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Efficiency of Transplanting Normal, Zona-Free, and Chimeric Embryos to One and Both Uterine Horns of Inbred and Hybrid Mice[1,2]

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Normal (zona intact), zona-free, and chimeric mouse embryos were cultured overnight and 1053 were transplanted to either one or both uterine horns of 106 pseudopregnant inbred and hybrid hosts. A higher percentage of transplanted embryos developed successfully when transplanted to hybrid hosts than to inbred hosts. Transplanting to both horns yielded a higher percentage developing and fewer resorptions ("moles") than transplanting to only one horn. Removal of the zona pellucida with pronase reduced the percentage of mice developing compared with intact embryos. Zona-free chimeric embryos were at least as viable as zona-free regular embryos.

Transplantation of preimplantation mouse embryos is a means of demonstrating that some treatment or manipulation of the embryos did not appear to reduce their viability. Cholewa and Whitten (1970), for example, demonstrated that culturing embryos in the absence of fixed nitrogen did not reduce their viability. It is also a means of obtaining animals that otherwise could not be produced, such as the chimeric or allophenic mice produced by Tarkowski (1961) and Mintz (1965).

McLaren and Michie (1956) obtained better results with transfers of 3½-day embryos to 2½-day pregnant hosts than with synchronous transfers. After this study was begun, McLaren (1969) reported that removal of the zona by untreated pronase reduced viability of transferred eggs at 10½ days of gestation and the reduction was accompanied by an increase in the proportion of sites occupied only by decidua. Bronson and McLaren (1970) reported that when dialyzed pronase (Mintz, 1967b) was used to remove the zonae from blastocysts, their viability was not reduced compared with controls with zona intact. This, however, was based on small numbers (6 or 24 transferred blastocysts developing on Day 10 of gestation). Similarly, Bowman and McLaren (1970), also using dialyzed pronase, found no significant difference between viability of zona-free embryos (21% developing) and controls (29% developing). They also found chimeric embryos to be as viable as controls. McLaren (1970) transferred small numbers of eggs and found neither a systemic nor a local luteolytic effect when one uterine horn was empty.

Experiments reported here were designed to compare the efficiency of inbred

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and hybrid hosts, the use of one and both uterine horns, and the transplanting of whole and zona-free embryos. Viability of chimeric embryos derived from the fusion of zona-free embryos is compared with nonchimeric zona-free embryos.

MATERIALS AND METHODS

Embryos were collected at the 8-cell stage on Day 2 of pregnancy (plug = Day 0) from (C57BL/10GnDg × SJL/J)F_1, or reciprocal females that had been mated with similar F_1 males. They were cultured overnight in drops of medium under paraffin oil and transplanted the next day (blastocyst stage) to pseudopregnant hosts that had mated with vasectomized males the day after the mating of donor females. Thus, 3½-day embryos (blastocysts) were transplanted to 2½-day pseudopregnant hosts. Host females were either C57BL/10GnDg, (C57BL/10GnDg × SJL/J)F_1, or reciprocals. All females were judged to be in proestrus by the external appearance of the vagina (Champlin *et al.*, 1973) and placed singly in pens with one stud male. The zona pellucida was removed by placing the embryos in an 0.5% pronase solution until the zona had almost completely dissolved. The 0.5% pronase solution contained 100 mg pronase (B. grade, Calbiochem) and 20 mg polyvinylpyrrolidone (PVP, Plastone C, General Aniline and Film Co.) per 20 ml of culture medium without albumin. The solution was allowed to stand for 1 h, filtered (0.20 μm filter), frozen, then thawed, centrifuged, and incubated at 37°C for about an hour before use. Details of culture medium and method of producing chimeric embryos is presented elsewhere (Mullen and Whitten, 1971).

Embryos to be transplanted were removed from culture dishes and pooled in a cavity slide. After anesthetizing with Avertin solution (tribromoethanol in amylene hydrate) and plucking the hair from the back of the host, a single 1-cm incision was made through the skin along the dorsal midline. A second incision was made through the abdominal wall directly over the ovary, and the ovary and cranial end of the uterus were pulled through the incision by grasping the fat pad. A thread was passed through the mesometrium to aid in holding the uterus. The cranial end of the uterus was punctured with a 25-gauge needle and the embryos introduced with a small glass pipet attached to a micrometer control. When transplanting to both horns, a similar procedure was done on the other side through a third incision. The pipet was drawn from 4 mm o.d. Pyrex glass tubing to a tube of about 0.3 mm o.d., 0.2 mm i.d., and 7 cm long. The fine end was fire polished before use. To assure that no air was injected, medium was drawn 5–6 cm into the pipet before the embryos were picked up in an additional centimeter of medium. The medium was injected until the meniscus moved 3–4 cm. The volume injected was estimated to be 0.2–1.5 μl. Whether transplanting to one or both horns, 8–14 embryos were transplanted per host. The operation from plucking of hair to final suturing lasted 4–5 min for one horn and 6–7 min for two horns.

Some hosts were killed at either 16 or 17 days of gestation and the number of fetuses and resorptions ("moles") recorded. Others were allowed to deliver and the number of pups, resorptions, and placental scars recorded. The data were analyzed by chi-square analysis.

RESULTS

There was no significant difference between the number of fetuses observed at 16–17 days of gestation and the number born, so the data were pooled and are referred to in Tables 1 and 2 as number "developing." There were only two instances of the number of placental scars exceeding the number born and these were classified with "resorptions."

The efficiency of transplanting whole and zona-free embryos into one and both horns of inbred and hybrid hosts is shown in Table 1. If the two C57BL/10 hosts that did not become pregnant were included, the percentages would be 33.0% born and 18.8% resorbed. Higher percentages of embryos developed when transplanted to hybrid hosts than to inbred C57BL/10 hosts, although the differences are not highly significant ($P < 0.1$).

Regardless of the host, a greater percentage of embryos developed when transplanted to both horns than to only one horn ($P < 0.01$). With F_1 hosts, part of this difference appears due to the fewer number of resorptions ($P < 0.01$) when both horns are pregnant (6.9%) than when only one horn is pregnant (17.6%).

When the zona pellucida was removed with pronase, fewer ($P < 0.01$) embryos developed. Only 39.5% of the zona-free embryos developed compared with 64.9% of the whole embryos.

TABLE 1
EFFICIENCY OF EMBRYO TRANSPLANTS

	C57BL/10 Hosts		Hybrid hosts		
	One horn	Both horns	One horn	Both horns	Zona-free embryos both horns
a. No. of hosts	12	12	12	12	12
b. No. becoming preg.	10	12	12	12	12
c. No. of embryos trans.	112	112	125	131	129
d. No. of emb. trans. to hosts becoming preg.	90	112	125	131	129
e. No. developing[a]	37	55	51	85	51
f. % Developing (e/d)	41.1	49.1	40.8	64.9	39.5
g. No. resorbed	21	18	22	9	7
h. % Resorbed (g/d)	23.3	16.1	17.6	6.9	5.4
i. No. lost (d-e-g)	32	39	52	37	71
j. % lost (i/d)	35.6	34.8	41.6	28.2	55.1

[a] Number developing at 16–17 days gestation or number born.

In Table 1 "both horns" includes all animals that had received embryos in both horns, although they were not necessarily pregnant in both horns when examined. All the F_1 hosts receiving whole embryos were pregnant in both horns. However, three of the 12 C57BL/10 had only one pregnant horn, and, if these animals were excluded, the data would read 57.0% developing, 15.1% resorbed, and 27.9% lost. Similarly, five of the 12 F_1 hosts receiving zona-free embryos had only one pregnant horn, and, if these were excluded, the data would read 47.5% developing, 1.3% resorbed, and 51.2% lost.

Results of transplanting chimeric embryos of various genotypes of hybrid hosts are shown in Table 2. The embryos were handled in the same fashion as the zona-free embryos referred to in Table 1 (i.e., collected on Day 2 and zonae removed with pronase) except they were fused. The efficiency for chimeras with various component genotypes ranged from 37.0 to 51.3% born. Although a direct comparison is not possible because of differences in genotypes, the values compare well with that obtained for zona-free embryos (i.e., 39.5%, Table 1).

DISCUSSION

Of the 106 hosts used in this study, 100, or 94.3%, became pregnant. Earlier results (Whitten, unpublished data) indicated that C57BL/10 females were better hosts that BALB/c or SJL females. C57BL/10

TABLE 2
EFFICIENCY OF TRANSPLANTING CHIMERIC EMBRYOS TO HYBRID HOSTS

Component genotypes	No. of hosts[a]	No. born/no. transplanted	%
Both horns			
(C57BL/10 × CBA/J)F_1 ↔ SJL/J	8	58/113	51.3
BALB/cGnDgWt ↔ C57BL/10	8	30/ 81	37.0
One horn			
SJL/J ↔ C57BL/10	15	54/134	40.3
(BALB/c × SJL)F_1 ↔ C57BL/10	6	20/ 49	40.8
(SJL × 129/Rr)F_1 ↔ C3HeB/FeJ	5	14/ 32	43.8

[a] Does not include four hosts that did not become pregnant.

and B10SJLF$_1$ hybrids are resistant to pregnancy block, whereas BALB/c and SJL are susceptible (Chapman and Whitten, 1969). Thus, the very high rate of pregnancy in this study may be partly attributable to the use of hosts resistant to pregnancy block.

Hybrids were more efficient hosts than inbreds. The difference, though not highly significant, is undoubtedly due to the better maternal environment of the hybrids.

McLaren and Michie (1959) found an abrupt increase in embryonic death when the number of implants in a single horn exceeded eight. Since approximately the same number of eggs were transplanted into one horn as into both horns, part of the difference observed in this study could have been due to crowding. However, the average number of implants per horn for females receiving embryos in only one horn was six (5.8 average for C57BL/10; 6.1 for F_1 hosts) and only two of the 22 hosts had more than eight implants in a single horn. Also, there was no difference in number of resorptions between hosts receiving 10 or 14 embryos in one horn and hosts receiving only eight in one horn.

A nonpregnant horn could exert an effect, possibly luteolytic, that reduces the number of embryos that can be maintained throughout gestation. McLaren (1970) transplanted embryos to one horn and then removed the ovary from that side and found that the nonpregnant horn did not exert a luteolytic effect. However, only about three eggs were injected per horn and only five of nine hosts became pregnant.

The observation that it is advantageous to have both horns pregnant is similar to the observations of Biggers *et al.* (1962) that mice with only one ovary, though nearly as heavy and ovulating as many eggs as both ovaries in controls, had smaller litters.

Bowman and McLaren (1970) found that, although the percentage of zona-free embryos developing (21.1%) was less than that for embryos with zonae (29.4%), the difference was not significant. In this present study the difference was highly significant. Only 39.5% of the zona-free embryos developed compared to 64.9% of the whole (zona intact) embryos. Thus, the difference observed by Bowman and McLaren, though not statistically significant, might have been real. There was no difference in incidence of resorptions, so the loss must have occurred at or before implantation. Zona-free embryos occasionally stick to the pipets during transfer. This might explain why several hosts that supposedly received embryos in both horns had only one horn pregnant.

Mintz (1967a) has reported similar percentages born for chimeric mice, but did not compare survival with normal zona-free embryos. Our results and those of Bowman and McLaren (1970) leave little doubt that chimeric embryos are at least as viable as regular embryos. The somewhat poorer results with BALB/c ↔ C57BL/10 chimeras may be due to the slower development of BALB/c preimplantation embryos and occasional difficulties in culturing them.

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