CHANGES IN THE REPRODUCTIVE PROCESSES OF MICE SELECTED FOR LARGE AND SMALL BODY SIZE

D. S. ELLIOTT, J. E. LEGATES AND L. C. ULBERG

*Department of Animal Science, North Carolina State University, Raleigh,
North Carolina, U.S.A.*

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Summary. Low fertility in mice selected for small body size (L) at 6 weeks of age was due to fewer matings, more ovulation failures in females mating, a lower ovulation rate and a lower fertilization rate than in unselected mice (C₂) maintained as a control. The L males apparently had less libido than C₂ males. This may have contributed to fewer matings in the L line. Failure to mate or failure to ovulate, if mating, could not be explained by differences in age or in body weight. It is suggested that a thyroid deficiency was responsible.

Females of the line selected for large body size (H) at 6 weeks of age ovulated more ova than C₂ females. Essentially no foetal death occurred, after implantation, except in the H line. A lower percentage of the foetuses was living at 18 days of gestation in females of this line than in the females of the C₂ line.

INTRODUCTION

Reduction in reproductive ability occurred in lines of mice selected for small body size (MacArthur, 1944, 1949; Falconer & King, 1953; Falconer, 1953, 1955). Further, Fowler & Edwards (1960) noted that selection affected the reproductive processes differently in two populations. They suggested that these differences may have been associated with the length of time the two populations had been subjected to selection. However, Bateman (1966) found that selection for small litter size in mice had little effect on body size. Lack of a definite relationship between litter size and body size is also suggested by a study in swine (Dettmers, Rempel & Comstock, 1965) in which selection for small body size did not change litter size.

Since the reproductive processes have been characterized in but a few lines of mice selected for body size, it seemed essential to investigate, in detail, the reproductive impairment in a population of mice selected for small body size at this institution.

MATERIALS AND METHODS

The present investigation consisted of three trials. Mice were obtained from lines selected for large (line H) and small (line L) body size at 6 weeks of age

for thirty-five generations. An unselected line (C_1) has been maintained to serve as a control for the selected lines. A reconstituted control line (C_2) has been maintained for ten generations. The percentage whole body fat in the H line was consistently lower than in the C_1 line, suggesting that increased body weight had not been achieved by the deposition of fat (Lang, 1966). The origin of the lines has been reported by Cox, Legates & Cockerham (1959). The H, L and C_1 lines were approximately 33, 39 and 20% inbred, respectively, while inbreeding in the C_2 line approached 5%. The C_2 line served as a control in this investigation so that an estimate of inbreeding effects could be obtained.

All four lines were used in Trial 1. The C_1 line was deleted from Trial 2. To obtain an appraisal of the L males' contribution to the lowered efficiency of reproduction, a reciprocal cross was made between the C_2 and L lines in Trial 3. Mice from the selected lines were from Generations 39 and 40 for Trials 2 and 3, respectively.

All animals were maintained in a controlled environment of 21° C, 65% relative humidity and 12 hr of artificial light daily. Litters were standardized to six mice, three of each sex when possible, 5 days *post partum* and weaned at 21 days.

Data were analysed by Chi-square and, where applicable, analyses of variance and regression analyses were conducted according to procedures outlined by Steel & Torrie (1960). Data with unequal subclass numbers were analysed by least-squares, utilizing Duncan's multiple range test for mean differences (Harvey, 1960).

Trial 1

Mice were monogamously mated as soon after 8 weeks of age as possible. Before being placed with the males, attainment of puberty in the females was assessed by the presence of a vaginal opening (Asdell, 1964). Males were left with the females until a copulation plug (CP) was observed. Inspection for CP was performed twice daily.

Those females not exhibiting a CP by the end of a 7-day mating period were autopsied and examined for evidence of undetected copulation (pseudopregnancy, presence of implantation sites or fertile ova). Reproductive tracts were also examined for abnormalities.

Females from each line were randomly assigned for time of slaughter: (1) 40 to 56 hr after CP; (2) 10 days after CP or (3) allowed to go to term, and are designated as 2-day, 10-day or term, respectively.

The number of corpora lutea (CL) and cleaved ova obtained from 2-day females provided estimates of ovulation and fertilization rates, respectively. The 10-day females furnished further estimates of ovulation rate as well as rates of pre- and post-implantation embryonic mortality. Pre-implantation loss was estimated by comparing the actual number of implantation sites with the number expected, based on the number of CL, and corrected for fertilization rates. Post-implantation loss was estimated by comparing the number of implantation sites with the number of embryos present.

Females at term furnished an estimate of the rate of foetal survival from 10 days post-CP to term by comparing the number of living young at littering with the number of living embryos at 10 days.

Trial 2

Procedures were the same as those for Trial 1 with the following exceptions. Mating was as near to 10 weeks of age as possible. Mice were weighed just before mating. Time of autopsy for females at term was changed to 18 days of gestation, designated as 18-day, to eliminate the possibility of cannibalism at birth.

Trial 3

Forty females and forty males from each of the C₂ and L lines were used in this trial. Matings within and between lines were made, with equal numbers of pairs in each of all four possible combinations. Monogamous mating of mice began when they had reached the age of 8 weeks. Males were left with the females for a period of up to 25 days or until a cp was observed. Females for each type of mating were randomly designated for slaughter at either 40 to 56 hr or 16 days post-cp. Data similar to that in Trial 2 were obtained, except that no count of cl was made nor body weights taken.

RESULTS

Puberty

All females in this investigation exhibited vaginal opening at the time of mating, indicating that puberty had been reached. The average body weight for the females in Trial 2, at the time of mating, was 30.0 ± 2.2 g, 22.2 ± 3.0 g and 27.5 ± 2.7 g for the H, L and C₂ lines, respectively.

TABLE 1
FREQUENCY OF PAIRS MATING
(TRIALS 1 AND 2)

<i>Line</i>	<i>No. of pairs</i>	<i>% Mated</i>
H	75	100
L	62	81*
C ₂	74	95
C ₁	30	100

* $P < 0.05$ (L versus C₂).

Number mating

Data on the number of females mating include those with an undetected cp, but they are excluded from further analysis.

All females in Trial 1 mated during the 7-day period. In Trial 2, 100% (45/45) of the H line, 73% (33/45) of the L line and 91% (41/45) of the C₂ line mated. When the two trials were combined (Table 1), significantly ($P < 0.05$) fewer pairs mated in the L line than in the C₂ line.

The C₂ females which did not mate had infantile uteri (approximately half the normal size) and apparently non-functional ovaries, as no cl or follicles were observed. The average body weight for these females was 26.8 ± 1.7 g, less than a gram lighter than those which mated (27.5 ± 2.7 g). Females which did not mate were older at the time of autopsy than females which did mate (85.2 versus 76.2 days, respectively).

There were no statistically significant differences in either age or body weight between L females that mated (76 days and 22 g) and those which failed to mate (79 days and 22 g).

Data from Trial 3 indicate a significantly ($P < 0.01$) lower percentage of L males mating (Table 2). The L males that did not mate were older at the time of autopsy than L males which did mate (100.2 versus 79.7 days, respectively). Females in the non-mating pairs were also older than females which mated (97.5 versus 82.3 days for the L females, respectively, and 100.0 versus 79.1 days for the C₂ females, respectively).

TABLE 2
FREQUENCY OF PAIRS MATING IN LINE
CROSSES (TRIAL 3)

Type ♀ ♂	No. of pairs	% mated
L × L	20	80
C ₂ × L	20	80**
L × C ₂	20	100
C ₂ × C ₂	20	100

** $P < 0.01$ (L line males versus C₂ line males).

Ovulation rate

All females which mated in Trials 1 and 2 ovulated except females in the L line. In Trial 1, 66.7% (4/6) of the females ovulated in both 2-day and 10-day mice. Of the four females not ovulating in the two groups, three had infantile uteri. In Trial 2, 72% (8/11) and 63.6% (7/11) of the 2-day and the 10-day females ovulated, respectively. Of the seven females not ovulating, six had

TABLE 3
FREQUENCY OF OVULATION AND OVULATION RATE IN
MATED FEMALES (TRIALS 1 AND 2)

Line	No. mated	% ovulating	Ovulation rate
H	47	100	11.4*
L	34	67**	7.3**
C ₂	41	100	10.1
C	19	100	11.6

* $P < 0.05$ (H versus C₂).

** $P < 0.01$ (L versus C₂).

infantile uteri. When data were combined for the times of slaughter and for trials, the difference between the L and C₂ lines in the number of mated females which ovulated was significant ($P < 0.01$, Table 3).

There were no statistically significant differences in ages between those L females which ovulated after mating and those which did not (61.0 versus 57.3 days and 76.2 versus 76.8 days in Trials 1 and 2, respectively). Also in Trial 2,

there was no statistically significant difference in body weight between those L females which ovulated and those which did not (22.7 g versus 21.9 g).

The ovulation rate for females ovulating within lines, in Trials 1 and 2, is given in Table 3. The overall analysis for the H, L and C₂ lines indicated no differences between trial or time of slaughter for ovulation rate. The L line had significantly ($P < 0.01$) fewer CL than the C₂ line, and the H line had significantly ($P < 0.05$) more CL than the C₂ line.

Neither linear nor quadratic regressions for number of CL on body weight, within lines (H, L and C₂), were significant (Text-fig. 1). Nevertheless, a positive relationship between number of CL and body weight is evident when the total regression is considered.

TEXT-FIG. 1. Regression of number of CL on body weight within lines. Δ —, H, $\hat{y} = 12.05 - 0.02(x)$; $\times$ — —, L, $\hat{y} = 1.84 + 0.23(x)$; $\circ$ ·····, C₂, $\hat{y} = 1.47 + 0.29(x)$.

Fertilization rate

The fertilization rates are given in Table 4 for Trials 1 and 2. The H line had a significantly ($P < 0.05$) higher fertilization rate than the C₂ line in Trial 1. No significant differences were noted between the other lines. In Trial 2 the fertilization rate of the L line was significantly ($P < 0.01$) lower than that of the C₂ line. When Trials 1 and 2 were combined, the fertilization rate of the L line remained significantly ($P < 0.05$) lower than that of the C₂ line. If only females with at least one fertile ovum are considered, the L line remains significantly ($P < 0.05$) lower than the C₂ line (87, 75 and 87% for the H, L and C₂ lines, respectively, for Trials 1 and 2 combined). The fertilization rate of the C₁ line was 85% on this basis. The low fertilization rate of the L line was due to a

general reduction across females (range 38 to 100%) while in the other lines, the reduction was due to few fertile ova being recovered from one or two females.

In Trial 3, the L males were involved in significantly ($P < 0.05$) fewer matings than the C₂ males. Also, the females mated to C₂ males had a significantly

TABLE 4
FREQUENCY OF FEMALES WITH FERTILE OVA AND PERCENTAGE OF THOSE OVA FERTILIZED
(TRIALS 1 AND 2)

Line	Trial 1				Trial 2				Total % fertilized
	No. of females	% with fertile ova	No. of ova	% fertilized	No. of females	% with fertile ova	No. of ova	% fertilized	
H	9	100	93	89*	15	93	162	83	85
L	4	100	36	75	8	87	47	64**	69*
C ₂	10	80	90	77	9	100	75	88	82
C ₁	10	70	84	65					

* $P < 0.05$ (H versus C₂, Trial 1; L versus C₂, combined trials).

** $P < 0.01$ (L versus C₂, Trial 2).

($P < 0.01$) higher fertilization rate than the females mated to L males. Fertilization rates, recorded only from females with at least one fertile ovum, are given in Table 5.

Embryo survival at 10 days after copulatory plug

In the analysis, which included only the H, L and C₂ lines, no significant differences were found between Trials 1 and 2 for number of implantation sites

TABLE 5
FREQUENCY OF FERTILE MATINGS AND PERCENTAGE OF OVA FERTILIZED
FOR TYPE OF MATING AND WITHIN LINE OF MALE (TRIAL 3)

Type of mating ♀ ♂	Females mated				Line of males	
	No.	% with fertile ova	No. of ova	% ova fertilized	% of pairs fertile	% of ova fertilized
L × L	12	92	74	92		
C ₂ × L	11	73	70	88	83*	90**
L × C ₂	14	100	104	94		
C ₂ × C ₂	16	100	139	100	100	97

* $P < 0.05$ (L males versus C₂ males).

** $P < 0.01$ (L males versus C₂ males).

or the percentage of sites with living embryos. The combined data for females with at least one implantation site are given in Table 6. The average number of implantation sites in the L line was significantly ($P < 0.05$) lower than in the C₂ line. Although the C₁ line is not comparable, for it was not included in Trial 2, it did not differ significantly from the C₂ line in Trial 1.

There were no significant differences between the C₂ line and the other lines

for the percentage of living embryos at 10 days after mating when trials were combined (Table 6).

Survival at littering

Data on females littering from Trial 1 are summarized in Table 7. No statistical differences between the C₂ and the other lines for number of females littering were observed. Also, there were no significant differences between lines

TABLE 6
EMBRYONIC SURVIVAL AT 10 DAYS AFTER MATING
(TRIALS 1 AND 2)

<i>Line</i>	<i>No. pregnant females</i>	<i>No. of implant sites</i>	<i>% living embryos</i>
H	19	200	95
L	8	59	92
C ₂	16	150	97
C ₁ †	7	49	96

† Data obtained from Trial 1 only.

for average litter size. The H line had 10% more dead young than the C₂ line; however, this was not statistically significant. There was a significant ($P < 0.01$) loss of young between 10-day post-CP and term, but no line differences.

Foetal survival at 18 and 16 days after copulation

The H line had a significantly ($P < 0.05$) lower number of living foetuses at 18 days after copulation than the C₂ line (Table 8). Foetal losses between 10 and

TABLE 7
PERCENTAGE FEMALES LITTERING, AVERAGE NUMBER OF
YOUNG BORN AND PERCENTAGE LIVING YOUNG PER
LINE (TRIAL 1)

<i>Line</i>	<i>No. of females</i>	<i>% littering</i>	<i>Average no. of young born</i>	<i>% young living</i>
H	10	80	7.6	84
L	5	60	6.7	100
C ₂	10	60	8.2	94
C ₁	9	89	7.7	95

18 days of gestation were significant at the 10% level of probability. There was some loss in all lines during this period; however, the greatest loss occurred in the H line.

In Trial 3 there were no significant differences between the types of matings in the number of females with foetuses at 16 days after CP, but there was a significant ($P < 0.01$) difference between the C₂♀ × C₂♂ and C₂♀ × L♂ matings for the percentage of living foetuses present at this time (Table 8).

TABLE 8
PERCENTAGE FEMALES WITH FOETUSES AND FOETAL SUR-
VIVAL AT 18 AND 16 DAYS POST-CP (TRIALS 2 AND 3,
RESPECTIVELY)

<i>Line or type of mating</i>	<i>No. of females</i>	<i>% females with foetuses</i>	<i>No. of foetuses</i>	<i>% living</i>
H	14	64	92	86*
L	11	36	26	89
C ₂	12	83	77	99
♀ × ♂				
L × L	4	75	23	100
L × C ₂	5	100	25	96
C ₂ × L	5	80	32	72**
C ₂ × C ₂	4	100	40	98

* $P < 0.05$ (H versus C₂ in Trial 2).

** $P < 0.01$ (C₂ ♀ by L ♂ versus C₂ ♀ by C₂ ♂).

DISCUSSION

Vaginal opening has been used to indicate puberty in mice (Asdell, 1964). It has also been noted that puberty is more closely associated with body weight than with age in mice (Asdell, 1964) and in rats (Kennedy & Mitra, 1963). The results of this study, however, suggest that vaginal opening is not a good indication that full reproductive capacity has been reached, especially in highly selected lines, for a significant ($P < 0.05$) number of the L line females with vaginal openings did not mate. Also, of those which did mate, approximately 30% did not ovulate. Neither age nor body weight was associated with failure of L females to mate or to ovulate when mating.

The failure of mating females to ovulate, as found in the L line, was also noted by Fowler & Edwards (1960) in a line selected for small body size. They postulated a hypofunction of the anterior pituitary. However, Edwards (1962) found that the size and secretory activity of the pituitary in a population of mice selected for large and small body size was proportional to body weight. It therefore seems that, if selection for small body size was mediated through small or hypofunctioning pituitaries, females not exhibiting full reproductive capacity would either be younger and/or lighter in body weight than females having attained full puberty. Neither of these conditions was noted in the present study. Thyroidectomy of immature female mice (Saliman & Reineke, 1952) caused infantile uteri and non-functional ovaries later in development. Infantile uteri and non-functional ovaries were noted in this investigation in the L females which mated but had not ovulated. This suggests that selection for small body size, in this population of mice, may have resulted in an aberrant relationship between the thyroid gland and the reproductive system such as hypofunctioning thyroid glands. This hypothesis is further supported because iodinated casein in the diet of the L females from the age of 21 days to 6 weeks alleviated the incidence of ovulation failure (Elliott & Bazer, unpublished data). This was accompanied by less than a gram increase in body weight of the treated over the non-treated mice.

The L line females ovulated an average of two to three less ova and the H line females ovulated one to two more ova, based on CL count, than the C₂ line females. Body weight within lines had essentially no effect on ovulation, as the regressions of CL number on body weight did not differ significantly from zero. The above results are in accord with published data (Fowler & Edwards, 1960).

Data from Trial 3 indicated that the L male was less likely to mate regardless of the line of female. This suggests that the libido of the L males was lower than that of the C₂ males. This again could be explained as a possible thyroid hormone deficiency existing in the L line, and is in accord with the data presented by Maqsood (1951, 1952). Although inbreeding has been reported to reduce male libido in mice (Krzanowska, 1964a), there was no apparent lack of libido in the C₁ or H males in this study.

The low fertilization rates obtained for the L and C₁ lines may be explained as inbreeding depression (Krzanowska, 1964b); however, the high fertilization rate obtained for the H line leaves this point obscure. The low fertilization rate in the C₁ line apparently was the major factor in reducing the reproductive ability of that line. There were no differences of consequence in the other traits measured when compared with the C₂ line.

There was no difference between lines with respect to embryonic loss at 10 days of gestation. The H line lost a proportionately larger number of young (10%) than the other lines either at or before the time of littering. Results in Trial 2 indicated that this loss occurred before 18 days of gestation. The loss in the H line may have been due to intra-uterine crowding (Hafez, 1964). It was observed in the group killed at 10 days that there was a high incidence of embryos being grouped together rather than being randomly distributed throughout the horns as found in the other lines in this investigation and as reported by McLaren & Michie (1959).

The overall results indicate that both the male and the female contributed to the low reproductive efficiency observed in mice selected for small body size.

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