CURRENT AND POTENTIAL REPRODUCTIVE TECHNOLOGY

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SUMMARY

Embryo transplantation has enabled the development of new technologies associated with the manipulation of eggs and embryos. Techniques have been developed for the maturation and fertilization in vitro of farm animal oocytes and these extend the possibilities for experimentation and genetic manipulation. Identical twins and quadruplets have been produced by micromanipulation of embryonic cells and possibilities for further clonal reproduction through chimaerism or nuclear transplantation are becoming realistic.

INTRODUCTION

The upsurge in reproductive science which has occurred during the last few decades has enabled many methods for the control of animal reproduction to be developed and these have already led to some significant improvements in livestock production. New technology which is in train will also undoubtedly provide even greater opportunities in the future. The wide scale application of artificial insemination, particularly in dairy cattle, has probably had one of the greatest impacts on animal breeding and, when coupled with effective systems for progeny testing, has led to marked genetic improvements. Long-term preservation of spermatozoa by deep freezing has enhanced the flexibility of techniques and not only enabled the transfer of valuable genetic material between countries, but also provided a unique method for genetic conservation. In many species, oestrous cycles can be effectively controlled by a variety of techniques and simple and accurate methods for the detection of oestrus and diagnosis of pregnancy are leading to more efficient management systems. Methods for the induction of parturition are also available, although less widely applied. There is much current interest in techniques for increasing prolificacy by immunisation of animals against endogenous hormones and it is possible that these will lead to a better and more controlled improvement in reprodutive capacity than can be achieved by the administration of exogenous gonadotrophins. Techniques for superovulation and embryo transplantation provide new dimensions for exploiting the genetic potential of superior female animals. In some species a high survival rate of embryos can be obtained after deep freezing and this also facilitates genetic conservation, transport between countries and simplifies quarantine procedures. The basic practices for embryo transplantation have now been well worked out and can be applied effectively in most of the large domestic species, but it is the opportunities that this technology now provides for the manipulation of eggs and embryos in vitro prior to transplantation which is opening up new and exciting possibilities for future developments in animal breeding.

AVAILABILITY OF EGGS AND EMBRYOS

There are several constraints that limit the number of embryos that can be obtained from individual animals. In species such as sheep and pigs, in which surgical procedures are generally used for embryo collection, only a relatively few operations can be performed on any one individual. When non-surgical methods can be applied, as in cattle, superovulation and embryo recovery can be carried out repeatedly to produce quite large numbers of embryos over a period of time. In all species, however, the ovarian responses to stimulation by exogenous gonadotrophic hormones are characterized by a great degree of variability (Moor, Kruip and Green, 1984; Bindon, Piper, Cahill, Draincourt and O'Shea, 1986). Alternative methods for extracting more eggs from the large pool of immature oocytes which are present within the ovaries of animals or for obtaining a cheap and plentiful supply of eggs of the large domestic species which could be used for manipulation would therefore be attractive.

Ever since the observations of Chang (1955) and Edwards (1965) that oocytes obtained from immature antral follicles resumed meiosis when cultured in vitro, many experiments have been carried out on in vitro maturation of oocytes. The problem has been, however, not so much to induce nuclear maturation but to establish culture conditions in which oocytes undergo the equally important cytoplasmic changes analagous to those that occur in vivo. Recently, a simple and reliable method has been developed for producing large numbers of fully matured sheep oocytes in an in vitro system (Stagmiller and Moor, 1984). The oocytes collected from small follicles are cultured with their cumulus and coronal cells intact in a medium containing hormones and supplementary follicle cells. A large proportion have then been shown to be fully competent to undergo fertilization and normal embryonic development leading to the birth of live young. Equally effective systems have yet to be developed for other species, but since a plentiful supply of ovaries from which oocytes can be obtained is available from slaughterhouses, the opportunities for experimentation are great.

The exploitation of techniques for the maturation of oocytes in vitro and for the production of large numbers of eggs and embryos for further manipulation would be greatly increased by the development of reliable and effective techniques for fertilization in vitro. Until recently, however, it was only in a relatively few laboratory animals and in the human that satisfactory techniques had been worked out for fertilization of oocytes in vitro (Wright and Bondioli, 1981) and in farm animals there had been very few reports of the birth of live offspring following such procedures (Brackett, Bousquet, Boice, Donawick, Evans and Dressel, 1982). By contrast, highly reproducible techniques have now been developed for fertilization in vitro of pig and sheep oocytes leading to the birth of piglets and lambs (Cheng, 1985; Cheng, Moor and Polge, 1986). Although experiments in the pig were mainly confined to fertilization of oocytes that had been collected shortly after ovulation, in the sheep over 80% of oocytes that had been matured in vitro were also fertilized in vitro.

MANIPULATION OF EMBRYOS

During recent years, microsurgical techniques for the manipulation of early embryos, which in the past had been mainly confined to the mouse for basic studies in mammalian embryology and development, have been very successfully applied to the large domestic species. The developmental potential and regulatory capacity of embryos that contain less than the normal number of cells, achieved by separation of blastomeres of early cleavage-stage embryos, has been studied in cattle, sheep, pigs and horses (Willadsen, 1985). The viability of embryos that contain half the usual number of cells appears to be equivalent to that of normal embryos and even embryos containing one quarter of the usual cell number retain a high developmental potential, but further reduction of cell number is not generally compatible with normal development (Willadasen, 1981). Early cleavage-stage embryos subjected to radical microsurgery which severely damages the zona pellucida require protection, such as embedding in agar, to permit further development in the female reproductive tract (Willadsen, 1979), but embryos at the late morula or early blastocyst stage can be split into two halves and returned directly to definitive recipients (Willadsen and Godke, 1984). These procedures have resulted in the birth of genetically identical twins, triplets and occasionally quadruplets of preselected parentage.

Chimaeric embryos can be made by the aggregation of cells from two or more embryos and, if cells from both parent embryos are represented in the inner cell mass, the resultant fetuses and animals are generally chimaeric. By contrast, if chimaeric embryos are made to contain less than the normal number of cells, the allocation of cells to the inner cell mass is reduced and these may be derived from only one of the parent embryos. Preferential selection of daughter cells from one of the embryos to form the inner cell mass may be deliberately directed by lineage engineering such as by mixing one cell from an 8-cell embryo with one cell from a 4-cell embryo and the animals produced then tend to be non-chimaeric and be derived from the blastomeres of the 8-cell embryo. The largest number of genetically identical animals produced from a single 8-cell embryo by using chimaeric techniques has been five (Fehilly, Willadsen and Tucker, 1984).

Chimaerism may also be used to rescue embryonic cells of defective genotype such as those from parthenogenetic embryos which are unable to develop to term. In the mouse, chimaeric embryos formed by mixing cells from parthenogenetic embryos with those from normal embryos have given rise to animals containing cells of both parthenogenetic and normal origin (Surani, Barton and Kaufman, 1977) and in some instances the parthenogenetic cells may enter the germ line (Stevens, 1978). It would be interesting if such an approach could be used to produce farm animals of special merit such as highly prolific females by rescuing cells of parthenogenetic embryos in which the genome had not been diluted by fertilization.

The production of several animals from individual embryos by micromanipulative techniques increases the number of offspring that can be obtained from a limited supply of embryos. The genetically identical animals also represent small clones, but perhaps the most significant advance towards cloning on a larger scale will come from nuclear transplantation. In mammals it has not been possible to obtain normal embryonic development from eggs into which adult nuclei from differentiated somatic cells have been

transplanted. By contrast, development to term has been achieved from eggs into which nuclei obtained from early embryonic cells, which still retain their totipotential characteristics, have been transplanted. In mice live young have only been produced following the transplantation of pronuclei from recently fertilized eggs into enucleated fertilized or activated single-cell eggs (Surani, Barton and Norris, 1984). In sheep, on the other hand, the transplantation of nuclei from more advanced embryos, achieved by the fusion of a blastomere from 8- or 16-cell embryo into the cytoplasm of an unfertilized enucleated egg, has led to the development of some normal embryos and lambs (Willadsen, 1986). From these early results it has been suggested that it may indeed be realistic to forsee opportunities for the large-scale cloning of domestic animals.

The application of modern technology for the manipulation of eggs and embryos of farm animals will obviously have a major impact on animal breeding in the future. Opportunities for genetic engineering by the introduction of foreign genes into the mammalian genome and the production of transgenic animals are also becoming a practical reality which could lead to major advances in the improvement of animal productivity.

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