection etc.) resembles that of a station specialized in artificial insemination techniques it may be advantageous to affiliate embryo transfer stations with an insemination station or a research laboratory. This means that at least part of the equipment may be shared and that, for example, cleaning and sterilization of equipment used for embryo transfer can be carried out in the AI station.

The basic requirements for a stationary ET station are as follows:

- office for collecting and processing information
- laboratory facilities providing microscope(s), heat plate(s), cryoequipment, sterilization facilities
- storage rooms for drugs, cooling equipment, instruments
- livestock housing for donors and recipients.

There are two different ways for handling embryo transfer programmes in stationary ET stations:

a) The donor dam is prepared where it is normally housed and transported to the station for flushing. This approach has several disadvantages: if the owner has to transport the cows over long distances expenses accumulating for the farmer may be very high. Transportation itself is also an important stress factor for the cow. In addition preparation of the donor cow is problematic if the animal has to be transported over long distances. Long distance travel of the highly paid transfer team cannot be regarded as an optimal use of their special training. If the cow is prepared by local veterinarians or by the owner this may lead to an increased rate of drop-outs during the stimulatory treatment and may also decrease insemination success. The biggest advantage of this procedure is the greatly reduced hygiene risk for the donor animals. If flushing is carried out in the ET station all equipment that might be required will be at hand, and the station itself is capable of handling all sorts of risks. It may also be very convenient to be able to rely on a trained team. Expenses for housing the animals at the station are minimal.

b) The donor cow is transferred to the ET station after having calved, where it remains until the next pregnancy. This is the most suitable approach in view of establishing gene reserves. Donor animals can be kept and fed under optimal conditions. In addition, all data required for successful embryo transfer such as heat periods, oestrous cycle, diseases etc. can be collected and will be available. The optimal time for starting superovulation can also be determined.

Embryo collection may be repeated if too few embryos are available or if the quality of the embryos is insufficient. The problem of the animals adapting to the new micro-and macroenvironment of the station may be a disadvantage. This approach would also require the establishment of a suitable infrastructure providing, for example, milking parlours. Therefore this method of obtaining embryos will require sufficient personnel and will also be very cost-intensive. An advantage is the fact that unfavourable conditions possibly prevailing at the farm might be compensated, which would, in turn, increase the success rates of the embryo transfer programme.

5.2 Mobile Embryo Transfer Teams

Provided that conditions are appropriate embryos may also be collected by visiting the farmer on site. This approach to obtain embryos is most easy on the donor animals. A disadvantage is that the ET team may have to travel long distances to prepare the animals and flush them, which also makes the availability of a mobile laboratory mandatory. Again, two different strategies may be pursued:

a) The transfer unit is installed in a bus, caravan or similar type of vehicle. Such a car may be autonomous and may have all the necessary equipment installed to produce for example electricity and pure water. A mobile laboratory can be equipped according to the specific needs of embryo transfer; as with stationary ET the precision work can be carried out in a standard environment. Special equipment such as micromanipulators or cooling equipment could also be carried along at all times. All equipment carried by a mobile unit would have to be stored under dust-free anti-skid and shock-proof conditions which means that selection of equipment should also be guided by considerations of robustness. An important disadvantage of such a unit may be a limited mobility. Because of the space required, the car would be relatively unwieldy, which might be problematic in relatively impassable terrains such as mountains or steppe. Another difficulty lies in positioning the vehicle. The area must be level or facilities must be available to adjust the work bench horizontally because a slanted table makes the work at the microscope impossible. Practical experience shows that this latter point in particular hinders work in a mobile unit in Alpine countries. However, if conditions at the farms do not allow a temporary laboratory unit to be set up (See b) a mobile unit is an imperative necessity.

b) The laboratory is set up temporarily for flushing in a clean room provided by the farmer. Certain requirements must be met because the room must be dust-free and dry, and it must possess water and electricity supplies. If needed water and electricity may also be supplied by the ET team (tanks, batteries). The room should not be too hot or too cold in order to minimize adverse conditions for the embryos. A mobile unit has the great advantage that a large amount of equipment, even delicate instruments, may be stored in a small place which allows for mobility even in unfavourable terrains. A disadvantage is the time required for setting up the equipment before use and dismantling the temporary laboratory after use. In addition there is the problem of having to carry out laboratory work under permanently changing conditions.

In this section we have described different ways of organizing the collection of embryos. We have not gone into details because individual conditions are too variable. It will be necessary to decide upon the most appropriate way by taking into account the conditions met on location. Apart from the availability of personnel and financial means decisions will be influenced essentially by the organization and the hygienic situation found at the farms providing the donor animals.

5.3 Donor Cows

Before genetic reserves are established in the form of embryo banks it is necessary to consider the number of animals available and to decide whether their genetic potential will allow collection of a sufficient number of unrelated embryos for subsequent reactivation of a breed. Model calculations carried out by Schütz (1988) will serve as an example of factors and conditions that must be taken into consideration when planning to establish an embryo bank for an endangered species of cattle.

The starting point is the number of desired female animals which are fit for breeding if the embryo bank is reactivated. The following calculations which are based on genetic considerations discussed above require reactivation from an efficient embryo bank of at least 25 female cattle fit for breeding. Considering success rates of embryo reactivation this number allows the calculation of the number of embryos that must be stored initially and the number of the corresponding donor animals.

None of the organizational and biotechnical steps connected with the establishment of an embryo bank is carried out with 100 percent efficiency. The entire process can be subdivided into 14 different steps, each characterized by a parameter describing its efficiency. The success rate of each step provides information about the number of cows, embryos, or calves that will be available to be used in the next step. The individual steps of establishing an embryo bank are listed in Figure 27; steps associated with reactivation of the embryo bank are shown in Figure 28. These figures do not only contain information concerning the way in which individual efficiency parameters have been calculated, but also data used in subsequent model calculations. Multiple superovulation rates and flushing rates constitute a special situation because these two steps are associated with a reduction of animal numbers.

The first step provides information about the control rate which indicates the number of cows for which information about performance parameters and herd-book data is available. Apart from performance data genealogical information is most important in the establishment of embryo banks because all attempts should be made to employ embryo donors which are as unrelated as possible. Only a fraction of all animals available are usually registered for gene conservation programmes. The willingness of animals holders to make their animals available for the collection of embryos depends upon the organization engaged in establishing the embryo bank and also on the individual interests of the animal holders.

Not all cows that are available are suitable for embryo collection. Their suitability is judged by the ET team according to information obtained from preliminary reports and by gynaecological investigations.

In principle it is possible to stimulate and to use cows repeatedly for embryo collection. This approach yields information collected under the heading multiple superovulation rate.

The reaction rate provides information about the fraction of stimulated cows that showed a positive reaction to superovulation. The mean number of embryos obtained by flushing is known as flushing rate. Because only a fraction of the embryos can be cryoconserved (and thawed) successfully this last step is also associated with a further reduction in the number of available embryos. Cryoconservation and storage of embryos are the final steps in establishing the embryo bank.

Reactivation of an embryo bank begins with the supply of suitable recipients derived from live populations and thawing and transfer of stored embryos. The retrieval rate can be considered very high. The survival rate is the proportion of embryos that are transferred in the number of embryos that were thawed. This value may vary considerably depending upon the quality of the embryos and the techniques employed for cryoconservation. The same degree of variability will be observed in the number of pregnant animals obtained after transfer of thawed embryos. On the other hand approximately 95 percent of all pregnant animals will give birth to live calves if the animals are treated with appropriate care. Approximately 90 percent of the female calves born should reach breeding age.

Model calculations are easily performed to estimate the number of cows, embryo flushings and stored embryos required to obtain, through reactivation from the embryo bank, a minimum number of female cattle that have reached breeding age. The examples given below assume that a total of 25 female breeding animals are to be reactivated from the embryo bank. The number of embryos that have to be cryoconserved for this purpose may be obtained from equation 9. The corresponding values for the number of superovulations (10) and cows take into consideration the factors described above.

$$ES = NH \times (RR \times SR \times CVR \times PR \times SVR \times TRR)^{-1} \qquad (9)$$

ES	= Minimal number of embryos that must be stored
NH	= Number of heifers required for breeding
RR	= Rearing rate
SR	= Sex ratio (number of female calves)
CVR	= Calving rate (number of calves per pregnancy)
PR	= Pregnancy rate
SVR	= Survival rate
TRR	= Retrieval rate (after thawing stored embryos)

Figure 27: Factors Influencing the Establishment of an Embryo Bank

Figure 28: Factors Influencing the Reactivation of an Embryo Bank

$$NS = ES \times (FR \times ECR \times DRR)^{-1} \qquad (10)$$

NS = Number of superovulations required

FR = Freezing rate (number of embryos frozen per number of animals collected)

ECR = Embryo collection rate (number of embryos collected per donor)

DRR = Reaction rate (number of animals responding to superovulation)

The data given in Figures 27 and 28 have been used to calculate, as an example, the number of embryos that must be stored in an embryo bank, the number of superovulations required, and the minimum population size of an endangered cattle species. These values are given in Tables 13–15. Corresponding calculations will have to be adjusted to the special situation encountered in a particular country, especially taking into consideration the breed of cattle.

$$SK = NS \times (MSR \times SR \times AR \times AAR \times CR)^{-1} \quad (11)$$

SK = Minimal size of cattle population

MSR = Multiple superovulation rate

SR = Rate of suitable animals

AR = Number of available animals

AAR = Number of registered animals

CR = Performance control rate

Embryo survival rate (SVR) (%)	Pregnancy rate (PR) (%)				
	20	30	40	50	60
50	616	411	308	247	206
60	513	342	257	206	171
70	440	293	220	176	147
80	385	257	193	154	129
90	342	228	171	1347	114

Table 13: Number of Embryos to Be Stored in an Embryo Bank to Produce 25 Heifers Fit for Breeding by Reactivation from the Bank

The values in Tables 13–15 demonstrate that the establishment of a complete embryo bank requires a great deal of expense and effort.

5.4 Costs Associated with Establishment of Embryo Banks

The establishment of genome reserves is rather expensive. Table 16 gives an overview of the estimated costs, the number of animals, and the time required for reactivation for several alternative approaches (Brem, Graf and Kräuβlich, 1982, 1984).

To a large extent the costs of establishing an embryo bank will depend upon the organizers. Institutions which are already actively engaged in embryo transfer

Flushing rate E/F	Freezing rate (FR)	No. of embryos to be cryoconserved (ES)

	(%)	114	154	220	342	616
6	20	116	157	224	348	626
	30	78	105	149	232	418
	40	58	79	112	174	313
	50	47	63	90	139	251
8	20	87	118	168	261	470
	30	60	79	112	174	313
	40	44	59	84	131	235
	50	35	47	67	105	188
10	20	70	94	135	209	376
	30	47	63	90	139	251
	40	35	47	67	105	188
	50	28	38	54	84	151

Table 14: Number of Embryos in an Embryo Bank Required for Reactivation of 25 Female Animals in Relationship to the Minimal Size of the Bank (See also Table 13) and to Different Flushing and Cryoconservation Techniques

programmes may calculate additional costs of only US $ 250 per embryo (Brem, 1979). These figures do not include compensation for the owners of donor animals. The costs of storage are no higher than those for cryoconserved semen. Calculations were done by assuming standardized conditions for countries providing a suitable infrastructure. However, considerable changes in relative costs may be expected, depending upon the individual situations prevailing in the distribution areas of an endangered cattle breed.

For two reasons financial estimations do not include future costs caused by reactivation of a breed, for example, in 20 years. Firstly it is impossible to calculate costs even for the year 2000. Secondly, the decision when to reactivate a gene reserve must be left to those then in charge because it will depend upon the future economic situation.

Control rate (%)	Rate of registered animals(AAR) (%)	No. of superovulations required*				
		35	63	135	232	626
25	25	997	1793	3843	6604	17819
	50	453	897	1921	3302	8910
	75	302	598	1281	2202	5940
	100	227	449	961	1651	4455
50	25	453	897	1921	3302	8910
	50	227	449	961	1651	4455
	75	151	299	641	1101	2970
	100	114	225	481	826	2228
75	25	302	598	1281	2202	5940

	50	151	299	641	1101	2970
	75	101	200	427	734	1980
	100	76	150	321	551	1485
100	25	227	449	961	1651	4455
	50	114	225	481	826	2228
	75	76	150	321	551	1485
	100	57	112	241	413	1114

* each cow superovulated once

Table 15: Minimal Size of an Endangered Cattle Population in Relation to the Number of Superovulations (See Table 14) and to Several Performance Parameters

In each case reactivation of an embryo rather than a semen bank should be much cheaper because considerably less animals will have to be kept (upgrading for five generations would also require five times the number of animals).

Technique	No. of animals required	Time required (years)	Cost unique (US $)	 running (US $)
small populations	5 m 25 w	-	28000	8500
cryoconserved semen (500 samples)	25 m	10	1400	280
cryoconserved embryos and semen (100 samples)	25 w 25 m	2 2	22500 1400	280 280

Table 16: Costs and Other Parameters for Alternative Approaches in Establishing an Embryo bank

The more serious disadvantage of conserving a breed as a population of live animals is current and recurring expenses. In addition, small populations may easily suffer from problems associated with recessive lethal factors and inbreeding depression due to the relatively high degree of inbreeding.

On the other hand conserving a breed as a population of live animals offers the advantage of analysing animals at all times. If cultural reasons come into play only live animals can be used for conserving a breed.

Reactivation of a pure breed from cryoconserved semen takes a long time, for example at least 10 years for cattle. The low costs for establishing and storing semen and the possibility to prevent the loss of genes by inbreeding or genetic drift certainly favour this approach.