GENETIC AND UTERINE EFFECTS ON SURVIVAL IN MICE SELECTED FOR BODY WEIGHT

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Summary. Intra- and inter-line transfers of blastocysts were performed between two lines of mice selected for high (H_6) and low (L_6) body weight at 6 weeks of age. A greater percentage of L_6 blastocysts survived to term than did H_6 blastocysts (7.8%, $P < 0.01$) and more L_6 recipients littered than did the H_6 recipients (19.7%, $P < 0.01$). A greater percentage of the mice born to the L_6 recipients was found alive at birth (8.5%, $P < 0.05$), as was a greater percentage of the young carried in the uteri of the opposite line (8.3%, $P < 0.05$).

INTRODUCTION

Major differences in reproductive performance exist between two lines of mice selected for body weight at 6 weeks of age (Elliott, Legates & Ulberg, 1968). When compared with the control line, fewer females in the line selected for small body size (L_6) mated. Of those L_6 females which did mate, fewer ovulated and they had lower ovulation and fertilization rates than control line females. These traits in the line selected for large body size (H_6) were similar to those in the control line, except that the H_6 line had a higher ovulation rate. It can be calculated from the mean ovulation rates (Elliott *et al.*, 1968) and the numbers born (White, Legates & Eisen, 1968) that the prenatal survival is 12.9% higher in the L_6 line than in the H_6 line. Embryo transfer studies were conducted to determine whether this difference in prenatal survival is due to a more viable embryo or to a physiologically superior uterus. The experimental procedure and results of the experiment pertaining to the growth of the transferred blastocysts are reported in detail in another paper (Moore, Eisen & Ulberg, 1970).

MATERIALS AND METHODS

Experimental animals were obtained by random mating within two lines of mice which had been selected for high (H_6) or low (L_6) 6-week body weight for at least forty generations. Four different types of embryo transfer were carried out: (1) H_6 early blastocysts into H_6 recipient uteri, (2) H_6 into L_6 , (3) L_6

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into H₆, and (4) L₆ into L₆. Early blastocysts were recovered 3½ days after coitus from donors that had been superovulated with 10 to 15 i.u. of PMSG, followed 48 hr later by 10 i.u. of HCG. Blastocysts were injected into both uterine horns of the non-treated recipients 3½ days after coitus with a vasectomized male. Part of the experiment was performed at one time (Analysis 1) and then repeated at a different time (Analysis 2). Means and standard errors of numbers of blastocysts injected per recipient were 10.02 ± 0.29 and 14.95 ± 0.46 in Analyses 1 and 2, respectively. Statistical significance of the differences between lines for the traits studied was tested by the maximum likelihood procedure of Dunlop & Tallis (1964).

RESULTS

Table 1 shows the proportions of the total number of transferred blastocysts that survived to term within transfer types. The overall survival in the whole experiment was 21.5%. A significantly ($P < 0.01$) greater percentage of L₆

TABLE 1
PROPORTION OF TOTAL TRANSFERRED BLASTOCYSTS THAT SURVIVED TO TERM

Transfer type Blastocyst Uterus		Analysis 1 S/T* %		Analysis 2 S/T %		Total S/T %	
H ₆	H ₆	34/210	16.2	55/403	13.6	89/613	14.5
H ₆	L ₆	25/164	15.2	89/352	25.3	114/516	22.1
L ₆	H ₆	25/116	21.6	95/443	21.4	120/559	21.5
L ₆	L ₆	34/121	28.1	113/372	30.4	147/493	29.8

Analysis 2 > Analysis 1 by 2.4% (Not significant, $P > 0.05$).

L₆ blastocysts > H₆ blastocysts by 7.8% ($P < 0.01$).

L₆ uteri > H₆ uteri by 6.5% ($P < 0.01$).

Blastocyst × uterus interaction of 1.2% (Not significant, $P > 0.05$).

* Total survived/total transferred.

TABLE 2
PROPORTION OF RECIPIENTS WHICH LITTERED

Transfer type Blastocyst Uterus		Analysis 1 L/R* %		Analysis 2 L/R %		Total L/R %	
H ₆	H ₆	11/20	55.0	13/27	48.1	24/47	51.1
H ₆	L ₆	11/17	64.7	21/26	80.8	32/43	74.4
L ₆	H ₆	7/11	63.6	18/28	64.3	25/39	64.1
L ₆	L ₆	10/13	76.9	21/24	87.5	31/37	83.8

Analysis 2 > Analysis 1 by 5.1% (Not significant, $P > 0.05$).

L₆ blastocysts > H₆ blastocysts by 10.9% (Not significant, $P > 0.05$).

L₆ uteri > H₆ uteri by 19.7% ($P < 0.01$).

Blastocyst × uterus interaction of -1.5% (Not significant, $P > 0.05$).

* Total littered/total number of recipients.

TABLE 3

PROPORTION OF TOTAL NUMBER OF TRANSFERRED BLASTOCYSTS THAT SURVIVED TO TERM IN RECIPIENTS WHICH LITTERED

Transfer type Blastocyst Uterus		Analysis 1 S/T* %		Analysis 2 S/T %		Total S/T %	
H ₆	H ₆	34/110	30.9	55/198	27.8	89/308	28.9
H ₆	L ₆	25/104	24.0	89/290	30.7	114/394	28.9
L ₆	H ₆	25/78	32.1	95/281	33.8	120/359	33.4
L ₆	L ₆	34/94	36.2	113/338	33.4	147/432	34.0

Analysis 2 > Analysis 1 by 0.6% (Not significant, $P > 0.05$).L₆ blastocysts > H₆ blastocysts by 5.5% ($P < 0.01$).L₆ uteri > H₆ uteri by 0.1% (Not significant, $P > 0.05$).Blastocyst × uterus interaction of 1.9% (Not significant, $P > 0.05$).

* Total survived/total transferred into recipients which littered.

blastocysts survived to term than did H₆ blastocysts. Also, a significantly ($P < 0.01$) greater percentage of blastocysts went to term within L₆ uteri than within H₆ uteri. The data in Table 1 can be partitioned into two components: the proportion of recipients which littered (Table 2) and the proportion of blastocysts injected which survived to term in those recipients which littered (Table 3). A significantly ($P < 0.01$) greater percentage of L₆ recipients littered than did H₆ recipients. Further, in females which littered, a significantly ($P < 0.01$) greater percentage of L₆ blastocysts survived to term. However, there was no significant difference between the two types of uteri in maintaining survival.

Table 4 shows the proportion of young born (number survived to term) and found alive (number viable) within the different transfer types. There was a significantly ($P < 0.05$) greater percentage of live-born young from the L₆ recipients compared to the H₆ recipients. This was probably related to the relatively high frequency with which H₆ females desert or eat their litters (White *et al.*, 1968). There was a significantly ($P < 0.05$) greater proportion of live-born mice when the embryos were carried in the uterus of the opposite genetic line.

TABLE 4

PROPORTION OF YOUNG BORN WHICH WERE FOUND ALIVE

Transfer type Blastocyst Uterus		Analysis 1 A/B* %		Analysis 2 A/B %		Total A/B %	
H ₆	H ₆	28/34	82.4	37/55	67.3	65/89	73.0
H ₆	L ₆	24/25	96.0	69/79†	87.3	93/104	89.4
L ₆	H ₆	22/25	88.0	87/95	91.6	109/120	90.8
L ₆	L ₆	32/34	94.1	97/113	85.8	129/147	87.8

Analysis 2 < Analysis 1 by 7.1% ($P < 0.05$).L₆ blastocysts > H₆ blastocysts by 6.6% (Not significant, $P > 0.05$).L₆ uteri > H₆ uteri by 8.5% ($P > 0.05$).Blastocyst × uterus interaction of -8.3% ($P < 0.05$).

* Total alive/total born.

† Ten mice delivered by Caesarean section were not included in this sub-class.

DISCUSSION

Differences between the H_6 and L_6 lines in the viability of blastocysts and the ability of the endometrium to maintain embryos are evidently a correlated response to the selection for body weight. The difference in the viability of the two genotypes of blastocysts is not believed to be due to a gross difference in the stage of development at the time of transfer, as only those embryos which were at approximately the same morphological stage were transferred.

The greater total survival rate of embryos in L_6 recipients (Table 1) can be attributed to the higher proportion of L_6 recipients littering. This result can possibly be explained as a line difference in the rate of development of the endometrium of the recipient in receptivity to implantation. In this study, both the donors providing the blastocysts and the recipients were used $3\frac{1}{2}$ days after coitus (the combination giving optimum survival, see Doyle, Gates & Noyes, 1963). Brumby (1960) transferred $3\frac{1}{2}$ -day blastocysts into $2\frac{1}{2}$ -day recipients and found a higher littering rate following transfer into a line of mice selected for high body weight than into one selected for low body weight. It has been shown by Doyle *et al.* (1963) that if the uteri were more advanced than the embryos, it was fatal to survival. In this study ($3\frac{1}{2}$ -day blastocysts transferred into $3\frac{1}{2}$ -day uteri), the H_6 uteri were possibly more often in a more advanced state of development compared to the embryos than the L_6 uteri, and this led to a lower littering rate. In Brumby's experiment ($3\frac{1}{2}$ day blastocysts transferred into $2\frac{1}{2}$ -day uteri), the embryos may have been too advanced for the slower developing, low line uteri. This may simply indicate the importance of the genotype of the embryo and endometrium in transfer studies.

The suggested slower development of the L_6 uterus could be due to an alteration in the rate of production of gonadotrophins which, in turn, could lead to a change in the steroid levels acting on the uterus. This suggestion is supported by the fact that the natural ovulation rate of the H_6 line was significantly higher than the L_6 line (11.4 versus 7.4, Elliott *et al.*, 1968). However, when both lines of donors were injected with 10 i.u. of PMSG, there was no significant difference in the total number of embryos collected $3\frac{1}{2}$ days after coitus (21.9 versus 20.6). Alterations of gonadotrophin levels were noted in a line of mice selected for low body weight (Fowler & Edwards, 1960).

Other explanations for the physiologically superior endometrium of the L_6 line are possible. There was a statistically significant ($P < 0.01$) positive correlation (0.55) between numbers transferred and numbers born for the L_6 uterus, but no such significant relationship existed for the H_6 uterus. Thus, the H_6 uterus may be putting a more severe restriction on prenatal survival than the L_6 uterus. Elliott *et al.* (1968), working with the H_6 line, noted a tendency for embryos to cluster into certain areas of the uterus, causing localized intra-uterine crowding.

An interesting feature of the data is that there were no greater survival rates when the uterus and the blastocyst were of the same genetic line, as shown by the non-significant blastocyst $\times$ uterus interactions in Tables 1, 2 and 3. Fekete (1947) carried out an embryo-transfer experiment using DBA and C57 inbred mouse strains, and showed higher overall survival and littering rates for inter-

strain than for intra-strain transfers. No such difference for survival rates was noted when only mice which littered were considered.

In all possible combinations of matings between strains of mice containing the genes + and a^t there was a significant deficiency of offspring of the maternal genotype (Hull, 1964), but the total litter size was not affected. Hull suggested that "a selective elimination is taking place at an early stage *in utero*, when there is an excess of embryos over those which the females would be able to carry to term in any case." It is suggested that specific genetic differences between mother and offspring affect the relative survival rates of different genotypes of embryo when they are competing in the same uterus, but may have little effect on survival when all of the embryos in the uterus are of a similar genotype.

Difference in viability at birth (Table 4) as measured by the proportion of young born and found alive favours embryos carried in the uteri of the opposite line, which can be attributed to a corresponding difference in birth weight (Moore *et al.*, 1970). The difference in birth weight may be explained by the observation that a genetic dissimilarity between mother and foetus leads to the growth of a larger placenta and foetus (for review see Billington, James & Kirby, 1968).

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