

## DEVELOPMENTAL POTENTIAL OF SINGLE BLASTOMERES OF THE RABBIT EGG

N. W. MOORE,\* C. E. ADAMS AND L. E. A. ROWSON

*A.R.C. Unit of Reproductive Physiology and Biochemistry,  
University of Cambridge†*

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**Summary.** Single blastomeres of two-, four- or eight-cell rabbit eggs devoid of the zona pellucida, enclosed in their own zona, or injected into an evacuated host zona were transferred to the Fallopian tubes of recipient does.

No single blastomeres devoid of the zona survived, while 30%, 19% and 11% of those of two-, four- and eight-cell eggs enclosed in their own zona developed into normal viable young.

Thirty-three out of ninety-seven single blastomeres of four-cell eggs injected into host zonae showed limited development, undergoing one or more cleavages, during the first 2 days after transfer. However, none implanted or developed into 6-day blastocysts. Failure to survive may have been due to leucocytic invasion of the zona and subsequent destruction of the blastomeres.

The developmental potential of individual blastomeres is briefly discussed in relation to the production of identical offspring.

### INTRODUCTION

Recently fertilized eggs of the mouse, rat and rabbit in which one or more of the blastomeres have been destroyed are capable of further development (Nicholas & Hall, 1942; Seidel, 1952, 1956, 1960; Tarkowski, 1959a, b; Mintz, 1962; Daniel & Takahashi, 1965). In most instances the single blastomeres were either cultured *in vitro* for short periods or transferred to recipient females which were autopsied within a few days of transfer. On the rare occasions when recipients were allowed to survive to term, Seidel (1952, 1960) reported the birth of living young from two-cell rabbit eggs in which one of the blastomeres had been destroyed, and Tarkowski (1959a, b) reported live births in three mice which had received either two- or four-cell eggs in which one blastomere had been destroyed. Tarkowski & Wroblewska (1967) have recently presented a detailed account of the development of isolated blastomeres of four- and eight-cell mouse eggs maintained under *in vitro* conditions for 36 to 48 hr.

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

† Postal address: 307 Huntingdon Road, Cambridge.

The objectives of the present experiments were first, to examine the developmental potential of single blastomeres of two-, four- and eight-cell rabbit eggs, and second, to investigate the possibility of producing identical offspring.

## MATERIALS AND METHODS

Eggs were obtained from superovulated donors which were mated with fertile males. Fifty sexually mature does were used as recipients. Ovulation was induced by the intravenous injection of 50 i.u. HCG (Lutormone, Burroughs Wellcome).

The apparatus used consisted of a de Fonbrune micromanipulator equipped with needles made of either glass or silver steel, together with a No. 0010 micro-syringe (Hamilton Co., Whittier, Calif.) connected to fine bore glass pipettes which were attached to the manipulator. When isolated single blastomeres were required, the needles were used to remove the zona pellucida and to separate the blastomeres. When single blastomeres enclosed within their own zona were required, the unwanted blastomeres were destroyed and removed from the zona by the following method. A pipette of 3 to 5  $\mu$  internal diameter was passed through the zona and each unwanted blastomere was punctured with the pipette. Careful injection of rabbit serum through the pipette dispersed the cytoplasm of the punctured blastomeres which then passed out of the zona through the small space surrounding the pipette. All manipulations were carried out under a dissecting binocular microscope ( $\times 80$  to 120 magnifications) with the eggs held in a welled glass slide containing homologous serum to which had been added 300 to 500 i.u. penicillin (sodium salt) per millilitre.

### *Experiment 1*

Single blastomeres of two- and four-cell eggs either enclosed in, or devoid of, the zona pellucida were transferred to the Fallopian tubes of seventeen synchronized recipients which were autopsied 5 days later.

### *Experiment 2*

Single blastomeres of two-, four- and eight-cell eggs enclosed in their own zona pellucida were transferred to fifteen synchronized recipients. Eight or 9 days after transfer the recipients were examined by laparotomy and those with implantations were observed for the outcome of pregnancy.

### *Experiment 3*

Isolated single blastomeres derived from four-cell eggs were drawn up into a fine bore pipette (20 to 25  $\mu$  internal diameter) and injected singly into evacuated zonae (Pl. 1, Fig. 1). These zonae were freshly prepared by destroying and evacuating their contents: zonae and blastomeres came from the same donor. The single blastomeres enclosed in their host zonae were transferred to the Fallopian tubes of fourteen synchronized recipients, which were autopsied 1, 2 or 5 days after transfer, or examined by laparotomy 9 days after transfer.

Some of the eggs recovered 1 or 2 days after transfer were re-transferred to the uteri of four synchronized recipients.

## RESULTS

*Experiment 1*

The results are shown in Table 1. None of the single blastomeres devoid of the zona survived, whereas 5% and 24% of the single blastomeres of two- and four-cell eggs enclosed in the zona were recovered as normal 6-day blastocysts.

TABLE 1  
SURVIVAL OF SINGLE BLASTOMERES OF TWO- AND FOUR-CELL RABBIT EGGS, FOLLOWING TRANSFER TO SYNCHRONIZED RECIPIENTS, AUTOPSIED 5 DAYS AFTER TRANSFER

Cell stage	Zona pellucida	No. of recipients	No. of blastomeres		Survival (%)
			Transferred	Developed into blastocysts	
2	Present	5	37	2	5
	Absent	4	31	0	0
4	Present	4	29	7	24
	Absent	4	31	0	0

*Experiment 2*

Data on the survival of single blastomeres derived from two-, four- and eight-cell eggs and allowed to remain within the zona pellucida are given in Table 2. Thirteen of the fifteen recipients contained a total of fifty-three implantations 9 days after transfer (Day 10), and forty-three of these survived to term in twelve recipients. The remaining recipient had only one implant on Day 10 and this subsequently underwent resorption.

TABLE 2  
SURVIVAL OF SINGLE BLASTOMERES OF TWO-, FOUR- AND EIGHT-CELL RABBIT EGGS, ENCLOSED IN THEIR OWN ZONA PELLUCIDA, FOLLOWING TRANSFER TO SYNCHRONIZED RECIPIENTS (FIVE RECIPIENTS PER GROUP)

Cell stage	No. single blastomeres transferred	Survived to Day 10		Survived to term	
		No.	%	No.	%
2	70	24	34	21	30
4	72	18	25	14	19
8	72	11	15	8	11

All except four of the litters were killed at birth when macroscopic examination of the young failed to reveal any gross abnormalities. The remaining four litters containing eleven young, including four males and seven females, were

retained and those that survived to 6 to 7 months (four males and five females) were mated. Both the males and females proved fertile.

### Experiment 3

The results are shown in Table 3. No implantations were observed 9 days after transfer, nor were there any blastocysts present in the '5-day' group. In recipients autopsied 1 or 2 days after transfer there were thirty-nine zonae, either 'empty' or containing only dispersed cellular material and leucocytes

TABLE 3  
FATE OF SINGLE BLASTOMERES INJECTED INTO EVACUATED ZONAE AND TRANSFERRED TO SYNCHRONIZED RECIPIENT DOES

No. of 'eggs' transferred	No. of recipients	Treatment of recipients (days after transfer)	No. and condition of eggs recovered		
			'Empty' zonae*	No development	Limited development
40	4	Autopsy—1	10	7	15
57	4	Autopsy—2	29	5	18
22	2	Autopsy—5	No blastocysts observed		
53	4	Laparotomy—9	No implants observed		

\* Empty zonae or zonae containing only dispersed cytoplasm.

(Pl. 1, Fig. 2); twelve eggs whose appearance remained unchanged from the time of transfer, and thirty-three eggs which showed limited development. Of the fifteen eggs which showed some development 1 day after transfer, two contained two cells, six had three cells, six had four cells and one had six cells. In recipients examined 2 days after transfer, thirteen of the eighteen eggs contained eight or more cells (Pl. 1, Fig. 3), while the remainder possessed only two or three cells. The thirteen most advanced eggs plus the fifteen two- to six-cell eggs, already referred to, were re-transferred to the uteri of four synchronized recipients. However, no recipient had implantations at laparotomy 7 or 8 days later.

### DISCUSSION

The present experiments show that at least up to the eight-cell stage individual blastomeres of rabbit eggs are capable of developing into normal, live offspring. However, we are not able to say that each and every blastomere has this ability. In fact, the progressive decrease in the survival rate of single blastomeres with increasing cell stage of the parent egg might suggest that every blastomere is not totipotent. Tarkowski & Wroblewska (1967) reported that the proportion of single blastomeres that developed into blastocysts after culture *in vitro* fell from about 40% with 1/4 forms to about 15% with 1/8 forms. At the same time they observed a slight increase in the incidence of false blastocysts and a very marked increase in the incidence of trophoblastic vesicles. In their opinion, 'the available evidence does not suggest the existence of a primary developmental inequality of blastomeres'. In our experiments it

PLATE 1

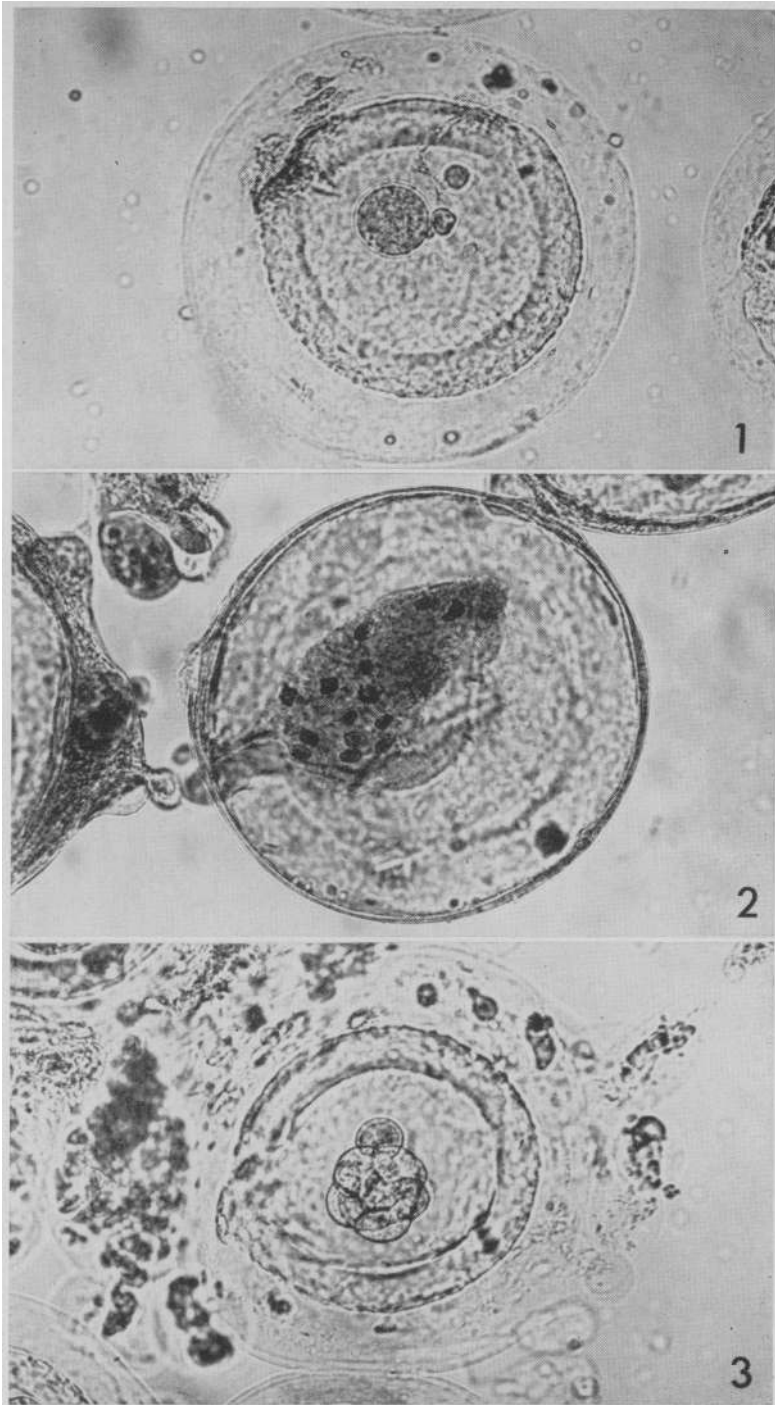


FIG. 1. Single blastomere injected into evacuated zona.  $\times 200$ .

FIG. 2. Zona containing dispersed cytoplasm and leucocytes. Recovered 1 day after transfer.  $\times 300$ .

FIG. 3. Zona containing more than eight cells. Recovered 2 days after transfer.  $\times 240$ .

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is also possible that greater damage may have resulted to 1/4 and 1/8 forms, as manipulation became more difficult with advancing development and decreasing blastomere size. The improved results obtained in the second experiment may simply reflect greater skill having been achieved with practice, thereby resulting in less harmful treatment during manipulation of the blastomeres.

In the first experiment we failed to recover naked blastomeres after transfer to the Fallopian tubes: Tarkowski has reported a similar experience with naked blastomeres in the mouse. Hence, in attempting to produce identical offspring in the third experiment, the naked single blastomeres were injected for their protection into a host zona. Although some of these underwent three and possibly more cleavages, none survived to implantation. It appeared that the failure may have been due to leucocytic invasion of the zona followed by digestion of the blastomeres.

The further development of eggs which did not contain leucocytes at recovery requires more investigation. Also, the technique should be applied to species, e.g. the mouse, pig, sheep, etc., which shed the zona pellucida within a few days of fertilization. In the rabbit the zona normally persists until implantation and blastocysts from which it has been removed do not survive (Adams, unpublished data).

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