Appendix II-C

Animal Fertilization Technologies

Sperm storage

DEFINITION

The freezing of semen to – 196° C, storage for an indefinite time, followed by thawing and successful insemination.

STATE OF THE ART

Conception rates at first insemination with frozen sperm average between 30 to 65 percent for most species. This technology is not a key to the success of artificial insemination (AI), but because of the convenience it is now an essential ingredient. Current operational procedures are adequate for the dairy industry.

ADVANTAGES

1. Greater use of selected bulls as AI studs.
2. Elimination of the need to maintain expensive and dangerous bulls on dairy farms.
3. Sperm can be tested for disease and treated for venereally transmitted diseases.
4. Ease of transport and therefore of increasing potential offspring.

FUTURE

Little change is anticipated in semen processing. Freeze-dried semen is unlikely to be successful enough to use. Sperm banking can be expected to increase, especially on AI studs. Banking provides cheap storage while bulls (slaughtered) are being progeny tested, and insurance against loss of bulls through natural causes. For preservation of semen from bulls of less populous breeds, banking can be completed in about a year, after which the-bull can be slaughtered.

Artificial insemination

DEFINITION

Manual placing of sperm into the uterus.

STATE OF THE ART

Highly developed for most species. Representative use rates in the United States are: dairy cattle, 60 percent; beef cattle, 5 percent; turkeys, 100 percent. The major limitation to the use of AI is the low national average conception rate at first service, around 50 percent. The success or failure of AI is determined by a multiplicity of factors including estrus detection, quality of semen, timing of insemination, and semen handling.

DISADVANTAGES

1. With increased herd size, estrus detection has become a major problem.
2. Inexperienced dairymen are buying semen and inseminating their own cows, resulting in lowered fertility and no feedback on semen fertility.

ADVANTAGES

1. Widespread use of genetically superior sires.
2. Services of proven sires at a lower cost.
3. Elimination of cost and danger of keeping bulls on the farm.
4. Control of certain diseases.
5. Use of other breeding techniques including crossbreeding.
6. Continued use of valuable sire after his death.

FUTURE

Greater use of AI in beef cattle will depend on the availability of successful and inexpensive estrus synchronization technology, on relaxed restrictions of the various breed associations, and on accurate progeny records,

Estrus synchronization

DEFINITION

Estrus (“heat”), is the period during which the female will allow the male to mate her. This sexual behavior is subtle and varies widely among individuals. Thus the synchronization of estrus in a herd, using various drug treatments, greatly enhances AI and other reproduction programs.

STATE OF THE ART

Effective methods for synchronization of estrus periods for large numbers of animals have been available for more than two decades, and several approaches are now available which result in normal fertility. Several schemes involve use of prostaglandin F_2 (PGF_2) for the cow and ewe. However, FDA approves usage only for controlled breeding in beef cows and heifers, nonlactating dairy heifers, and in mares.

ADVANTAGES

1. Time a heifer’s entry into a milking stream.
2. Increase productivity by breeding heifers earlier in life.

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3. Ability to breed large numbers of cattle over a shorter calving interval.
4. Increase use of AI, especially in beef cattle, sheep, and swine.

FUTURE

Estrus cycle regulation should allow selected sires to be more widely used to improve important traits in beef cattle. It should also gain widespread and rapid acceptance among dairymen as well.

Superovulation

DEFINITION

Superovulation is the hormonal stimulation of multiple ovarian follicles resulting in release from the ovary of a larger number of oocytes (ova) than normal.

STATE OF THE ART

Superovulation with implantation into surrogate mothers increases the number of offspring, usually from highly selected dams. Adequate procedures are presently available for superovulation of laboratory and domestic animal species, except the horse. The drugs used to induce superovulation are the gonadotropins, pregnant mare's serum gonadotropin (PMSG) and follicle stimulating hormone (FSH), in some instances followed by other treatments to stimulate ovum maturation and ovulation. Superovulated ova result in normal offspring with the same success rates as achieved with normally ovulated ova.

DISADVANTAGES

1. Greatest drawback is that degree of success cannot be predicted for an individual animal.
2. Batches of hormones for ovulation treatment vary widely in quality.
3. PMSG is scarce, and has been declared a drug by the Food and Drug Administration (FDA). Thus, most use of PMSG is now illegal.
4. There is insufficient data to judge the effect of repeated superovulation.

FUTURE

Methods for superovulation will improve consistency of results. Additional understanding of basic physiological mechanisms will facilitate such efforts. New work in superovulatory technology involves active immunization against adrostenedione (a hormone involved in regulation of follicular development). This treatment prevents atresia and reliably increases the frequency of multiple ovulations. The technology has definite commercial potential for cattle husbandry and limited potential for sheep husbandry, and much current effort is directed towards developing and testing a commercial procedure.

Embryo recovery

DEFINITION

The collection of the fertilized ova from the oviducts or uteri. Collection of embryos is a necessary step for embryo transfer or storage, and for many experiments in reproductive biology. Both surgical and nonsurgical methods are used.

STATE OF THE ART

Surgical. -Methods are available for recovering 40 to 80 percent of ovulations from cattle, sheep, goats, swine, and horses. The development of adhesions and scar tissue following surgery limits these techniques. Surgical recovery is the only method for sheep, goats, and pigs. It is presently practiced almost exclusively when a suspected pathology of the oviducts renders an individual subfertile, or when embryos must be recovered before the individual reaches puberty.

Nonsurgical.- Non-surgical embryo recovery techniques are preferred for the cow and horse. Fifty to eighty percent of cow ovulations can be recovered, and 40 to 90 percent of the operations on horses to recover the single ovulation are successful.

ADVANTAGES

1. Nonsurgical embryo transfer can be performed an unlimited number of times.
2. Requirements for equipment, personnel, and time are low in nonsurgical recovery. This is especially important in milk cattle: since the nonsurgical procedure is performed on the farm, milk production is not interrupted.
3. A single embryo can be obtained between superovulation treatments.
4. Embryos can be obtained from a young heifer before it reaches puberty.
5. The technology is especially important for research, e.g., in efforts to produce identical twins, embryo biopsies for sex determination, etc.

FUTURE

Methods of collecting embryos have not changed appreciably since about 1976, nor are significant advances predicted for the future.

Embryo transfer

DEFINITION

Implantation of an embryo into the oviduct or uterus.

STATE OF THE ART

Surgical.—Pregnancy rates of 50 to 75 percent are achievable in cows, sheep, goats, pigs, and horses. Surgical transfer is the only practical method in sheep, goats, and pigs, and is the predominant method for cows and horses. A number of factors determine the success of surgical transfer: age and quality of embryos, site of transfer, degree of synchrony between estrous cycles of the donor and recipients, number of embryos transferred, in vitro culture conditions, skill of personnel, and management techniques. The 50- to 60-percent success rate in cattle compares with AI success rates at first service. (Pregnancy rates should not be confused with survival rates, which may be much lower.)

Nonsurgical.—This method is an adaptation of AI. Reported success rates are much lower than those with surgical transfer. Nonsurgical transfer is not used in sheep, goats, or pigs.

ADVANTAGES

1. Obtaining offspring from females unable to support pregnancy,
2. Obtaining more offspring from valuable females.
3. With a homozygous donor, undesirable recessive traits among animals used for AI can be rapidly detected.
4. Introducing new genes into specific pathogen-free swine herds.
5. Coupled with short- or long-term embryo storage, transportation of animals as embryos.
6. Increasing the population base of rare or endangered breeds of animals by use of closely related breeds for recipients.
7. Separation of embryonic and maternal influences in research.

DISADVANTAGES

1. Personnel requirements in surgical transfer account for a large share of high costs and thus limit applicability in animal agriculture.
2. Provision of suitable recipients is the greatest single cost in embryo transfer.

FUTURE

Surgical transfers will remain the method of choice for sheep, goats, and pigs in the foreseeable future. For cows and horses, however, nonsurgical methods will be increasingly used rather than surgical techniques (and this will be apparent) within the next year or two. It is likely that half of the commercial transfer pregnancies in cattle in North America in 1980 will be done nonsurgically, even if success rates are only 60 to 80 percent of those obtainable with surgical transfer. Among future applications, a role for embryo transfer can be predicted in progeny testing of females, obtaining twins in beef cows, obtaining progeny from prepubertal females, and in combination with in vitro fertilization and a variety of manipulative treatments (e.g., production of identical twins, selfing, genetic engineering, etc.)

Embryo storage

DEFINITION

Maintenance of embryos for several hours or days (short-term) or for an indefinite length of time (freezing).

STATE OF THE ART

Short-term.-The requirement for embryos from farm animal species has not been defined, although adequate culture systems for the short interval between recovery and transfer have been developed by trial and error. Whereas the important parameters of culture systems have been identified (e.g., temperature, pH, etc.), optimal conditions have not been determined. Cow embryos may be stored for three days in the ligated oviduct of the rabbit.

Long-term (freezing).-No completely adequate protocol exists for freezing embryos of farm species. One-third to two-thirds of embryos are killed using present methods. Pregnancy rates of 32 to 50 percent for cattle, sheep, and goats have been reported after freezing. No successful freezing of swine or horse embryos followed by development to term has been reported. Despite disadvantages (one-half of embryos are often killed) advantages are such that in some' situations embryo freezing, and embryo selling, are already profitable.

ADVANTAGES

1. Amplification of advantages of embryo transfer.
2. Elimination of requirements for large recipient herds when embryo transfer is being used.
3. Reduction of costs in animal transport.
4. Control of genetic drift in animals over prolonged time intervals.

FUTURE

Anticipated development of embryo culture technology would be of significance in efforts toward in vitro maturation of gametes, in vitro fertilization, sex determination, cloning, and genetic engineering, all of which involve prolonged manipulation of gametes and embryos outside of the reproductive tract.

As freezing rates improve, nearly all embryos recovered from cattle in North America will be frozen. Probably as many as half of the embryos will be deep-frozen for 2 to 3 years. It is unlikely that success rates will ever approach 90 percent of those without freezing. However, 70- to 80-percent success rates may be attainable within several years. It appears that embryos can be stored indefinitely with little deterioration.

Sex selection

DEFINITION

Tests to determine the sex of the unborn or determination of sex at fertilization by separating x- bearing from y-bearing sperm.

STATE OF THE ART

Sexing of embryos.—Through karyotyping nearly two-thirds of embryos can be sexed. Techniques using identification of the condensed X chromosomes are unreliable. A third method, identification of sex-specific gene products, is under development.

Sexing of sperm.—A 100-percent method has not been achieved in any mammalian species; and no standard protocol for farm species exists.

FUTURE

Before this technology can be applied commercially, it must be simple, fast, inexpensive, reliable, and nonharmful for embryos. Such techniques could undoubtedly be developed. There would be numerous medical and experimental applications.

There is much interest in research in this area because of its use in understanding male fertility with AI in humans, and in enhancing sperm survival after frozen storage.

Twinning

DEFINITION

Artificial production of twins, either using embryo transfer or hormone treatments,

STATE OF THE ART

Currently, embryo transfer is the most effective method for inducing twin pregnancies in cattle, resulting in pregnancy rates of between 67 to 91 percent, of which 27 to 75 percent deliver twins. Other methods include transferring one embryo into a cow which has been artificially inseminated, and hormonal induction of twinning, which is a modification of superovulation. This latter method is not reliable.

ADVANTAGE

The advantage of twinning in nonlitter-bearing species is the improved feed conversion ratio of producing the extra offspring.

DISADVANTAGE

The major disadvantage of twinning is intensive management necessary for periparturient complications, unpredictable gestation periods, depressed lactation, etc.

FUTURE

Technical feasibility for twinning, in conjunction with embryo transfer, management adjustments, and selection for good recipients, can be predicted. A reliable procedure for twinning in sheep can also be expected. The technology would most likely be first used in Europe and Japan, where there are shortages of calves to fatten for beef.

In vitro fertilization

DEFINITION

The union of egg and sperm outside the reproductive tract. For some species, the technology includes successful development of the embryo to gestation and birth.

STATE OF THE ART

In vitro fertilization has been accomplished in several laboratory animal species, including the rabbit, mouse, rat, hamster, and guinea pig and nine other mammalian nonlaboratory species, including man, cat, dog, pig, sheep, and cow. However, normal development following in vitro fertilization and embryo transfer has only been accomplished in the rabbit, mouse, rat, and human. Consistent and repeatable success with in vitro fertilization in farm species has not yet been accomplished.

None of the cases of reported success of in vitro fertilization, embryo transfer, and normal development in man is well documented.

Most of the in vitro fertilization work to date has concentrated on the development of a research tool so that the physiological and biochemical events in fertilization and early development could be better understood. More practical application of in vitro fertilization techniques would include:

1. a means for assessing the fertility of ovum and/or sperm;
2. a means to overcome female infertility with embryo transfer into a recipient animal; and
3. when coupled with ovum and/or embryo storage and transfer, a means to facilitate combination of selected ova with selected sperm for production of individuals with predicted characteristics at an appropriate time.

FUTURE

Rapid progress in research is anticipated and many of the potential applications of in vitro fertilization to animal breeding should become practical within the next 10 to 20 years. With further development of in vitro fertilization methodology, along with storage of unfertilized oocytes (gamete banking), fertilization of desired crosses should become possible. In the more distant future, genetic engineering and sperm sexing along with in vitro fertilization may become possible.

Parthenogenesis

DEFINITION

The initiation of development in the absense of sperm.

STATE OF THE ART

Parthenogenesis has not been satisfactorily demonstrated or described for mammalian species. The best available information leads to the conclusion that maintenance of parthenogenetic development to produce normal offspring in mammals approximates impossibility.

Cloning: production of identical twins

DEFINITION

The production, using a variety of methods, of genetically identical individuals.

STATE OF THE ART

There are several ways to obtain genetically identical livestock. The natural way is identical twins, although these are rare in species other than cattle and primates. Both natural and laboratory methods depend on the fact that the blastomeres of early embryos are totipotent (i.e., each cell can develop into a complete individual if separated from the others.) For practical purposes, highly inbred lines of some mammals are already considered genetically identical; F_1 crosses of these lines are also considered genetically identical and do not suffer from the depressive effect of inbreeding.

ADVANTAGE

An advantage of identical twins is the experimental control provided by one animal through which two sets of environmental conditions can be compared for effects on certain end points, e.g., native v. surrogate uterine environments for gestational development, nutrition on milk production, etc.

Cloning: nuclear transplantation

DEFINITION

The production of genetically identical mammals by inserting the nucleus of one cell into another, before or after destroying the original genetic complement. These occur by separation of embryos or parts of embryos early in development but well after fertilization has occurred.

STATE OF THE ART

Experimentalists have found in certain amphibia that transplantation of a nucleus from a body cell of an embryonic (tadpole) stage into a zygote following destruction or removal of the normal nucleus can lead to development of a sexually mature frog.

FUTURE

The ideal technique for making genetic copies of any given outstanding adult mammal would involve inserting somatic (body) cell nuclei into ova, which may take years of work to perfect if indeed it is possible, There is some evidence that adult body cells are irreversibly differentiated.

How identical will clones be? They can be expected to be fairly similar in appearance. They would be less similar than identical twins, however, which share ooplasm and uterine and neonatal environments. Furthermore, certain components are inherited exclusively from the mother, e.g., the mitochondrial genome and perhaps the genome of centrioles. The random inactivation of one or the other of the X chromosomes may also limit similarities. Other differences among clones would result from the prenatal environment: in litter-bearing species even uterine position can affect offspring. In single-bearing species the maternal effect may be pronounced. Environmental differences in later life may greatly affect certain traits, even if those traits have a strong genetic component.

Serious technical barriers must be overcome before realistic speculation of possible advantages in animal production can be foreseen.

cell *fusion*

DEFINITION

The fusion of two mature sex cells or the fertilization of one ovum with another. An analogous scheme for the male would be accomplished by microsurgical removal of the female pronucleus and substitution of nuclei from two sperm. Combining sex cells from the same animal is called "selfing."

STATE OF THE ART

Combination of ova has led to early development to the blastocyst stage in the mouse but no further development following transfer has been reported. Initial success in experimentation with manipulation of pronuclei has been reported.

FUTURE

Cell fusion technology may someday prove useful for getting genetic material from a somatic cell into a fertilized l-cell embryo for the purpose of cloning. In conjunction with tissue culture technology the technology would have a role in gene mapping of chromosomes for the cow and perhaps other species.

Combining ova of the same animal, selfing, would rapidly result in pure genetic (inbred) lines for use as breeding stocks. The technique would also lead to rapid identification of undesirable recessive traits which could be eliminated from the species.

Chimeras

DEFINITION

A chimera is an animal comprised of cell lines from a variety of sources. They can be formed by fusing two or more early embryos or by adding extra cells to blastocysts.

STATE OF THE ART

Live chimeras between two species of mouse have been produced. Such young have four parents instead of two; hexaparental chimeras have also been produced.

FUTURE

Practical applications of chimera technology to livestock are not obvious at this stage of development. The main objective of this research is to provide a genetic tool for better understanding of development, and maternal-fetal interactions.

Recombinant DNA

DEFINITION

The introduction of foreign DNA into the germplasm.

STATE OF THE ART

The mechanics of changing the DNA molecules of farm animals directly have not yet been worked out. The plasmid methods used in bacteria may not be applicable,

FUTURE

None of these techniques, no matter how great the potential, will be of any use in animal breeding until knowledge of genetics is greatly advanced. Before one can alter genes, they must be identified,

Prior to exploitation of recombinant DNA technology in animal breeding, it is necessary to identify gene loci on chromosomes, i.e., genetic mapping. Work toward this goal has only recently been initiated and rapid progress cannot be anticipated. Multivariate genetic determinants of characteristics of economic importance are anticipated to be the rule.