Effect of implantational delay on transfer of rat embryos to mice

A. J. Copp and J. Rossant

Department of Zoology, South Parks Road, Oxford OX1 3PS, U.K.

Summary. Rat and mouse blastocysts were transferred to the uteri of ovariectomized mice, maintained by progesterone for a few days and induced to implant by oestradiol administration. Mouse implantation sites contained normal embryos; all rat embryos were retarded, although some had developed to the egg-cylinder stage.

Introduction

Mammalian embryos can sometimes complete their preimplantation development after transfer to the oviduct or uterus of another species (Briones & Beatty, 1954; Averill, Adams & Rowson, 1955; Tarkowski, 1962; Chang, 1965, 1966; Brinster & TenBroeck, 1969; Lawson, Adams & Rowson, 1972; Beyer & Zeilmaker, 1973). However, implantation has only rarely been observed after hetero-specific transfers, and postimplantation development has not proceeded beyond the earliest stages (Tarkowski, 1962; Blaha & De Feo, 1964; Chang & Pickworth, 1969) except for transfers between sheep and goats when advanced embryos have been obtained (Warwick & Ferry, 1949; Lopyrin, Loginova & Karpov, 1951). Similarly, interspecific aggregation chimaeras between rat and mouse (Rossant, 1976) and bank vole and mouse (Mystkowska, 1975) show limited development, although when rat inner cell masses are injected into mouse blastocysts improved postimplantation development results (Gardner & Johnson, 1975), suggesting that trophoctoderm–uterine incompatibility may be responsible for the peri-implantation embryonic death occurring after heterospecific transfer. Kirby (1969) briefly outlined a method for obtaining postimplantation development of rat embryos in recipient mice which involved the transfer of rat blastocysts into mouse uteri held in implantational delay. After several days of progesterone administration, implantation was induced by oestradiol injection. Kirby reported that a normal 14-day rat embryo was obtained in this way, but gave no experimental details. Since such a method would be of great value in the study of the development of interspecific chimaeras, we have attempted to repeat Kirby's experiment.

Materials and Methods

Blastocysts were obtained from random-bred CFLP mice and CFHB rats (Anglia Laboratory Animals Ltd) as described previously (Rossant, 1976). Two experiments were performed. In the first, female CFLP mice were bilaterally ovariectomized and subsequently given a daily s.c. injection of 1 mg progesterone (Intervet) in 0.05 ml arachis oil. On the day after ovariectomy, 4–6 rat blastocysts were transferred to one uterine horn of each recipient mouse, the contralateral horn receiving an equal number of mouse blastocysts. On the 4th and 5th days after transfer, 10 ng oestradiol benzoate (Intervet) were included in the daily injection. Implantation began on the day following the second oestradiol injection, and the mice were killed on the 8th day after transfer, when early egg-cylinder stage embryos would be expected. In the second experiment, the method described by Kaufman, Barton & Surani (1977) for improving the postimplantation development of mouse parthenogenones was followed. Mice on Day 3 of pseudopregnancy were bilaterally ovariectomized immediately after receiving rat and mouse embryos. Two days later, daily s.c. injections of 1 mg progesterone in arachis oil were initiated and 20 ng oestradiol benzoate were included on Days 6, 7 and 8 after transfer. Recipients were killed the following day. Any implantation sites in either experiment were fixed in formol–acetic–alcohol, processed and embedded in paraffin wax (Orsini, 1962), serially sectioned at 6 µm, and stained with haemalum and eosin.

Results

The results are summarized in Table 1. All mouse embryos were normal egg-cylinders (Pl. 1, Fig. 1). Although some rat embryos developed to the egg-cylinder stage (Pl. 1, Fig. 2), these appeared retarded and disorganized in comparison with those obtained after transfer of rat blastocysts to the rat uterus (Rossant, 1976). Some rat embryos consisted only of vacuolated endoderm plus trophoblast giant cells (Pl. 1, Fig. 3) while others showed no recognizable structure and were classified as disorganized blastocysts.

Table 1. The results of transfer of rat and mouse blastocysts to ovariectomized mice treated with progesterone and oestradiol (see text)

	No. of embryos transferred	No. of recipients	No. of recipients pregnant	No. of implantations	State of embryos			
					Normal egg cylinders	Retarded egg cylinders	Endoderm plus trophoblast	Dis-organized blastocysts
Experiment 1								
Mouse	25			5	5	—	—	—
Rat	25	5	4	9	—	3	2	4
Experiment 2								
Mouse	24			12	12	—	—	—
Rat	24	5	4	8	—	2	1	5

Proportion of normal mouse embryos implanted (17/17) is significantly greater than proportion of normal rat embryos (0/17), $\chi^2 = 30.1$, 1 d.f., $P < 0.001$.

Combined implantation rates for Experiments 1 (14/50) and 2 (20/48) do not differ significantly, $\chi^2 = 1.46$, 1 d.f., $0.2 < P < 0.3$.

Discussion

Rat blastocysts induced decidualization of the mouse uterus as frequently as did mouse blastocysts (Table 1) and all implantations contained trophoblastic giant cells. Nevertheless, normal rat post-implantation development did not occur, and the range of structures seen (Pl. 1, Figs 2 and 3) was very similar to that found previously after transfer of rat blastocysts to oviducts of mice on Day 1 of pseudopregnancy (Tarkowski, 1962) or to uteri of mice on Day 3 of pseudopregnancy (Rossant, 1976). In a review of implantation Surani (1977) mentioned a similar failure to promote interspecific development after transfer of rat embryos to ovariectomized mice: development was not observed beyond the implanting blastocyst stage. Although Kaufman *et al.* (1977) have successfully used the method employed here in Exp. 2 to promote the postimplantation development of parthenogenetic embryos in the mouse, it is clear that implantational delay does not permit prolonged postimplantation development of rat blastocysts transferred to the mouse. This suggests that the inability of rat blastocysts to develop normally in the mouse uterus is not simply due to insufficient time for adjustment to the foreign environment but may indicate a more fundamental species-specific interaction. Failure to establish intimate trophoblast-uterine contacts at the normal time could be involved (Tarkowski, 1962), perhaps due to the presence of species-specific trophoblast and uterine epithelial cell surface components. A role for the maternal immune system cannot be ruled out, however, since the transfer of mouse blastocysts to the uteri of irradiated, pseudopregnant rats has produced normal 7-day mouse embryos (Zeilmaker, 1971).

In conclusion, we have been unable to confirm Kirby's observation and believe that the failure of postimplantation development after interspecific transfer, at least in rodents, is due to some as yet undefined trophoblast-uterine incompatibility which cannot be overcome by prolonging the preimplantation existence of the blastocyst in the foreign uterine environment.

PLATE 1

Fig. 1. Section of normal mouse egg-cylinder produced in Exp. 1.

Fig. 2. Retarded rat egg-cylinder produced in Exp. 2.

Fig. 3. Rat embryo produced in Exp. 1 showing a trophoderm vesicle lined by scattered distal endoderm cells and containing a central group of vacuolated endoderm cells.

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