THE SURVIVAL OF SINGLE BLASTOMERES OF PIG EGGS TRANSFERRED TO RECIPIENT GILTS

By N. W. Moore,*† C. Polge,* and L. E. A. Rowson*

[*Manuscript received February* 4, 1969]

Summary

Single blastomeres of four- and six-celled pig eggs, either enclosed in their own zona pellucida, or injected into a foreign zona pellucida, were transferred to recipient gilts which were autopsied 5–6 days later. A control group in which the zona pellucida was penetrated without disturbing the blastomeres was included in the experiment.

At autopsy the following percentages of transferred eggs were recovered as normal blastocysts: zona penetrated, 77% (22 eggs transferred); single blastomeres in own zona, 35% (48 eggs transferred); single blastomeres in foreign zona, 0% (30 eggs transferred).

The possible reasons for partial failure of single blastomeres to develop in their own zona pellucida and complete failure of those in a foreign zona pellucida are briefly discussed.

I. Introduction

Identical twins, particularly in cattle, have been used successfully for numerous experiments in nutritional, physiological, and environmental studies (Hancock 1954). Their value lies in the fact that each pair of twins provides an almost pure genetic background. However, such studies are severely limited by the low natural incidence of identical twins and it has been calculated that monozygotic twins in cattle account for only about 4% of twin births (Meadows and Lush 1957). The artificial production of identical offspring, particularly in farm animals, would be of great value, but to date no success has been achieved in any vertebrate. In addition, there is little information available on the events which occur during the natural production of identical offspring.

It has been shown that single blastomeres of rat, mouse, and rabbit eggs are capable of development (Nicholas and Hall 1942; Seidel 1952; Tarkowski 1959; Daniel and Takahashi 1965). Recently, Moore, Adams, and Rowson (1968), in further studies on the rabbit, have shown that two-, four-, or eight-celled eggs, in which all but one of the blastomeres had been destroyed, could develop into normal living young when transferred to recipient does. However, the survival and development of the single blastomeres was dependent upon the presence of a relatively intact zona pellucida. Single blastomeres devoid of their zona pellucida, or single blastomeres placed within the zona pellucida of another egg, were rapidly destroyed when transferred to recipient does.

* A.R.C. Unit of Reproductive Physiology and Biochemistry, University of Cambridge, Animal Research Station, 307 Huntingdon Rd., Cambridge, CB3 OJQ.

† Present address: Department of Animal Husbandry, University of Sydney, McCaughey Memorial Institute, Jerilderie, N.S.W. 2716.

The choice of the rabbit as the experimental animal by Moore, Adams, and Rowson (1968) may have been unfortunate since the zona pellucida of the rabbit egg, unlike that of many other species, persists up to the time of implantation, and Adams (personal communication) has shown that normal rabbit blastocysts from which the zona pellucida had been removed do not survive when returned to the reproductive tract.

In the present study, the development potential of single blastomeres from four- and six-celled pig eggs was investigated. The pig was chosen as an experimental animal because pig blastocysts, like those of many other species, "hatch" from their zonae pellucidae several days before implantation.

II. Materials and Methods

(*a*) *Experimental Animals*

Eighteen Large White gilts (9 donors and 9 recipients) were used and all had shown at least one oestrous period before the experiment was started. The time of ovulation in respective donors and recipients was controlled and synchronized by treatment with "Methallibure" (I.C.I.) followed by injections of pregnant mares' serum gonadotrophin and human chorionic gonadotrophin (HCG) as described by Polge, Day, and Groves (1968). On the day after the injection of HCG, 16 hr before the expected time of ovulation, the donors were inseminated with 100 ml of freshly collected semen. Recipients remained unmated. On the morning of the third day after insemination eggs were recovered from the donors *in vivo*, by flushing the Fallopian tubes and a portion of the uterine horns with sterile Tyrode's solution containing 1 mg/ml bovine serum albumin (Dziuk, Polge, and Rowson 1964). This regime invariably provided fertilized eggs at the four- to six-cell stage of development.

Immediately after recovery, the eggs were treated as described below and then transferred at a rate of at least seven per animal to the uterine horns of recipients. The recipients were killed 5–6 days later and the reproductive tracts flushed with saline in order to collect developing embryos.

(*b*) *Treatment of Eggs*

The apparatus used for the various treatments (see Table 1) consisted of a de Fonbrune micromanipulator (Beaudouin, Paris) equipped with glass and steel needles, and a microsyringe (Hamilton Co., Wither, California) attached to fine-bore glass pipettes.

In the first group of eggs the zona pellucida was penetrated with a pipette (3–5 μ internal diameter) and a small volume of Tyrode's solution was injected into the perivitelline space. In the second group the zona pellucida was penetrated with a pipette of similar size and all except one of the blastomeres was ruptured. The cytoplasm of the ruptured blastomeres was then dispersed and forced out of the zona pellucida through the small gap surrounding the pipette, by the injection of Tyrode's solution. In the third group of eggs the zona pellucida was removed by means of the steel needles and the blastomeres were separated. The isolated blastomeres were then drawn up into a pipette (20–25 μ internal diameter) and injected singly into evacuated zonae pellucidae. The evacuated zonae pellucidae were prepared by dispersion and removal of all the blastomeres in eggs as previously described and they were obtained from the same donors as provided the single blastomeres. All manipulations were carried out under a dissecting microscope ($\times 80$ to $\times 120$ magnification) with the eggs held in sterile Tyrode's solution at room temperature.

III. Results

At autopsy, six of the nine recipients were pregnant and yielded a total of 34 blastocysts, all of which appeared normal (Table 1). In the first group, simple penetration of the zona pellucida with a fine pipette did not appear to affect survival

and development of the eggs; 77% of the eggs transferred were recovered as blastocysts. In the second group, 17 of 48 single blastomeres which were enclosed within their own zona pellucida developed into blastocysts. There was no apparent difference between these blastocysts and those which developed from four- or six-celled eggs which had been penetrated with a pipette. In all blastocysts a germinal disk was visible. By contrast, in the third group, none of the single blastomeres transferred after injection into evacuated zonae were recovered as blastocysts. At autopsy, 20 degenerate eggs were recovered from the three recipients, but it was impossible to distinguish between transferred eggs and the recipients' native unfertilized eggs. The degenerate eggs were either fragmenting or contained dispersed cytoplasm.

TABLE 1

SURVIVAL OF TREATED FOUR- AND SIX-CELLED PIG EGGS TO 7- AND 8-DAY BLASTOCYSTS FOLLOWING TRANSFER TO RECIPIENT GILTS

Treatment	Number of				Percentage of Eggs Survived
	Eggs Transferred	Recipients	Blastocysts Recovered	Recipients Pregnant	
Zona penetrated	22	2	17	2	77
All but one blastomere removed	48	4	17	4	35
Single blastomere injected into evacuated zona pellucida	30	3	0	0	0

IV. DISCUSSION

These results show clearly that single blastomeres from four- or six-celled eggs in the pig are capable of further development at least to 7- or 8-day blastocysts, but they do not provide direct evidence of their potential for full development. However, the normal appearance of the blastocysts, and the findings of Moore, Adams, and Rowson (1968) that single blastomeres of the rabbit transferred under similar conditions were capable of development to term, suggest that single blastomeres of these early-stage pig eggs do have the potential for full development.

The difference in survival of eggs which were penetrated only, and those in which all but one blastomere was destroyed (77 cf. 35%) could be due either to damage inflicted on blastomeres by the manipulation procedures, or to the possibility that not all of the blastomeres possess the potential for further development. If only one blastomere had this potential, then the theoretical maximum survival of individual blastomeres from four- or six-celled eggs would be 25% or less. The recorded 35% survival would suggest that more than one, if not all, of the blastomeres possess the potential for full development.

Several explanations can be offered to account for the complete failure to develop of single blastomeres injected into evacuated zonae pellucidae. Perhaps, an extremely critical relationship exists between blastomeres and their own zona pellucida and thus blastomeres enclosed in a foreign zona pellucida would have no chance of survival. However, there is no evidence for or against such a theory.

Slight damage *per se* to the zona pellucida cannot be implicated. The survival of eggs in which the zona pellucida was penetrated by a fine pipette was comparable to that of untreated eggs (Dziuk, Polge, and Rowson 1964). From results of studies in the rabbit, Moore, Adams, and Rowson (1968) suggested that failure of single blastomeres to develop in a foreign zona pellucida was due to leucocytic invasion through the large hole made by the blastomere-injection pipette and subsequent digestion of the blastomeres. If this suggestion is true for the pig (and other species), then marked chronological changes must occur in either the uterine environment or in the properties of the developing egg. In most species the zona pellucida is lost within a few days of fertilization, thus exposing naked cells to the uterine environment. In the pig the zona pellucida is lost about 6 days after ovulation (Polge, unpublished data). Therefore, for blastocysts to survive, either the uterine environment must lose its hostility towards cells of the developing egg, or the cells must acquire resistance against attack by the components of the uterine environment.

The possibility of artificially producing identical offspring probably depends on the discovery and use of suitable methods for protecting single blastomeres during their early stages of development within the uterus.

V. References

Daniel, J. C., and Takahashi, K. (1965).—Selective laser destruction of rabbit blastomeres and continued cleavage of survivors *in vitro*. *Expl Cell Res.* **39**, 475–82.

Dziuk, P. J., Polge, C., and Rowson, L. E. (1964).—Intrauterine migration and mixing of embryos in swine following egg transfer. *J. Anim. Sci.* **23**, 37–42.

Hancock, J. (1954).—Monozygotic twins in cattle. *Adv. Genet.* **6**, 141–81.

Meadows, C. E., and Lush, J. L. (1957).—Twinning in dairy cattle and its relation to production. *J. Dairy Sci.* **40**, 1430–6.

Moore, N. W., Adams, C. E., and Rowson, L. E. A. (1968).—The developmental potential of single blastomeres of the rabbit egg. *J. Reprod. Fert.* **17**, 527–32.

Nicholas, J. S., and Hall, B. V. (1942).—Experiments on developing rats. II. The development of isolated blastomeres and fused eggs. *J. exp. Zool.* **90**, 441–59.

Polge, C., Day, B. N., and Groves, T. W. (1968).—Synchronisation of ovulation and artificial insemination in pigs. *Vet. Rec.* **83**, 136–42.

Seidel, F. (1952).—Die Entwicklungspotenzen einer isolierten blastomere des Sweizellenstadiums in Säugetierei. *Naturwissenschaften* **39**, 355–6.

Tarkowski, A. K. (1959).—Experiments on the development of isolated blastomeres of mouse eggs. *Nature, Lond.* **184**, 1286–7.