Journal of Reproduction and Fertility (2000) **118**, 205–210

Effect of long-term selection for early postnatal growth rate on survival and prenatal development of transferred mouse embryos

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Reciprocal embryo transfer procedures were performed among mouse selection lines to examine prenatal maternal effects on survival and development of transferred embryos. Mice were from generations 28 and 29 of an experiment to select for (i) increased body weight gain from 0 to 10 days (E+); (ii) decreased body weight gain from 0 to 10 days (E–); or (iii) a randomly bred control line (C). A total of 118 embryo transfer procedures performed 12 h after conception resulted in 983 progeny born to 89 litters. There was a 39% overall embryo survival rate and 75% overall pregnancy success rate. Response to superovulation and oestrous synchronization was significantly lower ($P < 0.01$) in the E+ line. E+ individuals that did superovulate produced an average of 37 oocytes per flush, which was significantly higher than in the control line mice (29 oocytes per flush; $P < 0.01$). The ability to complete pregnancy successfully was not influenced by uterine environment or embryo–uterine interaction. In contrast, embryo survival in successful pregnancies was significantly affected by uterine environment. There were large maternal effects for body weight and tail length at birth; E+ recipients produced pups that were significantly larger than E– recipient pups ($P < 0.01$), which in turn were significantly larger than pups gestated by control recipients ($P < 0.01$)

Introduction

Survival and development of mammalian embryos is regulated by a complex of interacting causative agents, including the genetic make up of the embryo, the genotype of the female gestating the embryo, the uterine environment and uterine–fetal interactions. The uterine environment and uterine–fetal interactions are components of what are commonly called maternal effects. Uterine–fetal interactions occur between maternal and progeny genomes during ontogeny. Developing embryos are influenced by maternal effects at many levels of developmental organization, including cytoplasmic inheritance, maternal age, maternal body size, utero–placental blood supply, litter size, duration of gestation, hormonal status and intrauterine position (Cowley, 1991a,b and references therein). Maternal effects are often classified as epigenetic factors because they condition progeny gene expression and therefore potentially alter the relationship between genotype and phenotype in the progeny (Cowley *et al.*, 1989; Atchley and Hall, 1991; Atchley *et al.*, 1991).

Embryo transfer conducted 3 to 4 days after conception has been used for many years to evaluate prenatal maternal effects on mouse development (Brumby, 1960; Moore *et al.*, 1970; Moler *et al.*, 1981; Cowley *et al.*, 1989; Pomp *et al.*, 1989). However, improvements in embryo transfer technologies now permit successful completion of zygotic transfers as early as 12 h after conception (Hogan *et al.*, 1994). Minimizing the time embryos spend in their donor uterus maximizes the effects of the recipient uterine environment. Consequently, it is possible to refine estimates of the relative importance that the maternal uterine environment plays in the range of causal factors known to influence fetal development.

Previous research has demonstrated significant uterine maternal effects from transferring embryos between unrelated inbred lines (Pomp *et al.*, 1989) and on pups born to dams selected for body weight at 6 (Moore *et al.*, 1970) or 10 weeks (Güneren *et al.*, 1996). Estimating correlated maternal effects resulting from selection for growth rate early in ontogeny, or using infundibular transfers, has not been accomplished.

The aim of the present study was to partition and quantify donor and recipient maternal effects in a replicated 3×3 factorial transfer experiment. Embryo transfers were performed 12 h after fertilization to minimize donor effects on the embryo and to optimize exposure to the recipient uterus during gestation. Reciprocal embryo transfers were performed between mice derived from a long-term selection experiment for changes in the rate of early body weight gain. These analyses were used to evaluate the null hypothesis that selection for early postnatal rate of gain has no significant effect on prenatal maternal effects.

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Revised manuscript received 12 April 1999.

0022–4251/2000

Materials and Methods

Experimental design

Female mice derived from generations 28 and 29 of an ongoing long-term restricted index selection experiment were evaluated for prenatal maternal ability. Mice derived from three selection regimens were used in this experiment: (i) E+ mice, selected to increase early body weight gain (rate of change from birth (day 0) to 10 days of age), holding late weight gain (28–56 days) constant; (ii) E– mice, selected to decrease early gain, holding late gain constant; and (iii) C mice representing a randomly selected control line. Each line was replicated three times. These selection lines were originally derived in 1988 from ICR mice obtained from Harlan Sprague–Dawley (Indianapolis, IN). Each replicate in each selection treatment comprised 12 litters. Selection line matings were performed to keep inbreeding to a minimum. Inbreeding is accumulating across the lines at similar rates of approximately 1.25% per generation. A more detailed description of these lines and direct results of selection up to generation 15 can be found in Atchley *et al.* (1997).

Mice were housed in opaque cages on hardwood chips with food and water supplied *ad libitum*. Room temperature was maintained between 23 and 26°C, with a controlled 14 h light:10 h dark photoperiod.

Reciprocal embryo transfers were performed between embryos and infundibuli of E+, E– and C mice. Animals were randomly chosen across replicates within each line to obtain a sufficiently large experimental population. Embryos were reared in a 3×3 factorial design in which the three genetic (donor) lines were developed in three uterine (recipient) lines. This design provides tests of donor and recipient selection effects on survival and growth of transferred embryos.

Experimental protocol

Embryo recovery and transfer protocols were as described by Hogan *et al*. (1994) and the timeline of the present experiment is shown (Fig. 1). Donor and recipient females were 100 ± 14 days of age at the time of transfer. Donor females were superovulated without regard to stage of the oestrous cycle with an intraperitoneal injection of 5 iu equine chorionic gonadotrophin (eCG) (purchased as PMSG; Sigma, St Louis, MO) between 11:00 and 12:00 h, followed by an intraperitoneal injection 5 iu hCG (Sigma) 48 h later. After the second injection, females were placed individually with breeder males of the same line, replicate and generation and observed the next morning for the presence of a copulatory plug. Breeder males had mated successfully before they were used in this experiment. Recipient (uterine) dams were placed with vasectomized males of proven sterility on the same evening and observed the next morning for the presence of a copulatory plug. Donor or recipient females without copulatory plugs were not used in the experiment. Injected (donor) females were weighed and killed by cervical dislocation beginning at 12:00 h on the same day. Recipient females were concurrently weighed and anaesthetized with avertin (0.017 ml g^{-1} body weight; Aldrich, Milwaukee, WI) (Hogan *et al.*, 1994).

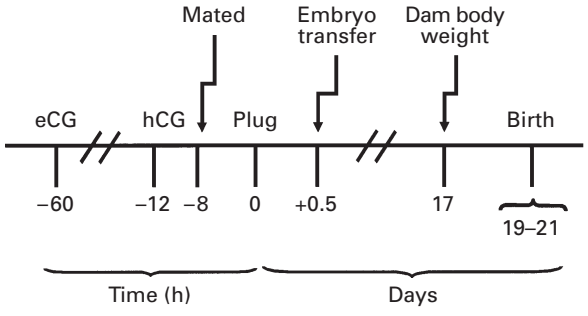

Fig. 1. Timeline of the major events in the reciprocal embryo transfer experiment in mice. Conception is arbitrarily established as 00:01 h on the day the copulatory plug is observed. eCG: equine chorionic gonadotrophin (purchased as PMSG from Sigma, St Louis, MO).

Zygote–cumulus complexes were harvested from each oviduct into M2 medium (Sigma) to which hyaluronidase (300 mg ml^{-1}; Sigma) had been added. Cumulus cells were removed by short incubation in this hyaluronidase solution. Zygotes were washed three times in M2 medium to dilute traces of hyaluronidase. The number and quality of zygotes were recorded for each oviduct. Fertilization was confirmed by observation of two pronuclei in at least one-third of the zygotes. Immature and degenerating oocytes were discarded.

Recipient females were prepared simultaneously for transfer by surgically exposing their oviducts (Hogan *et al.*, 1994). Zygotes from an individual donor were transferred into the oviducts of a single recipient in small amounts (2–5 µl) of M2 medium via a drawn out glass capillary pipette. Each litter of zygotes was transferred; one half of the litter was transferred to the left oviduct and the other half to the right oviduct. The number of zygotes transferred was not standardized in this experiment, as fertility of individual zygotes had not been ascertained. Litter integrity was maintained throughout the experiment. The entire procedure took $\leqslant 20$ min per transfer. Recipient dams provided the uterine environment for the duration of gestation (19–20 days).

Recipient females were caged individually at 17 days after transfer and observed for parturition. Birth date, total litter size (live and dead) and total litter mass (live and dead) were recorded, and average pup body weight was calculated. Individual sex, body weight and tail length were recorded at birth for all pups born alive.

Statistical analysis

An inverse sine transformation (Steel *et al*., 1997) was applied before analysis to binomial data expressed as percentages. Analyses using weighted logistic regression, or linear models were carried out using the SAS procedures CATMOD and GLM, respectively (SAS Institute, 1988). Transformed variables included successful impregnation of donor, proportion of recipients producing litters and proportion of transferred embryos carried to term.

For the purposes of the present study, replicate females

were pooled within treatment to create offspring, therefore, the subsequent analyses infer line and not replicate effects. Differences between lines were tested statistically for the following traits: (i) successful impregnation of donor; (ii) hormone-induced ovulation rate of donors; (iii) body weight of donors at the time of transfer; (iv) body weight of recipients at the time of transfer; (v) proportion of recipients producing litters; (vi) proportion of transferred embryos carried to term; (vii) percentage of transferred embryos carried to term from successful pregnancies; and (viii) body weight and tail length of young at birth. Sex ratio, embryos recovered from left or right ovary, and percentage of degenerating or immature oocytes were tested and found to be non-significant.

The statistical model used in this study included fixed effects of donor and recipient line and their interaction, as well as a random residual component. Statistical models for body weight and tail length of pups at birth included sex as a fixed effect and litter size at birth as a covariate.

Table 2. Least squares means ± SE of hormone-induced ovulation rate and body weight of female mice used for embryo transfers

	Donors			Recipients	
Line	*n*	Body weight (g)	Ovulation rate	*n*	Body weight (g)
E+	35	37.30 ± 0.45	37.29 ± 2.82	38	37.02 ± 0.53
C	48	30.97 ± 0.39	28.75 ± 2.41	45	30.28 ± 0.49
E–	35	30.85 ± 0.45	31.06 ± 2.82	35	31.13 ± 0.55
LSD[a]		E– C E+	C E– E+		C E– E+

Mice were from generations 28 and 29 of an experiment to select for increased body weight gain from 0 to 10 days (E+), decreased body weight gain from 0 to 10 days (E–), or a randomly bred control line (C).
Donor females were superovulated without regard to stage of the oestrous cycle with an intraperitoneal injection of 5 iu equine chorionic gonadotrophin (eCG) between 11:00 and 12:00 h, followed by an intraperitoneal injection 5 iu hCG 48 h later
[a]Least significant difference: for each trait, underlined values identify means that are not significantly different from each other.

Results

Response to oestrous synchronization

A total of 138 (50%) of 277 donor females that superovulated were successfully impregnated, as determined by the presence of a copulatory plug (Table 1). Control line mice had the highest success rate of synchronized females showing a copulatory plug the next morning. Animals selected for increased growth rate from 0 to 10 days (E+) had a significantly lower response rate (32%; $P < 0.01$) than either E– or control line mice.

Ovulation rate

Embryos at 12 h after conception were transferred to 118 recipient female mice. Twenty donor females had no recipient counterpart available for embryo transfer. Their ovulation rates were not determined and they were removed from the experiment.

Donor hormone induced ovulation rate (Table 2) was greatest in E+ line. Comparatively, control line ovulation rate was significantly less, averaging 29 zygotes per donor (29 versus 37; $P < 0.01$). Overall, ovulation rate ranged from 0 to 87 zygotes recovered per donor. The variability in ovulation rate may be due in part to administration of hormones without regard to the stage of the oestrous cycle, the use of mature animals (> 70 days of age), or the potential for reproduction to be correlated with selection for growth rate.

The body weight of donor and recipient mice was similar within line. For both donor and recipient groups, the body weight of mice of the E+ line was significantly greater than the body weight of mice in the other two lines. Body weights of E– and control mice were similar for both donor and recipient groups, indicating that E– mice experienced compensatory growth after the suppressed growth selection interval. This result is consistent with previous findings in this colony (Atchley *et al.*, 1997; Ernst *et al.*, 1998, 1999).

Table 1. Response to oestrous synchronization in mice synchronized without regard to the stage of the oestrous cycle

Line	Number of mice synchronized	Number of mice with copulatory plug	Percentage successful impregnation
E+	122	39	32.0
C	77	53	68.8
E–	78	46	59.0

Mice were from generations 28 and 29 of an experiment to select for increased body weight gain from 0 to 10 days (E+), decreased body weight gain from 0 to 10 days (E–), or a randomly bred control line (C).

Prenatal embryo survival

The proportion of embryo transfers resulting in pregnancies that were carried to term for all embryo transfer recipients is presented (Table 3). Overall, pregnancy success rate was 75%. Pregnancies established by transferring

Table 3. Successful pregnancies for female mice, within embryo transfer category

	Donor			Total number of litters
Recipient	E+	C	E–	
E+	7/12[a] (58.3)[b]	9/16 (56.3)	9/10 (90.0)	25/38 (65.8)
C	8/12 (66.7)	15/20 (75.0)	12/13 (92.3)	35/45 (77.8)
E–	10/11 (90.9)	9/12 (75.0)	10/12 (83.3)	29/35 (82.9)
Total	25/35 (71.4)	33/48 (68.8)	31/35 (88.6)	89/118 (75.4)

Mice were from generations 28 and 29 of an experiment to select for increased body weight gain from 0 to 10 days (E+), decreased body weight gain from 0 to 10 days (E–), or a randomly bred control line (C).
[a]Numerator is number of recipients producing litters, denominator is total number of recipients.
[b]Values in parentheses indicate percentages.

Table 4. Proportion of total embryos that survived to term in successful pregnancies, within transfer category

Recipient	Donor			Overall embryo survival
	E+	C	E–	
E+	76/280[a] (26.6)[b]	106/252 (42.1)	89/228 (39.0)	271/766 (35.4)
C	112/239 (46.9)	161/384 (41.9)	139/341 (40.8)	412/964 (42.7)
E–	108/283 (38.2)	107/232 (46.1)	85/258 (32.9)	300/773 (38.8)
Overall	296/808 (36.6)	374/868 (43.1)	313/827 (37.8)	983/2503 (39.3)

Mice were from generations 28 and 29 of an experiment to select for increased body weight gain from 0 to 10 days (E+), decreased body weight gain from 0 to 10 days (E–), or a randomly bred control line (C).
[a]Numerator is number of pups successfully developed, denominator is total number of oocytes transferred.
[b]Values in parentheses indicate percentages.

embryos within the same selection line were slightly less likely to succeed than pregnancies established across selection lines. Genetic lineage of embryos was significant ($P < 0.05$) for litter survival; zygotic litters derived from E– donors were most likely to survive. Furthermore, E– recipients were most likely to carry zygotes to term ($P < 0.05$), regardless of zygotic genotype.

Zygote survival was calculated from transferred litters that successfully completed gestation. In recipient females producing litters, overall survival rate of transferred zygotes was 39% (Table 4), and the overall average litter size was 11 pups. The ratio of males:females was 515:468 and did not deviate significantly from a 1:1 expectation. Significant differences were observed for main effects and the interaction term ($P < 0.01$). Analyses of marginal terms reveal control line zygotes had significantly higher survival rates ($P < 0.01$) regardless of uterine environment compared with selection line counterparts. Zygotes transferred into control mice experienced improved survival rates compared with other lines ($P < 0.05$) and zygotes transferred into E+ recipients had a significantly lower chance ($P < 0.01$) of survival than zygotes transferred into E– or C lines. In the evaluation of fetal–uterine interactions, there was a significantly greater ($P < 0.01$) proportion of E+ zygotes that survived to term in E– uteri. The reciprocal response was not significant.

Prenatal growth of transferred embryos

Body weight and tail length at birth for pups derived from reciprocal embryo transfer are presented (Table 5). Trends for least squares means for growth traits at birth demonstrate the impact of uterine environment on genetically derived control embryos; body weight was significantly different for pups that developed in E+, C and E– lines (Table 5). Genetically derived mice retained birth weight order (E– < C < E+) regardless of the genotype of the dam that gestated the pup. Mouse pups were largest when gestated in E+ (1.55 g; $P < 0.05$) dams and smallest when gestated in C dams (1.42 g; $P < 0.05$); E– dams produce pups of intermediate size, but significantly different from the other two lines (1.47 g; $P < 0.05$). The same trend was observed for the correlated trait, tail length. Pups gestated in E+ dams had the longest tails (11.72 mm; $P < 0.05$), E– recipients produced pups with intermediate yet significantly different tail length from the other lines (11.44 mm; $P < 0.05$) and control females produced pups with the shortest tails (11.24 mm; $P < 0.05$).

Discussion

The present experiment evaluated the null hypothesis that 28 to 29 generations of restricted index selection for early divergent growth rate would have no significant effect on prenatal maternal effects. The results of this study provide evidence that selection on growth rate asymmetrically affected prenatal maternal effects and thus the null hypothesis is rejected.

Mice selected for increased early body weight gains (E+) showed a decreased response to superovulation and oestrous synchronization. A reduction in superovulation response associated with selection for increased growth rate has not been reported elsewhere. The effects of age and timing of injections on ovulatory rate are well documented for prepubescent females (Hogan *et al*., 1994). In the present study, the average ovulation rate for mice > 70 days old ranged from 29 to 38 oocytes per donor, which is comparable to an average prepubescent rate. E+ donors that did respond to hormonal stimulation responded with a high ovulation rate, which was similar to E– line donors and significantly higher than control line mice. Further research is planned to evaluate the effect of selection for increased growth on superovulatory response.

The observation that interline transfers tend to be more successful than intraline transfers and that higher survival rates are achieved by recipients selected for decreased gain in body weight is consistent with other studies (Moore *et al.*, 1970). The 75% successful pregnancy rate reported in the present study is 7% higher than that reported by Moore *et al.* (1970) in a similar experiment involving selected mice, and that reported in the study of Pomp *et al*. (1989) in which embryo transfers were performed between inbred lines. Furthermore, the 39% survival rate reported in this study represents an 8% improvement in survival over the 31 and 30.5% survival rates reported by Moore *et al.* (1970) and Pomp *et al.* (1989), respectively. A key difference between this and previous studies may be the stage at which embryos were transferred. In the present study, zygotes were

Table 5. Least squares means ± SE for (a) body weight and (b) tail length at birth of all mice produced by reciprocal embryo transfer

(a)

	Donor				
Recipient	E+ Body weight (g)	C Body weight (g)	E– Body weight (g)	Overall body weight (g)	LSD
E+	1.75 ± 0.02	1.56 ± 0.02	1.34 ± 0.02	1.55 ± 0.01	E– C E+
C	1.49 ± 0.02	1.41 ± 0.02	1.37 ± 0.02	1.42 ± 0.01	E– C E+
E–	1.53 ± 0.02	1.46 ± 0.02	1.41 ± 0.02	1.47 ± 0.01	E– C E+
Overall	1.59 ± 0.01	1.48 ± 0.01	1.37 ± 0.01		E– C E+
LSD[a]	C E– E+	C E– E+	E+ C E–	C E– E+	

(b)

	Donor				
Recipient	E+ Tail length (mm)	C Tail length (mm)	E– Tail length (mm)	Overall tail length (mm)	LSD
E+	12.12 ± 0.09	11.46 ± 0.08	11.59 ± 0.09	11.72 ± 0.05	C E– E+
C	11.40 ± 0.08	11.03 ± 0.06	11.30 ± 0.07	11.24 ± 0.04	C E– E+
E–	11.40 ± 0.08	11.34 ± 0.08	11.59 ± 0.09	11.44 ± 0.05	C E+ E–
Overall	11.63 ± 0.05	11.28 ± 0.05	11.49 ± 0.05		C E– E+
LSD[a]	C E– E+	C E– E+	C E+ E–	C E– E+	

Mice were from generations 28 and 29 of an experiment to select for increased body weight gain from 0 to 10 days (E+), decreased body weight gain from 0 to 10 days (E–), or a randomly bred control line (C).

[a]Least significant differences: for each trait, underlined values identify means that are not significantly different.

transferred 12 h after conception and before the first mitotic cleavage. In the other studies, blastocysts were transferred directly into the uterus at 3.0–3.5 days after conception.

Brumby (1960) reported that transfer of day 3.5 blastocysts into day 2.5 uteri increased survival potential. Doyle *et al.* (1963) demonstrated that transfer of embryos into uteri in an advanced state of pregnancy was fatal. Transfer of day 3.0–3.5 blastocysts into uteri at the same or earlier developmental stage has become common (Hogan *et al.*, 1994). There is strong evidence that the uterine maternal environment affects survival and development of transferred embryos (Fekete, 1947; Brumby, 1960; Moore *et al.*, 1970; Al-Murrani and Roberts, 1978; Moler *et al.*, 1981; Pomp *et al.*, 1989; Ernst *et al.*, 1998). The present results indicate that even greater gains in survival might be achieved by minimizing the time spent in the reproductive tract of the donor. The potential for embryo–uterine interactions that affect the initiation of development well before implantation, which, when disturbed, reduce embryo survival, should be considered. If this is the case, transferring embryos before initiation is preferable.

Previous research on reproductive performance in this colony demonstrated a significant delay in vaginal opening for E– females compared with E+ females and controls (Ernst *et al.*, 1999). E+ individuals are significantly larger with respect to body weight at day 56 and transfer than either the C or E– lines. Both control and E– dams were of similar size at the time of transfer, in spite of selection for decreased growth early in E– ontogeny. E– individuals experienced compensatory body weight gains and had body weight phenotypes that converged with those of control line mice by 56 days. When evaluating recipients main effects adjusted for litter size, E+ line recipients produced pups that were significantly larger with respect to body weight and tail length ($P < 0.05$) compared with litters gestated in E– or C line females. Effect of early selection for suppressed growth is apparent when the abilities of the C and E– selection lines to gestate pups are compared. After adjusting for differences in litter size, pups born to E– recipients have significantly greater body weight ($P < 0.05$) and significantly longer tails ($P < 0.05$) than their control line counterparts. It is possible that retarded pre-pubescent reproductive development results in a reproductive tract that is more conducive to sustaining pregnancy, fetal development and growth.

This research was generously supported by NIH grant 5-46472 to W. R. Atchley. The training and advice of Ricky L. Monson is gratefully acknowledged.

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