Rev. sci. tech. Off. int. Epiz., 1985, **4** (4), 843-858.

Embryo transfer in the cow: general procedures*

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Summary: Since the production of the first calf by embryo transfer in 1951, the method has been progressively improved so that it can now be used for commercial ends.

The applications of this method are numerous in the field of research, but its main economic interest is in bovine selection programmes. Selection intensity in females can be doubled in elite herds, thus increasing the possibility of producing high yielding bulls and heifers.

The embryo transfer technique can also be used for detecting recessive traits, the production of twins in beef cows, the salvage of genetic material from terminally ill cows or infected herds.

International transport of embryos has been made possible by progress in freeze-thaw procedures, but depends upon the resolution of regulatory problems and the training of transfer personnel.

Surgical methods for embryo collection and transfer are increasingly replaced by non-surgical methods, which are much more practical and less expensive in selection programmes.

Advances in research on in vitro *fertilization, production of twins or multiplets, sexing, manipulation of embryo genetic stock and other genetic engineering techniques are discussed.*

KEY-WORDS: Cow - Disease control - Embryo - Fertilisation - Freezing - Genetics - Micromanipulation - Preservation - Sex - Thawing - Transplantation - Twin birth.

INTRODUCTION

Walter Heape reported in 1890 that a litter of rabbits resulting from embryo transfer had been born in his laboratory (1). He could not have imagined the impact that discovery would have. Although considerable research on the collection and transfer of bovine embryos was conducted during the 1930's and 1940's (2), the first calf produced as a result of egg transfer was born in Wisconsin in 1951 (3). Interest in embryo transfer increased as studies involving mammalian eggs began to appear in the literature, starting in 1950; by 1977 there were nearly 1,000 articles on embryo transfer in the literature, with an increasing number concerned with cattle.

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* Report presented at the OIE/IETS Round Table Meeting on sanitary problems related to embryo transfers. Paris, 9 December 1985.

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It was in the early 1970's that great commercial interest in bovine embryo transfer developed. European dual-purpose breeds of cattle were imported to North America and, because of their relative scarcity, became extremely valuable. As a result, there were considerable economic incentives for the application of embryo transfer procedures : breeders wanted a method to increase the reproductive rate of these females for the profitable sale of their offspring. Thus, the technology of embryo transfer in farm animals was developed with money from cattle breeders rather than through traditional research funding (4).

APPLICATION

Over the years, techniques associated with embryo transfer have had many uses, especially in research. The widespread use of this technology in animal breeding schemes, however, is relatively recent. Genetic engineering and related technologies will only increase its utilization. In fact, many laboratories are presently using *in vitro* fertilization techniques to study the fertilizing capacity of sperm (5, 6, 7). A few of the more common uses of embryo transfer technology in animal production follow.

Genetic improvement

Genetic progress is considered to be slower through the use of embryo transfer than it is using conventional artificial insemination (AI), especially on a national herd basis (8). However, with increased selection intensity and shortened generation intervals, i.e. transferring female offspring, genetic gain can be made on a within-herd basis (9). The production of about six offspring per donor cow could double selection intensity and the rate of response to genetic selection for traits such as growth that can be measured in both sexes (10, 11, 12). This would be especially worthwhile in improving elite herds, the genetics of which could be spread over a large population through the use of AI. Although the rate of genetic improvement in dairy cattle may range from 2.5-8%, Seidel suggested that through the use of embryo transfer it could be increased three to four times if dairy replacements were selected from the top 10% of the herd (13). Embryo transfer is now commonly used to produce AI sires from the very best proven cows and bulls available. Economics would not at this time support the use of embryo transfer techniques for anything but seedstock production.

Planned matings

By far the most common use of embryo transfer in animal production programmes is the proliferation of so-called desirable genotypes (4). AI has permitted the widespread dissemination of a male's genetic potential. Embryo transfer provides the opportunity of disseminating the genetics of proven elite females (14). Embryo transfer also permits the development of herds of genetically valuable females, most of which may be sibs if not full-sibs. As AI has led to the very valuable bull, now embryo transfer has resulted in the very valuable female. Many breeders have identified individual females whose offspring are most saleable and used them exclusively in embryo transfer. Embryo transfer has also been used to rapidly expand a limited gene pool. The dramatic rise of the embryo transfer industry in North America is a direct result of the introduction of European breeds of cattle, which were then in short supply.

Genetic testing for Mendelian recessive traits

A common use of embryo transfer procedures is to genetically test AI sires for deleterious heredity traits (15). Some AI organizations keep known carriers of certain genetic defects on hand to serve as donors in testing new sires. Embryos are transferred into unrelated recipients and pregnancy may be terminated at various stages to examine fetuses for presence or absence of the defect. Depending on the heritability of the defect, generally eight to ten non-affected fetuses are sufficient to declare a bull free of that trait. Another alternative is to mate the bull in question to seven or eight of his superovulated daughters. Offspring will then represent all recessive traits that a bull may carry. A less attractive alternative would be to naturally mate the bull in question to 40-50 of his daughters.

Twinning in cattle

Compared to other domestic animals, beef production is somewhat less efficient because not all cows produce a calf each year. However, it has been estimated that unit beef production can be increased by 60% in intensively managed herds through twinning (16). Approximately 70% of nutrient intake by a beef cow is utilized for her own maintenance, whereas only about 30% is for growth and maintenance of the calf during pregnancy and lactation (13). Thus, it seems desirable to take advantage of the efficiency of gestation and lactation. Genetic selection for twinning in cattle has been largely unsuccessful (16) and gonadotrophin treatments to induce twinning have also been unreliable (17). Embryo transfer does provide a very real alternative in the production of twins (18).

The most limiting factor at this time is the cost of transfer which continues to exceed the average price for calves to be raised for meat (4). The nonsurgical transfer of a previously frozen embryo to a recipient that has already been serviced may become an economical method of producing twins. Other alternatives include the nonsurgical transfer of two or three frozen embryos to commercial beef cows (19).

It must be remembered that recipients carrying twins will require extra nutrition and management, especially around calving. Furthermore, recipients must be sufficiently large to carry twins and produce enough milk to feed twins (20). Freemartins, make twinning unattractive for purebreeding purposes. However, the incidence is relatively low and sexing of embryos would eliminate the problem entirely. Similarly, the production of identical twins by microsurgery would make twinning very feasible for embryo transfer programmes in purebred herds (21).

Disease control

Infectious diseases in the bovine species seem unlikely to be transmitted by the embryo (22). Consequently, it has been suggested that embryo transfer be used to salvage genetics in the face of a disease outbreak (23). This may be a useful alternative in the establishment of dairy herds that are free of bovine leukosis as this virus was not transmitted with embryos (24). However, much research has yet to be done on embryo-virus interactions before this technique can be carried out with complete confidence.

Import and export

The intercontinental transport of a live animal may cost $1,000 or more, whereas an entire herd can be transported, in the form of frozen embryos, for less than

the price of a single plane fare. This may be the single most important potential application of embryo transfer. Additional benefits of the export of embryos over that of live animals include a wider genetic base from which to select, the retention of genetics within the exporting country and adaptation. This is particularly true of tropical and subtropical climates where the embryo would have the opportunity to adapt both in the uterus and then suckling a recipient indigenous to the area.

There are several potential problems which must be overcome in order to make the international movement of embryos commonplace. Firstly, this use is dependent on the successful freezing of embryos. Secondly, the inadvertent introduction of disease into a herd and/or country with or within the embryo presents some very difficult regulatory problems. Well-defined methods of collection, handling and washing embryos must be followed to ensure that disease transmission is avoided. Finally, the international movement of embryos is heavily dependent on technology transfer as personnel within the importing country must be able to successfully thaw and transfer embryos.

Salvage of reproductive function

Embryo transfer procedures have been useful in the diagnosis, treatment and salvage of reproductive function in so-called infertile cows (25, 26, 27). Although it is recommended that the cause of the infertility not be of genetic origin, this is often difficult to determine. The marketplace should sort this out if a genetic problem were inadvertently propagated through embryo transfer.

Another very important use of embryo transfer is to salvage the genetics of terminally ill animals. It may be possible to produce an additional two or three offspring through embryo transfer before the animal dies. The fact that the animal may not be cycling can be overcome through the use of progestational steroids given by injections or implant (28, 29).

Research

Embryo transfer techniques have proven to be a very useful research tool. In fact, many technical developments in embryo transfer prior to 1970 were directed toward research purposes rather than for the propagation of superior livestock. These studies included natural limitations to twin pregnancies, uterine capacity, endocrine control of uterine environment, maternal recognition of pregnancy, embryo-endometrium interactions, and the endocrinology of pregnancy (30). Studies that were originally planned to answer basic physiologic questions are now being used to improve and increase the utilization of embryo transfer. Newer techniques have added an entirely new perspective to the utilization of embryo transfer for research purposes. The production of identical twins, clones, chimeras, to mention a few, will certainly advance many of these sciences (31).

DONOR SELECTION

Reasons for wanting to do embryo transfer on a given animal are more often economic than genetic. As optimal results will reduce costs, making the procedure much more economical, donor selection may involve a previous history of success in embryo transfer. In addition, it has been suggested that the potential donor animal be at its prime reproductive age, that it has a previous history of a high level of

fertility and that it has demonstrated superiority in traits of economic importance (19, 32). Strict selection criteria will not only ensure genetic superiority, but should also ensure a high level of success thereby making the procedure more economical.

EMBRYO TRANSFER IN THE COW

Embryo transfer technology has been most extensively applied in the bovine species. Not only is there an economic incentive, but the cow is well suited to complete utilization of nonsurgical techniques making the procedure more cost-effective. Finally, the cow is easy to work with making cattle excellent experimental animals.

Although it is possible to collect single embryos from the cow with good success and through the use of prostaglandin (PGF)2a to do so every 10 days, most cows are superovulated prior to embryo collection. The cow seems to superovulate best if gonadotrophin treatment is begun around day 10 of the cycle (33, 34, 35). Forty-eight to 72 h after initiation of treatment, the cow is treated with a luteolytic dose of PGF and she is normally in heat 36-48 h later. Recipients are treated with PGF 12-18 h before the donor cow so that oestrus will be synchronous between donor and recipients. As multiple ovulations have been shown to take place over as long as 24 h, multiple inseminations are usually done. Normally the donor cow is inseminated with a single vial of semen at 12, 24 and 36 h after the onset of oestrus.

Pregnant mares' serum gonadotrophin (PMSG) as a single injection of 2000-3000 IU (36,37), or twice daily injections of a total dose of 28-50 mg porcine pituitary follicle stimulating hormone (FSH-P) have been most commonly used to superovulate cows (32, 38, 39). Most have found superovulatory results to be superior with FSH-P than with PMSG (40). It seems that this may be associated with the shorter biological half-life of FSH-P.

Initially, embryos were collected surgically from the cow around day 4 after oestrus (41). However, three reports appeared in the same journal on the successful nonsurgical collection of bovine embryos in 1976 (42, 43, 44). Basically, techniques described involved the passage of a cuffed rubber catheter through the cervix and into one of the uterine horns on days 6 to 8 after oestrus. Embryos were then collected by a closed continuous or interrupted flow system (32), or by an interrupted syringe technique (38).

Embryos are located with a stereoscopic microscope after settling and syphoning or aspiration (38), or after filtering through a plankton filter (45). Although embryos are usually transplanted as soon as possible after collection, it is possible to culture embryos for several hours at 35-37°C. If transfers are to be done soon after collection, embryos may be maintained at room temperature. It is also possible to cool bovine embryos and to maintain them in the refrigerator for 2-3 days (46). As a final alternative, embryos may be frozen for use at a later date.

Embryos are normally cultured in the same or a similar medium to that in which they were collected. Media must be buffered to maintain a pH of 7.2-7.6 and have an osmolarity around 300 mO (7). Traditionally, embryos were collected in Tissue Culture Medium 199 (TCM 199) enriched with bovine serum albumin or heat inactivated fetal calf serum (FCS) and antibiotics. However, as TCM 199 requires CO_2 for its buffer system, it has largely given way to Dulbecco's phosphate buffered

saline (PBS) enriched with FCS and antibiotics. Glucose and pyruvate are often added for longer terms of culture. Normally, enriched PBS with 1-2% FCS is used for flushing embryos from the uterus, whereas, embryos are cultured in the same medium with 15% FCS. There is evidence that more complex media such as Ham's F-10, which has a carbonate buffer, yield superior results for long-term culture of bovine embryos.

Embryos are classified and evaluated by morphological examination at 50-100× magnification (32, 47, 48). The overall diameter of the bovine embryo is 150 to 190 μm including a zona pellucida thickness of 12-15 μm. The overall diameter of the embryo remains virtually unchanged from the one-cell stage until blastocyst expansion (48). Generally, the embryo is described as to its stage of development and quality. The best predictor of an embryo's viability is its stage of development relative to what it should be on a given day after ovulation. Embryos of good and excellent quality and at the developmental stages of late morula to blastocysts yield the best pregnancy rates. It is advisable to select the stage of embryo for the synchrony of the recipient.

Transfers of embryos in the cow can be made with good success only if the preceding oestrus in the donor and recipient occurred within 2 days of each other. Alternately, recipients must be synchronous with the stage of development of embryos that had been frozen previously. Recipients can be made available by maintaining a large herd to obtain natural heats or recipients may be synchronized with PGF which is much more economical. Most recipients are chemically synchronized regardless of whether embryo transfers are to be done at an embryo transfer centre or "on farm".

Until very recently, most embryo transfers in the cow were done surgically (49), whereas, at present, most are done using nonsurgical methods (50, 51). Surgical transfers were done initially by way of a mid-line incision which necessitated a general anaesthetic and rather elaborate facilities. During the mid to late 1970's, surgical transfers were done by way of a flank incision which was quicker and did not necessitate the same sophistication in facilities. This made "on farm" embryo transfer possible and added a whole new perspective to the use of embryo transfer in cattle production schemes. More recently, the use of nonsurgical embryo transfers has increased the utilization of this technology in cattle breeding schemes because of even less elaborate requirements (52, 53).

For surgical transfers, the uterine horn adjacent to the ovary bearing the corpus luteum (CL) is exteriorized and the embryo is deposited through the uterine wall into the uterine lumen with a Pasteur pipette (49) or an intravenous catheter (38). Nonsurgical embryo transfer techniques utilized today involve the use of a Cassou AI pipette or some similar apparatus (50, 51, 54). The embryo is placed in the uterine horn adjacent to the ovary bearing the CL by passing the pipette through the cervix, very similar to AI. Practice and dexterity seem to improve one's ability to achieve high pregnancy rates suggesting that trauma to the endometrium may be a limiting factor in this method of embryo transfer. Stimulation of the cervix and inadvertent introduction of bacterial contaminants do not seem to be major determinants under normal circumstances (51, 54). With practice and attention to detail, pregnancy rates with nonsurgical transfers can approach that of surgical transfers (51, 55).

With existing technology, an average for each donor cow superovulated would be eight to ten ova collected, six to seven embryos transplanted and three to four

pregnancies resulting. It must be emphasized that very few donor cows are average. Pregnancy rates are generally around 60% with fresh embryos and range from 30%-40% with frozen embryos. One can anticipate a fetal death loss of 10% from pregnancy diagnosis until the calf is six months old (4).

LONG-TERM PRESERVATION OF EMBRYOS BY FREEZING

Successful embryo freezing has many applications in embryo transfer programmes. Firstly, recipient management is improved and made more cost-effective. In addition, season of parturition can be controlled, even though embryo collection and freezing may take place year around. Embryo freezing also allows progeny and performance tests of sibs to be conducted more rapidly and efficiently. Further, full sibs or identical sibs can be frozen until the genetic worth of those transferred can be established. Finally, embryo freezing is necessary for international movement of embryos because it eliminates critical timing and allows disease testing while the embryos are held in quarantine.

Basic principles

Cellular freezing constitutes a complex physiochemical process of heat and water transport between the cell and its surrounding medium (56, 57). There exists an optimum cooling rate for each cell type. It is dependent on the size of the cell, its surface to volume ratio, its permeability to water, and the temperature coefficient of that permeability (58, 59).

Cells are injured during freezing and thawing primarily by solution effects and intracellular ice formation (57). At high cooling rates the dehydration of the cell falls behind that of the solution and intracellular ice forms. To avoid intracellular freezing, embryos must be cooled at 1°C/min or slower. However, too low a rate of cooling can also damage cells by what has been referred to as solution effect. This is especially harmful if cells are not allowed to rehydrate during thawing. The required thawing rate depends on the freezing regimen used. The more rapid techniques of freezing and thawing are preferred for field work.

Embryos are normally stored in liquid nitrogen at −196°C. The only reactions that occur at −196°C are direct ionizations from background radiation. Consequently, storage times of 200 years or so are unlikely to produce any detectable reduction in the survival of frozen embryos or cause genetic change (57).

Cryoprotectants such as glycerol or DMSO in concentrations ranging from 1.0 to 2.0 M are required to ensure embryo survival after freezing. It is thought that cryoprotectants act by reducing the amount of ice present at any temperature during freezing, thereby moderating the changes in solute concentration. During the addition and dilution of a permeating cryoprotectant, the cell undergoes osmotic changes in cell size. As a consequence, if the addition or particularly the dilution are carried out inappropriately, the viability of cells can be affected (57). There is clear evidence that the rate of removal of cryoprotectants is critical (60). The standard, empirical, method is to dilute it by step-wise addition of PBS or to pipette the embryos into decreasing concentrations of cryoprotectant solution, e.g. 0.25 M steps.

Leibo and Mazur suggested a modification in the procedure of cryoprotectant removal by including nonpermeable solutes like sucrose into the dilution medium

(60). The sucrose acts as an osmotic counterforce to restrict water movement across the membranes. As the cryoprotectant leaves the embryo, it will shrink in response to the extracellular hypertonic dilution medium. It regains its normal volume when at the end of the process the embryo is placed in normal isotonic culture medium. Using this information, practical methods of quickly removing glycerol from thawed embryos have been devised (61, 62). As a result, a one-step straw has been developed so that embryos can be thawed, solutions mixed within the straw and transfer done nonsurgically to the recipient.

Freeze-thaw procedures

The following protocol (57) has been proposed for the cryopreservation of day-7 bovine embryos in PBS supplemented with pyruvate, glucose, FCS and glycerol (1.0 M to 1.5 M). Embryos are pipetted into the freezing medium at room temperature (20°C) and kept for 8-10 minutes to permit the glycerol to equilibrate. During this equilibration period the embryo(s) are transferred in volumes of 0.2 to 0.5 ml of freezing medium into vials or French straws which are then securely sealed. The samples can be immediately transferred into the freezing chamber at −7°C and held for 5 minutes. Crystallization (seeding) of the extracellular medium is initiated by touching the outside wall of the straw or vial with cold forceps precooled in liquid nitrogen. The samples are kept at the seeding temperature for 5-10 min to allow the crystallization of the medium to progress to equilibrium. Next embryos are cooled at 0.3° to 0.8°C/min to between −30°C and −40°C, at which time they are immersed into liquid nitrogen and stored. Thawing is carried out by placing the straw or vial into a waterbath at 35°C.

The cryoprotectant must be removed without causing osmotic damage. The method of choice is dilution in a sucrose-PBS medium. A sucrose solution approximately isosmolal with the glycerol is added to the freezing medium at room temperature in a volume ratio of 10:1 for about 5-10 minutes. Alternatively, embryos are moved through decreasing concentrations of glycerol in 0.25 M steps of 10 minutes each. The embryos are then pipetted into PBS culture medium and evaluated prior to transfer.

Success rates

Similar procedures have resulted in the successful freezing of morula and blastocysts from cattle. Survival rates of cryopreserved embryos are steadily improving. Only a few years ago pregnancy rates from transferred frozen-thawed bovine embryos was about half of that obtained from transferred non-frozen embryos. At present, overall pregnancy rates of 40% are being reported for frozen-thawed cattle embryos (53). However, unless all steps are controlled closely, pregnancy rates from day 7 bovine embryos may vary considerably among different donors. The extent of variation can be an important factor in the cost effectiveness of a programme.

MICROSURGICAL PROCEDURES AND GENETIC ENGINEERING

Genetic engineering might be narrowly defined as modification of the chemical structure of DNA or RNA. However, Rutledge and Seidel have broadened the definition to include any directed change in the genetic make-up of a population (63).

AI and embryo transfer are clearly useful tools for genetic engineering. Microsurgery of the embryo is yet another generation of genetic engineering which could have profound effects on animal breeding.

In vitro fertilization

The most difficult aspect of *in vitro* fertilization is capacitation of sperm. Ejaculated mammalian spermatozoa seem to be incapable of fertilizing ova until they are capacitated, a process that normally takes place in the female reproductive tract over a variable period of time, depending on the species. Capacitation has also been accomplished *in vitro* in many species (64).

The procedures used for *in vitro* fertilization in farm animals have been reviewed (5, 6, 7). The most common measure of fertilization is division to the 2-cell stage, but parthenogenesis and fragmentation of the ovum are difficult to distinguish from cell division of fertilized embryos. The recent birth of a genetically marked calf was the first unequivocal evidence of successful *in vitro* fertilization in farm animals (65). It may be some years before *in vitro* fertilization with gametes of domestic animals becomes a routine procedure. However, this initial success has demonstrated its feasibility and the various potential applications are overwhelming.

Prenatal sex selection

Although considerable research effort has been directed toward sexing sperm over the past several years, there seem to be no reliable methods available at this time. The potential application of sperm sexing, especially in humans and cattle, makes this an intense area of research. The control of sex prior to fertilization is certainly preferable to control after fertilization. However, the embryo seems to be a more likely candidate for sexing at this time. Biopsy and karyotyping procedures are tedious, time-consuming and relatively inaccurate, making them impractical for routine commercial use (66, 67). An alternative in the sexing of embryos is to use an antibody to the H-Y antigen, a protein found on the cell membrane of male mammalian cells (68, 69). The 8-cell embryo apparently has sufficient H-Y antigen for detection if the ovum were fertilized by a Y-bearing sperm. Antibodies to H-Y antigen bind to the male embryos which are then detected by one of two methods (69). The first is to add complement, which results in death of the male embryos. The more practical method is the second antibody method which consists of producing a fluorescent-labelled antibody to the first antibody so that the male embryos will fluoresce in appropriate light. This procedure was about 80% accurate for sexing mouse embryos (69) and is being developed by several companies for use with bovine embryos.

Micromanipulation

The simplest method of making identical twins is to separate the cells of 2-cell embryos and allow each half to develop independently (70). Half and quarter embryos have also been used to produce identical quadruplet sheep, triplet cattle, and twin horses and pigs (70, 71). Success rates are fairly high for identical twins but drop off considerably when identical quadruplets are attempted. However, identical quintuplets have been produced by mixing cells of 4- and 8-cell embryos. The blastomeres of the 8-cell embryo apparently developed into fetuses while those of the 4-cell embryos formed placentas (31, 72).

A simpler method of producing identical twins from morulae and early blastocysts involves the microsurgical division of the embryo into two groups of cells followed by immediate embryo transfer (73, 74). This procedure takes approximately 10 minutes per embryo, and can be applied during embryo transfer procedures providing proper equipment and appropriately trained personnel are available (75). Pregnancy rates of 50% or more per demi-embryo were reported in cattle, which results in a net pregnancy rate in excess of 100% per original embryo.

Methods of making identical multiplets are, in many respects, methods of cloning, as genetically identical animals are produced asexually. Sometimes cloning is defined in a more narrow sense such as nuclear transplantation. Nuclear transplantation was apparently accomplished in a 1-cell mouse ovum utilizing the donor nucleus of an embryonic cell (76). Although very few mice have been born as a result of such procedures, one could theoretically make hundreds of genetically identical animals in this way by serial nuclear transplantation.

Chimeras

One of the most powerful embryological techniques ever devised is aggregation of cells of two or more preimplantation embryos to produce offspring that have cells from more than one cell line. The most common procedure is to aggregate two 4-cell mouse embryos which would result in a tetraparental mouse (77). Another method is to inject cells of one embryo into the blastocoelic cavity of another (78). Although some of the chimeric animals so produced are likely to be useful, there is no way, at this time, of ensuring that the intended genotype is incorporated into the embryo. The numbers of other possibilities with the production of chimeras are almost limitless. Considerable future research effort will undoubtedly be conducted in this area.

Addition or modification of DNA

Recently, in two separate studies, the DNA for rabbit beta-globulin (79) and for rat growth hormone (80), was injected into pronuclei of 1-cell fertilized mouse ova. In some instances, the injected DNA was integrated into the genome and expressed and transmitted to future generations. This procedure permits modification of genetic material in a fundamentally different way from conventional procedures. One might add genes for anatomical and production traits or disease resistance, directly to embryos rather than breeding for it by selection methods. Furthermore, genetic DNA does not seem to be species specific. Genetic engineering utilizing this technology has great potential in animal production schemes.

Future considerations

The sciences associated with genetic engineering technology are advancing at a rate that is practically inconceivable. The limits of this technology seem only to be bound by one's imagination. For example, sperm injection techniques, nuclear transplantations and the manipulation of individual genes and chromosomes are potentially very useful in animal breeding schemes. Whereas cloning is likely to only produce a copy of a genetically desirable animal, techniques associated with genes and DNA have the potential of producing a so-called super animal. As research tools the value of genetic engineering techniques are virtually limitless.

Seidel points out that genetic engineering may be overrated as a short-term means of improving animal production (77). For example, fewer than 5% of beef

cows in North America conceive by AI although this is proven technology that is inexpensive and easy to apply relative to most genetic engineering techniques. Because a technology is available, does not mean that it will be widely applied. However, a few spectacular successes are bound to capture the imagination of some and the incentive to apply these techniques in animal production schemes will be there. It is unlikely that anyone would have predicted 10 years ago that embryo transfer technology would have evolved to its present status.

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LE TRANSFERT D'EMBRYONS CHEZ LA VACHE : MÉTHODES GÉNÉRALES. — R.J. Mapletoft.

Résumé : *Depuis la production du premier veau par transfert d'embryon en 1951, la méthode a été progressivement améliorée et peut désormais être utilisée à des fins commerciales.*

Les applications de cette méthode sont nombreuses dans le domaine de la recherche mais son enjeu économique se situe surtout dans les programmes de sélection bovine. L'intensité de sélection des femelles peut être doublée dans les troupeaux d'élite, accroissant ainsi les possibilités de produire des taureaux d'insémination et des génisses à hautes performances.

La technique du transfert d'embryons peut être utilisée aussi pour la détection de caractères récessifs, la production de jumeaux chez les vaches allaitantes, la conservation du matériel génétique de vaches définitivement malades ou de troupeaux infectés.

Le transport international des embryons est devenu possible grâce aux progrès de la congélation-décongélation, mais nécessite que soient résolus les problèmes de réglementation et de formation du personnel de transfert.

Les méthodes chirurgicales de récolte et d'implantation des embryons sont de plus en plus remplacées par les méthodes non chirurgicales, beaucoup plus pratiques et moins coûteuses dans les programmes de sélection.

L'auteur présente l'avancement des recherches sur la fécondation in vitro, *la production de jumeaux ou de multiplés, le sexage, la manipulation du stock génétique des embryons et les autres techniques de génie génétique.*

MOTS-CLÉS : Congélation - Conservation - Décongélation - Embryon - Fécondation - Génétique - Micromanipulation - Naissance de jumeaux - Prophylaxie sanitaire - Sexage - Transplantation - Vache.

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TRANSFERENCIA DE EMBRIONES EN LA VACA : MÉTODOS GENERALES. — R.J. Mapletoft.

Resumen : *Desde que se produjo el primer ternero por transferencia de embrión en 1951, se ha ido mejorando progresivamente el método, por lo que se le puede utilizar en lo sucesivo para fines mercantiles.*

Son muchas las aplicaciones de este método en el campo de la investigación, pero su transcendencia económica se ubica sobre todo en los programas

de selección bovina. La intensidad de selección de las hembras puede llegar a ser doble en los rebaños selectos, al incrementarse así las posibilidades de producir toros de inseminación y terneras con altas características.

Se puede usar asimismo la técnica de transferencia de embriones para detectar carácteres recesivos, producir gemelos en las vacas lactantes, conservar el material genético de vacas definitivamente enfermas o de rebaños infectados.

El transporte internacional de embriones ha llegado a ser factible en base a los progresos de la congelación y descongelación, aunque es preciso que se solventen los problemas de reglamentación y capacitación del personal de transferencia.

La tendencia actual es que los métodos quirúrgicos de recolección e implantación de embriones están siendo sustituidos por métodos no quirúrgicos, aunque más prácticos y de costo menos elevado en los programas de selección.

Presenta el autor el avance de las investigaciones de la fecundación in vitro, *la producción de gemelos o de multillizos, sexaje, manipulación de la reserva genética de los embriones y demás técnicas de ingeniería genética.*

PALABRAS CLAVE : Congelación - Conservación - Control sanitario - Descongelación - Embrión - Fecundación - Genética - Micromanipulación - Nacimiento de gemelos - Sexaje - Transplante - Vaca.

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