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Chimaeric Rabbits from Embryonic Cell Transplantation (38371)

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In recent years, understanding the role of embryonic disc (inner cell mass) and trophoblastic cells during the preimplantation stages of mammalian development has increased appreciably while the homotypic and heterotypic interactions between individual embryonic cells have remained largely unknown. Thus, micrurgical techniques (1, 2) for isolating and transplanting specific types and number of cells have been used to provide information on such interactions.

Previous reports have described the development of chimaeric mice following micrurgical cell transplantation into blastocysts (3, 4). However, in spite of the obvious advantage of the rabbit blastocyst for biochemical or physiological experiments, the chimaeric rabbit is a mammal known in theory only. The purpose of this investigation was to produce the chimaeric rabbit. Specific genetic markers, such as coat color and ocular pigmentation, were utilized as indicators of successful cell transplantation and contributors to recipient organogenesis. An additional measure was the significantly disproportionate sex ratio favoring the male among the young developed from recipient blastocysts. A brief summary of embryonic and fetal survival is included in the present report.

Materials and Methods. Six to eight months old virgin does, New Zealand White (NZW), New Zealand Red (NZR), and Dutch-Belted (DB), were superovulated with 0.4 mg of follicular stimulating hormone-pituitary (FSH-P, Armour) in a 1% methyl cellulose carrier (5) given subcutaneously as a single dose on four successive days. Eight to nine hours after the last FSH-P injection, 2.5 mg pituitary luteinizing hormone (PLH, Armour) was administered intravenously. Does were then artificially inseminated with semen from the respective strain except in the case of DB does where NZW semen was used to obtain (DB×NZW) hybrid embryos. Pseudopregnancy was induced in recipient NZW females with an intravenous injection of 2.5 mg PLH, given 14–16 hr after donor does received their PLH injection. Recipients were scheduled for embryo transfer 77 (± 1) hr after PLH injection. Foster females (NZW) were injected with 2.5 mg PLH and artificially inseminated concurrently with donor does.

An average of 10 morulae, 11 early blastocysts, and 14 blastocysts with distinct embryonic disc were recovered from each donor, 88 (± 1) hr after PLH injection and insemination. Only the two blastocyst stages were used for this study. The medium used consisted of F-10 basic salt (6) containing 16.65 m*M*-glucose (as energy source) and 25.07 m*M* $NaHCO_3$. This medium was extended with either 1 mg/ml bovine serum albumin (BSA, Pentex) for collecting embryos, or 15 mg/ml BSA for embryo culture and micromanipulation. Every five (± 1) blastocysts were cultured in a 100 μl microdrop of culture medium maintained under mineral oil (335/350 sv) in a 15×60 mm tissue culture dish (Falcon). Culture dishes were incubated at 37.5° in a chamber under humidified 5% CO_2 in air environment.

The donor blastocysts to be used for isolated single cells, along with the micrurgical tools and two wells (one each for donor and recipient blastocysts) were prepared as previously described (1). Each donor blastocyst served as a source of cells for two recipient blastocysts. Seven (± 2) cells from the embryonic disc of the donor blastocyst were transplanted either into or adjacent to the embryonic disc of the recipient blastocyst. After completion of cell transplantation, blastocysts were removed from their well and cultured for 2–3 hr prior to transfer into pseudopregnant

recipient females.

Sham blastocysts were subjected to all phases of the micrurgical procedures except actual cell transplantation. Control blastocysts were pooled according to strain and maintained in culture until transferred into recipient females.

Five (± 1) blastocysts from the same experimental group were transferred into each uterine horn. Caesarian section was scheduled for the 31st day of gestation. The number of live, dead, and resorbed fetuses and embryos was recorded for each uterine horn. The sex ratio as well as ocular pigmentation and skin color was noted for both living and dead fetuses. Live fetuses were placed with foster mothers which had given birth within the previous 24 hr. The Chi square test (7) was employed to compare the sex ratio in control and experimental litters. The Mann–Whitney *U*-test (7) was used to compare the incidence of embryonic loss in all groups.

Results. Table I indicates that the rabbit blastocyst can tolerate micrurgical cell transplantation, since no statistically significant difference was observed between the control and experimental groups with regard to embryonic and fetal death. However, there was some evidence ($P = 0.05$) that the number of implanted embryos were reduced in experimental groups. Such micrurgical cell transplantation seemed to alter the sex ratio in the resultant litters. The percentage of male neonates developing from the experimental groups was significantly different ($P < 0.05$) than the corresponding value for the combined control and sham groups, being 66.5% and 55.4%, respectively. The work of Jost's group on sex determination in mammals (8) provides a possible explanation for the observed increase in the percentage of male neonates following cell transplantation. These studies suggest that the presence of the Y chromosome determines the sex phenotype of the fetus. Thus, the transplantation of XY donor cells into an XX recipient blastocyst would result in phenotypically male neonates. The expected incidence of these XX/XY males is 17% of all the male neonates. The validity of this hypothesis is awaiting chromosomal analysis.

Based on variegated coat color (Plate I) and/or ocular pigmentation, five neonates were overtly chimaeric. These five young (three males and two females) exhibited a mottled skin and fur coloration reflecting both the donor cells and recipient blastocyst genotypes (Table I). Five

TABLE I. Fate of Transferred Blastocysts at Term (Day 31).

Group	Recipient blastocyst (RB1)	Donor cells (DC)	Number of blastocysts transferred	Number of implanted embryos and fetuses			Number of young with pigmentation resemblance			Sex ratio
				Resorbed	Dead	Alive	RB1	DC	Mottled	(♂/♀)
Control	NZR	None	11	4	1	6	7	—	—	2/5
	NZW	None	68	7	1	47	48	—	—	27/21
Sham micrurgy	NZW	None	72	6	0	57	57	—	—	33/24
Cell transplantation	NZR	NZW	36	3	0	26	24	2	0	17/9
	NZR	DB × NZW	23	6	0	14	13	0	1	12/2
	NZW	NZR	89	17	0	46	42	1	3	27/19
	NZW	DB × NZW	68	11	0	39	36	2	1	26/13
	DB × NZW	NZR	6	0	0	5	5[a]	0	?[a]	3/2
	DB × NZW	NZW	64	4	0	46	46[a]	0	?[a]	32/14

[a] Skin and coat coloration not utilized because of the questionable purity of the DB supplies. See text for explanation.

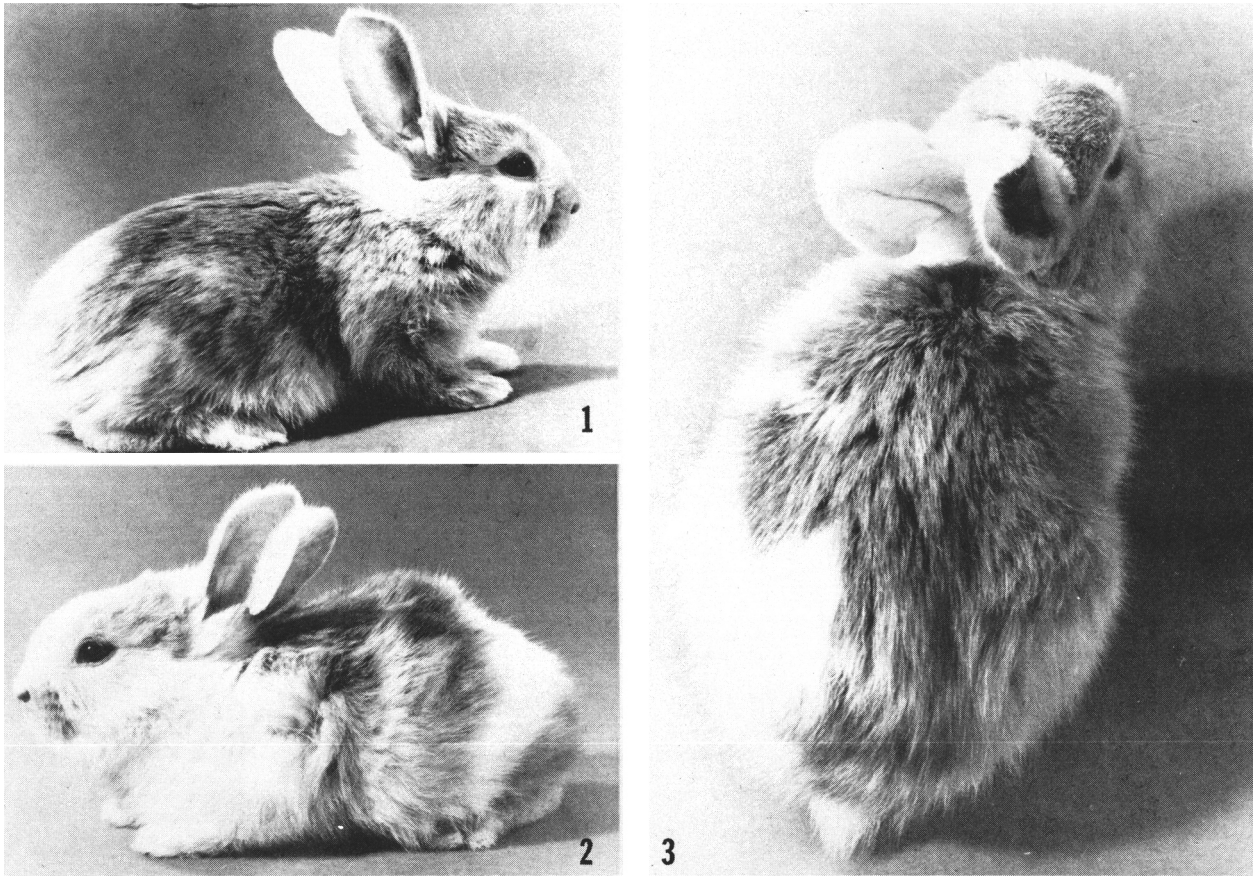

PLATE I. A four-week old male chimaeric rabbit. Seven cells from the embryonic disc of a NZR donor blastocyst were transplanted adjacent to the embryonic disc of NZW recipient blastocyst. (1) Right side of the chimaera. Note the dominating red pigmentation, only a few albino spots being present. (2) Left side of same chimaera. Note the dominating albino areas and the two red bands. (3) Dorsal view showing the sharp mid-dorsal determination line between right and left sides.

additional neonates exhibited skin, coat, and/or ocular pigmentation of the donor cells genotypes only. [This is in sharp contrast to reported incidents in chimaeric mice (9).] The skin and coat color of the DB × NZW hybrid ranged such as to suspect the closed breed of the DB strain. Skin colors ranged from solid black (with distinct white facial marks) to the classical DB marks. Coat colors varied from solid black to wild. As a result, the last two groups in Table I did not reflect skin and coat coloration as overt indicators of chimaerism. Concerning the young which phenotypically resembled the recipient blastocyst only, it is possible some carried a donor cell line which did not affect pigmentation (9). This rationale is corroborated by the disproportionate sex ratio mentioned earlier.

The production of rabbit chimaera provides us with a highly unique means for studying embryonic cell interactions, and determining to what extent we could correct for embryonic susceptibility to an extrinsic environmental factor(s). Also, whether an auto-immune disease of an embryo can be inhibited by transplanting specific types of cells.

Summary. Embryonic single cells were successfully transplanted into preimplantation intact rabbit blastocysts. Phenotypic expression of the recipient blastocyst-donor cells genetic differences in the eye, skin, and coat color evidenced successful participation of transplanted cells in recipients organogenesis. An additional measure was the significant disproportionate sex ratio favoring the male in experimental neonates. It is, therefore, clearly established that the rabbit is a suitable model for micrurgical cell transplantation and further studies of cell interactions.

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