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Micromanipulation of Sheep and Cow Embryos

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緬羊 및 牛受精卵의 微細操作手法

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Most cattlebreeders are now familiar with embryo transplantation as a means of increasing the calf crop from individual outstanding cows. However, the procedure is also an invaluable research tool. This is particularly true in studies of embryonic development. In the past such studies were confined to mouse embryos, but they are now increasingly being undertaken with embryos of the domestic animals. One of their principal aims is to discover new and more effective ways of livestock breeding and improvement. Here I shall deal with some recent experiments with early embryos, mainly sheep and cow embryos, at the ARC Institute of Animal Physiology, Cambridge.

These experiments were concerned with the mechanism whereby early embryonic development is regulated, so that each fertilized egg gives rise to a single young. We tend to take it for granted that a single individual develops from each fertilized egg. But of course, this is not always the case, for sometimes identical twins are born. This shows that at least some embryos have a surplus capacity for development, and suggests that perhaps any embryo could be induced to develop into twins. During the first two days of development the embryo is enclosed in a shell, the zona pellucida, and does not actually increase in size. The number of cells doubles at fairly regular intervals, in the sheep and the cow approximately every 24 hours, but the size of the individual cells is halved by each division. Only when the embryo consists of about 64 cells, i.e. after it has cleaved about six times, does it start to grow. At the beginning, the increase in size is due mainly to accumulation of fluid inside the embryo whereby it develops into a vesicle, the blastocyst, which soon completely fills the zona pellucida. In the blastocyst, two types of cells can be distinguished: those which form the wall of the vesicle - the trophectoderm - and those which form the inner cell mass - a small knob of cells which protrudes into the central cavity from the trophectoderm (Fig 1). These two cell types differ in the way they develop subsequently, the trophectoderm gives rise to fetal membranes only, while the fetus itself develops from the inner cell mass. Since only one inner cell mass is formed in each blastocyst, only one fetus is normally produced.

In younger embryos all the cells, blastomeres, belong to the same type, and if one or a few blastomeres are damaged early on, the remainder are able to regulate their development so that the embryo retains its viability. This regulatory ability of blastomeres becomes even more evident if the early embryo is taken apart by microsurgery and each of its cells allowed to develop on its own. The individual blastomere will then continue to develop according to the original time table, dividing every 24 hours or so, and will produce a blastocyst at the normal time, ca. 7 days after the egg was fertilized. However, such blastocysts are of reduced size for they contain fewer cells than

do normal blastocysts. Thus a blastocyst produced from a single blastomere of a 2-cell embryo contains about 32 cells instead of the usual 64. and a blastocyst produced from a single cell of a 4-cell embryo contains only about 16 cells, one quarter of the normal number. But even more importantly, the number of cells in the inner cell mass is disproportionately reduced, with the reduction in total cell number. A single cell from an 8-cell embryo, instead of producing a proper blastocyst, in most instances will develop into a vesicle with no inner cell mass at all. Due to the lack of an inner cell mass, no fetus develops, and hence the embryo is not viable. However, if four or even two blastmeres from an 8-cell embryo are allowed to develop together, they can form a proper blastocyst. So it is the total number of cells, and not the stage of development at which the blastomeres are separated, which decides whether a viable blastocyst is formed.

It has been shown that the position a cell occupies within the embryo when the blastocyst is formed determines whether that cell ends up in the trophectoderm or in the inner cell mass. Those cells which are in the outer layer of the embryo form the trophectoderm, while those which are inside the embryo, completely surrounded by other cells, give rise to the inner cell mass. Therefore, an inner cell mass is only formed if the embryo contains enough cells for some of them to be completely surrounded by others. In embryos containing half of the normal number of cells ('half embryos) or a quarter of the normal number ('quarter' embryos) this is practically always the case, but rarely in 'eighth' embryos.

Normally, the zona pellucida ensures that the blastomeres stay together so that a single blastocyst is formed. Once a blastocyst, the embryo no longer needs its zona pellucida. However, if the zona pellucida is removed before this stage, the embryo cannot survive in the female genital tract, whereas it could, at least in theory, survive and develop without its zona if it was grown in a test tube. The only problem is, that at present there is no satisfactory method for test-tube culture of the domestic species, and this has been an obstacle to the study of the development of separate blastomeres. However, the obstacle has recently been circumnavigated by the use of agar - a biological gel - for coating of the naked blastomeres. After being embedded in agar, microsurgically isolated blastomeres can survive and develop in the female genital tract, and this has opened the way for the study and thereby exploitation of twin developmental ability.

Due to the minute size of early embryos - the diameter of a cow embryo is only about 1/10 of a mm - special instruments and a good deal of practice are required for such studies. The general procedure used is outlined in Fig. 1. It consists of the following steps:

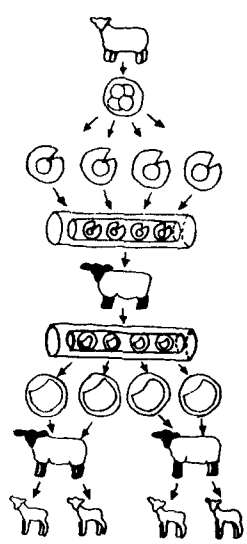

Fig. 1. Outline of procedure for micromanipulation of embryos

1. The zona pellucida is removed microsurgically.
2. The blastomeres are separated mechanically.
3. The separated blastomeres are inserted sing-

ly or in groups into the empty zona pellucidae of unfertilized eggs, recovered from slaughterhouse material.

4. Several micromanipulated embryos produced in this way are embedded in a small cylinder of agar (ca. 0.8 x 1.5 mm). The agar seals the hole in the zona pellucida.
5. The cylinder is transferred to the oviduct of a recipient ewe which functions as a temporary incubator. Up to about 20 micromanipulated embryos may be transferred to a single recipient.
6. When the embryos have developed into blastocysts they are recovered from the temporary recipient. They are then released from the agar and transferred to definitive recipients as in ordinary embryo transplantations.

With this procedure it is relatively simple to examine the development ability of every single blastomere of an early embryo. Such investigations have shown that 'half' embryos, whether produced from single blastomeres from 2-cell embryos, pairs of blastomeres from 4-cell embryos or groups of four blastomeres from 8-cell embryos are fully viable in all the domestic species. Consequently, it is now possible to produce identical twins virtually at will in these species, and unlike spontaneously occuring identical twins, those produced in this way are of preselected parentage. A pair of twin blastocysts may be transferred to two different recipients, and since sheep and cow blastocysts can be stored by deep-freezing, it is possible, in these two species, to produce pairs of identical twins in which the two animals are of different ages.

In the cow and sheep 'quarter' embryos, whether produced from single blastomeres from 4-cell embryos or pairs of blastomeres from 8-cell embryos, are also viable in many instances. It is therefore possible to produce identical quadruplets by micromanipulation of 4- and 8-cell embryos. This has been done in the sheep, whereas the largest number of calves produced from a single embryo so far is three. 'Eighth' embryos, produced from single blastomeres of 8-cell embryos, are generally not viable. Four is therefore the maximum number of genetically identical animals which can develop from a single embryo when the procedure of blastomere separation is used in cattle and sheep. Apart from having a considerable ability to develop on their own the blastomeres of an early embryo have another interesting ability. If mixed with blastomeres from another embryo of about the same stage of development they can develop into a complete blastocyst. The composite blastocyst is viable and can give rise to a composite animal. Such animals in which cells originating from two fertilized eggs co-exist are called chimaeras and are useful for various developmental, immunoligical and genetic studies. However, in the present context, the fact that composite blastocysts often give rise to animals which are not chimaeric is more interesting. The reason for this is that a chimaera is only formed if the inner cell mass of the composite blastocyst contains cells from both parent embryos. If the inner cell mass consists solely of cells from one parent embryo then the fetus will not be a chimaeric. Less than half of the cells of a blastocyst are allocated to the inner cell mass. Therefore a single cell from an 8-cell embryo although it cannot form a complete blastocyst, could, in theory, supply all the cells which are necessary for the formation of a functional inner cell mass if combined with sufficient cells of another embryo to restore the total cell number. In this way identical octuplets could be produced from a single 8-cell embryo. There are, however, very major technical problems in ensuring that the cells deriving from one particular blastomere end up forming the inner cell mass in a composite blastocyst, and so far the largest number of genetically identical animals produced from a single embryo in this way is five. This type

of experiment has only been carried out with sheep embryos.

Turning finally to the practical implication of micromanipulation of embryos, it would probably be unrealistic to suggest that they will have any direct impact in cattle breeding for the time being. However, identical twins can be produced so relatively easily and effectively that commercial embryo transplantation companies may see their advantage in using this technique. The general procedure could also be used to enable sexing of cow embryos, but this would be somewhat extravagant. On the other hand, identical twins, triplets and quadruplets are in high demand as experimental animals, and those produced in the course of the present work are already being used by other research workers in various investigations which, one hopes, will yield results of importance to the livestock industries. However, the most important aspect of the work which I have described here is perhaps that it has opened the way for other, much more radical, micromanipulative experiments which could have a very considerable impact in cattle breeding. These experiments are aimed at cloning of domestic animals by nuclear transplantation. They present rather formidable technical and more basic problems.