

Biotechnological Advances in Livestock Reproduction

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Introduction

Livestock products such as milk and meat depend on efficient reproduction. Therefore improvement in these products, at least, requires improvement in reproduction. Embryo transfer and other related technologies such as embryo sexing, *in vitro* fertilization, embryo manipulation and cryopreservation aim at improving animal reproduction and breeding performance.

Today, molecular biology and genetic engineering have advanced to such a high level and will likely offer a new dimension in research and development for future application in animal reproduction. However, a large gap between theoretical advances and practical application advances still exist. This paper reviews briefly the status of biotechnology of animal reproduction with reference to application in livestock production.

Embryo transfer

The development of embryo transfer technique has provided livestock producers such advantages as rapid multiplication of superior genotypes, cheaper ways of obtaining superior genotype, better control of animal disease arising from movement and better adaptation to environment. Genetic improvement can be accelerated by increasing the number of off-

springs that could be generated from genomic combination of elite female and progeny tested sires. Moreover, more intense selection can be carried out in a breeding program on females, particularly in nucleus herd, to supply sires for progeny testing.

Reliable superovulation is a key factor to embryo collection¹⁾. Either FSH or PMSG is used to induce superovulation. There are conflicting results with respect to which of the two hormones is superior with respect to the dose and regime of administration²⁾. Non surgical recovery and transfer of embryos are now considered as the method of choice in cattle. Unfortunately, these techniques have not been well developed for the ewe, doe and sow. It is generally accepted that the success of the non-surgical recovery method depends largely on the type of catheter used, skillful placement of the catheter, and manipulation of the uterus³⁾. Surgical transfer seems to be superior with respect to success rate and allow the technician to place the embryos in the most favorable position with certainty and assess the status of the corpus luteum. But non-surgical transfer has an advantage of being a simple field transfer technique. Its success rate has also been improved by the use of protective sheath which prevents contamination of the uterus with vagina micro-organisms.

Although embryo transfer together with artificial insemination has proved to be useful, tools for improving livestock production a lot remains to be done to increase the efficiency and safety of embryo transfer technique. Pregnancy rate has to be raised to apprecia-

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ble level. Testing protocol for most economic diseases is required since some diseases have been found to be carried by embryos.

Embryo freezing

Embryo freezing provides a means of conserving excess embryos especially from elite cows and rare breeds which may become economically important in the future. It allows control of the timing of transfer to recipient female and facilitates transport of genetic material cheaply and conveniently between countries via frozen embryos. With freezing of embryos, geneticists could also measure directly genetic drift. Conventional cryopreservation procedures for freezing mammalian embryos involve dehydration of embryos in the presence of freezing medium containing cryoprotectant, by slow cooling to temperature of -30°C before plunging into liquid nitrogen (Fig. 1). Survival of cryopreserved embryos has been found to depend on such factors as developmental stage of embryos at freezing, state of zona pellucida during freezing and thawing, freezing medium, cryoprotectant and cooling, and freezing and thawing rates⁶⁾. Compacted morula and blastocyst

stages with intact zona pellucida give better results. Glycerol in modified phosphate buffered saline (PBS) supplemented with serum has been one of the most popular freezing medium used. Addition of glycerol has developed from stepwise to one step addition and the stepwise removal of cryoprotectant is being replaced by a one step method which utilizes sucrose. Hence, allowing thawing and transfer to be made in the field.

Experiments are being conducted to reduce or eliminate lengthy cooling and freezing time, thus allowing embryos to be cryopreserved by direct transfer into liquid nitrogen (LN) vapor at -170° to -180°C before being plunged into LN⁸⁾ or by direct plunging into LN. This was made possible because the early-cleavage-stage embryos were initially predehydrated in hypertonic solution containing permeating (e.g. glycerol) and non-permeating (e.g. sucrose) cryoprotectant. Another new approach to preservation of embryos is vitrification. It refers to the physical process by which concentrated solution of cryoprotectants solidify into an amorphous glass-like solid form during cooling without crystallization⁹⁾. It is necessary that both extracellular and intracellular com-

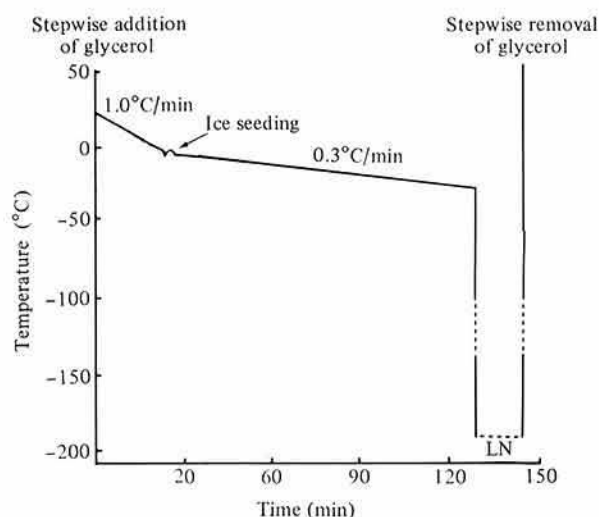


Fig. 1. Illustration of typical cooling curves during the conventional freezing and thawing procedures

ponents are vitrified simultaneously. This procedure is said to be suitable for field use because of its simplicity, however efficiency of this procedure is yet to be tested.

Embryo manipulation

Micro-surgical manipulation has made it possible not only to split embryos into several parts but also to inject genetic material into

embryos. Production of twins arising from embryo splitting has been reported in all kinds of livestock⁹⁾ and is being exploited commercially (Fig. 2). It provides a means of producing homozygous genotype.

Transgenic mice expressing exogenous genes have been produced successfully by injecting genes into the male pronucleus at the 1-cell stage or by introducing the genes by means of retroviral vectors at later em-

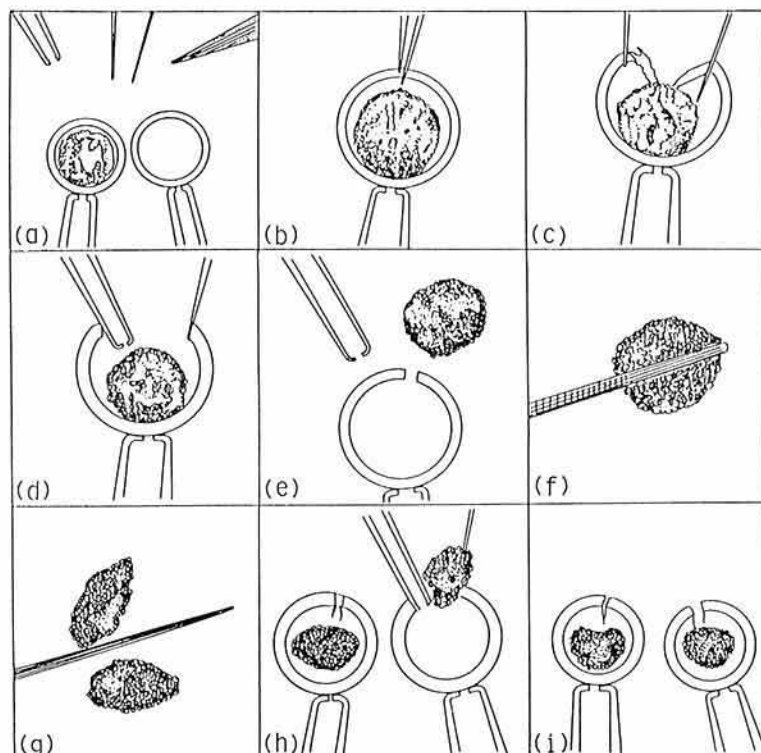


Fig. 2. Diagrammatic illustration of bissection of the cow blastocyst by micromanipulation

- (a) Six microinstruments are placed in the optical field of an inverted microscope. Embryo is immobilized by suction with a holding pipette.
- (b, c) Two sharp microneedles cut the zona pellucida along the middle. The blastocyst is subsequently rotated by 90° with the two microneedles to show the slit.
- (d, e) The micropipette is introduced through the slit inside the zona pellucida while a small volume of medium is injected to expel the embryo.
- (f, g) Bissection of the blastocyst is achieved using the microscalpel along the sagittal plane.
- (h, i) Using the suction of the microneedle each half embryo is put back into an empty zona pellucida.

bryonic stages or into embryonic cell lines. However, this is not the case of livestock due to the huge undertaking in injecting DNA into several hundred embryos and transferring them. In addition, there is the problem of dense cytoplasm that makes the visualization of pronuclei and nuclei very difficult. This problem is circumvented by centrifugation of the embryos to make the pronuclei visible. Transgenic rabbits, sheep and pigs have been reported but the frequency of

integration and expression of the gene injected was much lower than in mice. Estimate for the production of transgenic animals per 100 injected embryos transferred to recipient female is said to be 1 for pig and 0.1 for sheep¹⁾.

Chimeric animals are produced by aggregation of embryos during early cleavage after removal of the zona pellucida or by the injection of embryonic cells into the blastocoel cavity. Aggregation of embryos also provides

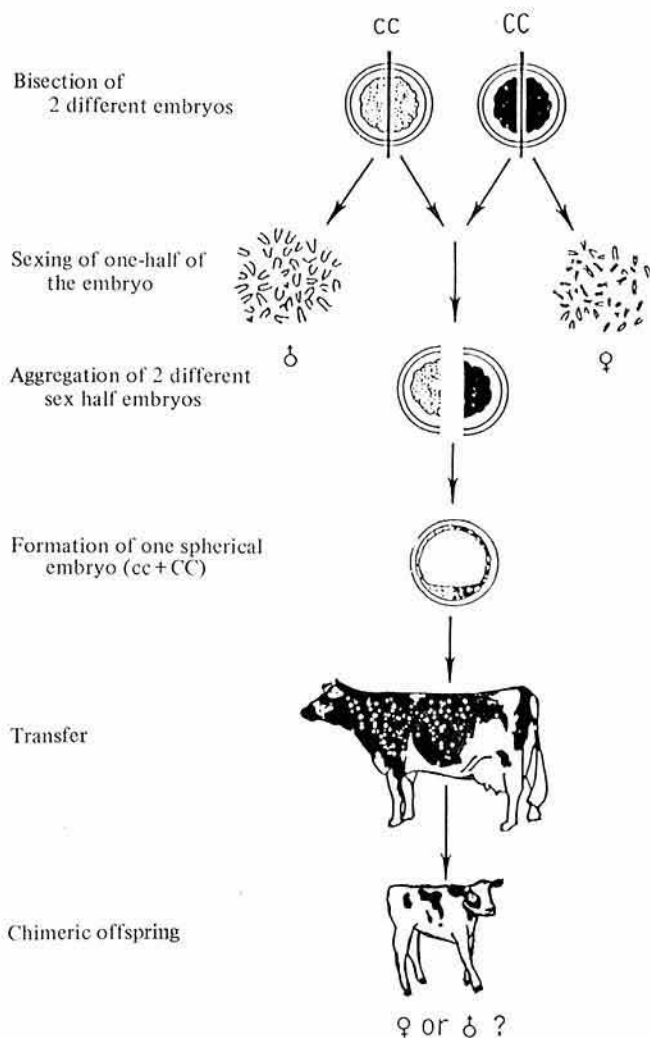


Fig. 3. Hypothetical protocol for the formation of chimeric cattle using sexed bisected embryos

What will the sex of the offspring be when two embryos of different sex were used for aggregation ?

a means of getting animal models which can be used for the study of embryology and developmental genetics since each cell can contain species-specific markers that distinguish it from cell of other species (Fig. 3). Chimeric rabbit, ovine, bovine and sheep-goat have been produced by inner cell mass transplantation. While the economic potential of chimera in domestic species is not obvious, the production of interspecific chimera may well be an important means of propagating endangered species especially if this can be done from embryonic cell lines.

Embryo sexing

Generally fewer males are required for reproduction, in livestock, than females. Therefore artificial sexing of progeny can make it possible to determine the sex of offspring as required. Determination of sex can be achieved either by inseminating females with a homogeneous population of either Y-bearing or X-bearing spermatozoa, or by identifying embryonic sex prior to transfer to recipients. Reliability of X- and Y-bearing spermatozoa separation techniques is very low in livestock and few alter the sex ratio sufficiently for the technique to be economically viable.

The various techniques currently being tested for sex determination of mammalian embryos include cytogenetic analysis (e.g. karyotyping) of some embryonic cells, measuring differences in metabolic activity (e.g. HPRT and G6PD) between male and female embryos, DNA hybridization by the use of a probe which is specific to Y-chromosome DNA and immunological detection of a sex-specific factor (H-Y antigen). Accuracy of identification of sex by chromosomal analysis of some cell removal from 8- to 17-cells bovine morula ranges from 30 to 60% while as for using measurement of metabolic activity of embryos, reported only in mice, is 64%. In the case of the H-Y antigen method, 84%, 81% and 85% accuracy rates have been reported for cattle, pigs and sheep, respectively. H-Y antigen is said to be readily detectable

on 8-cell and morula stages only and has been suggested that the fluorescent H-Y antibody techniques may become a useful tool in bovine sex determination in the future. However, more studies are required to refine the techniques so as to produce an efficient embryo sexing procedure for farm animals.

In vitro fertilization

This technique can provide the much needed livestock embryos for research pri-

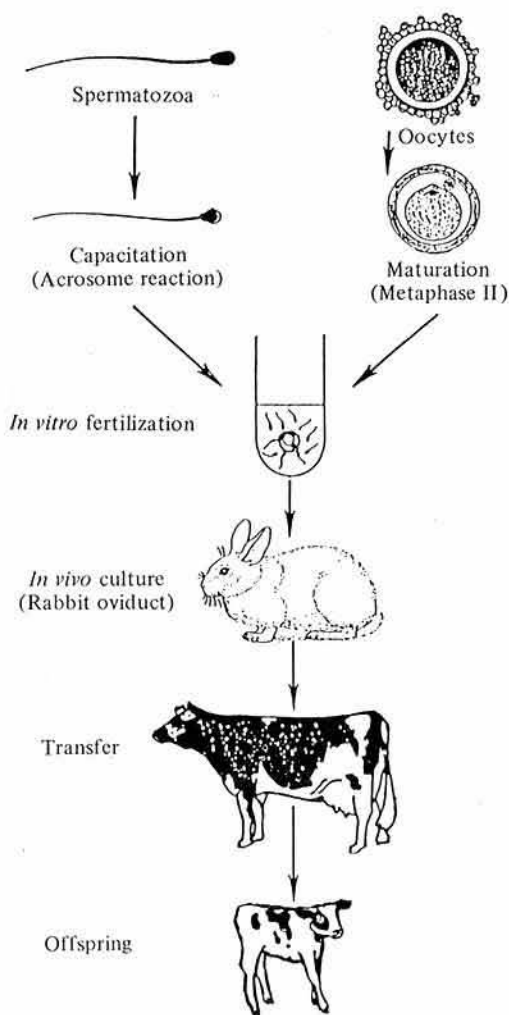


Fig. 4. Stages involved in bovine *in vitro* fertilization of embryos from capacitation of spermatozoa and maturation of oocytes to their final transfer into a recipient

marily and for transfer to recipients in countries where even a small genetic improvement is welcomed. It provides the potential use of ovarian material obtained from the slaughter house.

Reports on live young produced after *in vitro* fertilization have been made in cattle, goat²⁾, sheep and pig¹⁰⁾ (Fig. 4). However, the number of live young produced is still small and the overall success rate is variable. The oocyte maturation and capacitation of spermatozoa are the two technological barriers which cause difficulty in *in vitro* fertilization in farm animals rather than the type of oocyte and culture medium.

Application in developing countries

Other than poor nutrition, poor health and low management practices which exist in livestock industries of most developing countries, indigenous animals have generally low genetic potential in most traits of economic importance compared to those animals in developed countries. To improve these animals, it is generally recommended that indigenous animals be upgraded by crossing with exotic breeds from developed countries. Thus the primary application of embryo transfer is in the introduction of exotic germ-plasm through embryos. This approach has much reduced disease risk, ease of adaptation, ease of transport and possible reduced cost. The secondary application is in the preservation of indigenous breeds, which could be extinct as a result of indiscriminate cross-breeding, through the establishment of embryo banks and rapid multiplication in nucleus herds.

However, basic scientific infrastructure and

supporting facilities are necessary for successful implementation of embryo transfer program. These already exist in some developing countries and in countries where they do not exist every effort should be made to establish them.

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