LOWERED REPRODUCTIVE EFFICIENCY AS A CORRELATED RESPONSE TO SELECTION FOR INCREASED POST-WEANING GAIN IN LABORATORY MICE

by

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INTRODUCTION

Selection for increased post-weaning gain is widely practiced in economically important, meat-producing animals. In selecting for this trait, the direct and correlated responses must be studied in order to uncover any detrimental responses that occur with selection.

Of course, selection studies should be done, whenever possible, with the species concerned. Unfortunately, this is not always possible due to economic, time, and space limitations. Thus, this study was undertaken using mice as the experimental animals.

Response to a selected trait, such as post-weaning gain may be accompanied by lowered reproductive efficiency. Thus, if increased selection for a specific trait lowers reproductive efficiency to such an extent that the continuity of the line is jeopardized, the causes, and the means to correct them, must be identified. In short, selection for a specific trait will have no benefit if the animals are incapable of reproduction.

This study was undertaken to determine which phase(s) of reproduction were altered as a correlated response to selection for post-weaning gain in mice. Although the laboratory mouse in itself is of little concern to livestock producers, it is hoped that the results of this study will prove useful to these breeders in the future. If reproductive efficiency decreases as production increases in economically important livestock, perhaps the processes affected will be the same as in this study. Then the principles of this study can be

applied to the economically important species and selection will not jeopardize reproductive efficiency in these larger animals. Thus, the principles of this study may prevent a substantial economic loss to livestock producers.

This study encompassed three major objectives. First, to determine if the causes of the lowered reproductive efficiency were associated with the male, the female, or a combination of the two sexes. Second, to determine if the problem is of genetic origin. Third, to determine which reproductive processes have been altered.

REVIEW OF LITERATURE

Many authors have reported results of selection experiments for body weight and post-weaning gain. Reproductive fitness has been altered as a response to selection. However, the specific phase or phases that have been changed differ according to the trait involved and the selection pressure placed upon the trait.

Estrous Cycle

Both the length and continuity of the estrous cycle have been reported to be altered through inbreeding and selection. Fowler and Edwards (1960) found that females of a strain that had been selected for large size at 6 weeks of age had longer cycles than unselected controls. They reported 6 to 7 day cycles for large females compared to 4 to 5 day cycles for control females.

Although this change in the estrous cycle may be attributed to the "strange male effect" (Whitten, 1958), Chipman and Bronson (1968) found contrary results. They could demonstrate this effect neither in hybrid nor inbred animals. They associated the lengthened cycle to indirect selection for lowered reproductive efficiency which usually accompanies inbreeding.

The number of females per cage will also have a definite effect on cycle length and stage (Bronson and Marsden, 1964). It should also be noted that strain differences in cycle length have been reported (Fowler and Edwards, 1960).

The delayed or lengthened cycle may have a harmful effect upon the

viability of embryos and fertilizability of the egg. Fugo and Butcher (1971) found that embryos in which ovulation was delayed with Nembutal had a marked decrease in implantation rate when transfered to control recipients. Freeman, et. al., (1970) found that eggs from females after delayed ovulation were abnormal with respect to size and stage of development. The effect of aged eggs include decreased fertilization (Salisbury and Hart, 1970) and increased abortion, especially in pregnant women (Poland, 1971).

Male Effects

Although the female probably contributes more to the prenatal effect on embryonic development, those of the male cannot be ruled out. Whitten (1958) has described the need for the presence of a male, or the male stimulus, to prevent the occurence of irregular cycles in the female. It was also pointed out by Whitten (1958) that the male stimulus may be needed at two points in the cycle rather than the entire cycle. The male stimulus from one cycle does not carry over into the next cycle (Whitten, 1958).

The male does influence litter size of the dam he inseminates and this trait is characteristic of the individual male (Finn, 1964). Finn indicates that the influence is not large, but might be of some biological significance.

The effect of selection on male libido, and in turn, on reproduction has been reported by several authors. Both Fowler and Edwards (1960) and Elliot (1966) found that selection for high and low body

weight, respectively, decreased male libido. In both cases, this lowered sex drive accounted for a portion of the lowered reproductive efficiency in the particular lines they studied. McGill and Tucker (1964) also related male libido to the genotype of the male mouse.

Ovulation Rate

In most studies, ovulation rate has been determined by a count of corpora lutea (CL) on the ovary (Bradford, 1969). Fowler and Edwards (1960) reported that ovulation rate was increased in mice as a result of selection for large body size. Bradford (1969) also found an increase in ovulation rate as a direct response to selection for this trait. Other authors have reported a change in ovulation rate as either a direct or correlated response to selection (Falconer and Roberts, 1960; King and Young, 1958; McLaren, 1962; White, Legates and Eisen, 1968).

Embryonic Mortality

Boshier (1968) found that the extent of prenatal losses within the uterus was a function of the total number of embryos present. He also found the pre-implantation period to be the most critical in embryo survival. Falconer and Roberts (1960) found that pre-implantation losses increased with the degree of inbreeding.

Bradford (1969) found that embryo survival could be increased as a direct response to selection. In selecting for increased embryonic survival, Bradford recognized an increased ovulation rate as a corre-

lated response. The increased ovulation rate was thought to mask the pre-implantation losses and to account for the increased number of embryos at implantation.

Post-implantation losses may be due to several factors. Bowman and Roberts (1958) found that an increased number of eggs shed was a factor in increased post-implantation losses. When large numbers of eggs are shed, these increased losses may be due to overcrowding in the uterus (Adams, 1960). O'Grady and Heald (1969) found a totally random placement of implantation sites in the uterus, although the spacing between the sites was significantly nonrandom. Adams (1960) suggested the spacing of implantation sites may be due to a limited blood supply.

Moore, et. al., (1970) found a definite uterine effect as a result of selection for body weight. Moore's group found the uterus of females selected for large body size were faster developing than those from low line females. That is, embryos were able to implant earlier than in the low line uterus. The uterus of the high line females was also thought to provide a more favorable environment for blastocysts than the low line uterus.

Several workers have indicated that post-implantation losses may be negligible in animals selected for large body size. Falconer and Roberts (1960) found no difference in the post-implantation mortality of embryos when comparing inbred and non-inbred females. This was supported by Fowler and Edwards (1960) who found that females from both control and lines selected for large body size maintained post-implan-

tation pregnancies with little difficulty.

Litter Size

Litter size is determined by ovulation rate, fertilization rate, and embryonic mortality. Litter size is affected by both the male and female, although the male influence is primarily on fertilization rate and is not large (Finn, 1964; Bowman and Roberts, 1958).

The primary factor determining litter size is ovulation rate since the number of eggs determines the number of potential young that can be carried to term. Bradford (1969), in selecting for an increased ovulation rate, did not find a significant increase in litter size from unselected controls. He attributed this to an increase in post-implantation mortality. In contrast, selection for increased body weight is reported to bring about a correlated increase in litter size (Fowler and Edwards, 1960; White, et. al., 1968).

Litter size can also be affected by embryonic mortality. This can result either as a direct response to selection for embryonic survival as described by Bradford (1969), or be due to the effects of inbreeding (Falconer and Roberts, 1960).

Length of Reproductive Life

Roberts (1961) found that mice selected for small body size lived longer than mice selected for large body size and had a significantly longer reproductive life. Unfortunately, he also found that large stock did not have a more rapid litter production to compensate for the

shorter reproductive life.

Hormone Levels

Although the amount of information available on the effect of selection on hormone levels is less than that of its effects on other aspects of reproduction, the subject is of enough importance to warrant a discussion.

Selection for litter size has resulted in an increased ovulation rate (Bateman, 1966). The principal effect of selection was to increase the sensitivity of the ovary to follicle-stimulating hormone (FSH) and an alteration in the shape of the dose-response curve (McLaren, 1962).

Since FSH indirectly controls estrogen levels, an alteration in FSH level could cause an imbalance in the estrogen and progesterone ratio. This could result in a decrease or absence of female receptivity to the male (Edwards, 1970). The increase of estrogen beyond the endogenous level can also result in a partial abortion of fetuses (Poland, 1970).

Chai (1970) selected mice for increased ^{131}I uptake and found that these mice showed definite consequences of selection. These mice matured earlier and had an increased growth rate. Thus, mice selected for increased weight gain may also have an increased thyroid activity.

Robinson and Bradford (1969) found that mice selected for post-weaning gain showed a weight increase in all organs measured. Edwards (1962) supported this by finding an increase in pituitary weight in mice selected for large size. However, Edwards found that the primary re-

sponse to selection was the weight increase, not an increase in unit potency.

MATERIALS AND METHODS

Animals

The initial population of mice for this study were obtained from the Institute for Cancer Research's random bred, ICR-albino strain. The control lines, lines 01 and 02, have been maintained by random mating. Mass individual selection is practiced on the lines selected for increased 21 to 42 day weight gain, lines 11, 12, 13 and 14. All matings involving full-sibs and first cousins were avoided, where possible. Both control and selected lines were in the 12th generation of selection when this study was initiated.

A linecrossing program was also initiated to study the possible change in reproductive efficiency between the crossbreds and the parent populations. Two types of crossing schemes were used. First, males from each of the selected lines were crossed with females from the control lines. The crosses were: line 01 females X line 11 males; line 02 females X line 12 males; line 01 females X line 13 males; line 02 females X line 14 males.

The second crossing scheme involved the crossing of males from one selected line to females from the other selected line. There were space limitations on the number of animals that could be maintained, so all possible crosses could not be performed. At the time this study was planned, lines 11 and 13 had lower reproductive performances than lines 12 and 14. Thus, lines 11 and 13 were chosen for the crossbreeding program. These crosses were: line 11 females X line 12 males;

line 11 females X line 13 males; line 11 females X line 14 males; line 13 females X line 11 males; line 13 females X line 12 males; line 13 females X line 14 males.

Thus, 16 lines were available for experimental use. For analytical and discussion purposes, these lines were combined into four experimental groups and will be referred to throughout this thesis by the following group designations. Group C is comprised of control lines 01 and 02. Group S is comprised of the selected lines, 11, 12, 13, 14. Group CS consists of the four control line X selected line crosses previously described. Group SS is comprised of the six selected line X selected line crosses previously described.

General Treatment

The mice utilized in this study were housed in the Dairy Science Mouse Laboratory, Saunders Hall, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. All animals were housed in polypropylene cages using purified Sanicel as litter. A flea and mite powder was used along with the litter to prevent these parasite infestations. All cages were changed weekly.

Food and water were supplied ad libitum directly through the metal cage top. Animals were maintained on a diet of Purina Laboratory Mouse Chow until breeding. From time of breeding until litters were weaned, animals were supplied with Old Guilford Breeder's Chow. In order to reduce any seasonal and environmental variation, the laboratory was maintained at a temperature of 72°F and the light-to-dark ratio was

one (12 hr to 12 hr).

In all matings, there was one male per cage. All matings were for a 17-day period of cohabitation. In treatments where the females carried young to term, the females were separated and placed one female per cage on day 17 after introducing the male. In other treatments, females were removed when exhibiting a copulatory plug (CP) and caged separately. In breeding regimes where more than one female was exposed to the same male, males not plugging females in ten days were replaced with proven males.

Treatment Procedures

Estrous Cycle and Litter Size

Thirty females from each of the lines within groups C and S and 10 females from each of the lines within groups CS and SS were used to study estrous cycle and litter size. The estrous cycle was charted through the use of vaginal smears. The females were allowed to maintain their pregnancys to term and litter sizes recorded.

The day males were placed in cages with females was designated as day 0. Smears were made daily, between 9:00 and 10:00 A.M., from Day 1 until females exhibited a CP, or until Day 17, if no CP was observed. The vagina was flushed with 1 to 2 drops of distilled water. The water was deposited in the vagina with an eyedropper. The flushings were placed on a clean microscope slide and air dried. The smear was examined under a light microscope at 40X and the cellularity stage of the cycle recorded.

After the males were removed, females were checked for parturition at least twice daily. The number of young recorded at the first observation of the litter was used for litter size.

Copulation Plugs

Females assigned to all treatments were checked twice daily for copulatory plugs (CP's), at 8:00 A.M. and again between 3:00 and 4:00 P.M. Examinations were made with the use of a pair of curved dressing forceps to give the most optimum view of the vagina.

Ovulation Rate and Percent Fertilization

Ten females from each of the 16 lines comprising the four experimental groups were examined for ovulation rate and fertilization rate at 3 1/2 days after mating. These females were anesthetized with methoxyfluorane (Metofane) and the ovaries exposed through a midventral incision. Each ovary was examined for the number of corpora lutea (CL) as described by Bradford (1969). The number of CL present was used as an indication of ovulation rate since this is accepted as an accurate method (Bowman and Roberts, 1958; Bradford, 1969).

Immediately after the CL count was completed, the uterus including about 2 mm of each oviduct was carefully removed from the mouse. The fat and supportive tissue were removed from the uterus which made the flushing process easier to control. The uterus was blotted on a paper towel to remove any blood and flushed with warm physiological saline solution using a 1 cc syringe fixed with a blunt 20 gauge needle.

Each horn was flushed separately and collected in a 90 mm watch glass.

The flushings were examined under a stereomicroscope at 50X. The number of eggs were recorded as well as the number fertilized and the stage of development of embryos.

Pre-implantation and Early Post-implantation Mortality

Ten females from each of the 16 lines comprising the four experimental groups were examined at 7 days after mating. A CL count was made on each ovary as previously described while the animal was still under anesthesia. The uterus was then removed and the female sacrificed by severing the spinal cord in the neck region. An incision was made in the uterus along the mesosalphigial border and the uterus opened up with forceps. The number and position of both live and dead fetuses were recorded.

Late Post-implantation Mortality

Each of the 16 lines comprising the four experimental groups contributed 10 females for examination 17 days after mating. In this treatment, the females were examined as before. The number and position of both live and dead fetuses were recorded.

Statistical Analysis

The data was analyzed according to a nested model which included effects due to groups, lines within groups, and the residual term (Steel and Torrie, 1960). ANOVA for significant traits may be found in the Appendix.

RESULTS

Ovulation Rate

The number of corpora lutea (CL's) found on the ovary during gestation has been shown to be an accurate measure of ovulation rate (Bowman and Roberts, 1958; Bradford, 1969). Ovulation rate in this study was determined at two stages of gestation, 3 1/2 days and 7 days after finding a copulatory plug (CP). The number of CL's found at 3 1/2 days and 7 days (Table 1) after breeding was found to be nonsignificant when comparing the four experimental groups.

In selecting for ovulation rate as a direct response, Bradford (1969) found significant differences between control and selected lines with selected lines having a higher rate. An increase in ovulation rate as a correlated response to selection for a particular trait has also been noted (Fowler and Edwards, 1960; Bradford, 1969; Bateman, 1966).

The number of eggs collected at 3 1/2 days after breeding was usually less than the number of CL's for all experimental groups. An analysis of variance for percent recovery was performed to determine if the difference could be attributed to errors in technique. Differences in recovery rate was not significant among groups.

Differences in the number of eggs recovered was found to be nonsignificant between groups (Table 2). These results contrast those of Fowler and Edwards (1960) and Elliot, et. al., (1968) who found that lines selected for high six-week body weight ovulated a larger number

TABLE 1. AVERAGE NUMBER OF CORPORA LUTEA (CL'S) AT 3 1/2 DAYS AND 7 DAYS AFTER MATING

Experimental Group	n	3 1/2 Days	Number CL*** n	3 1/2 Days*	n	7 Days	n	7 Days**
Group C	17	10.35	15	11.53	17	11.06	13	14.46
Group S	38	9.05	30	11.20	29	10.76	20	15.60
Group CS	38	11.37	38	11.37	36	9.61	21	14.43
Group SS	59	9.53	51	10.96	55	12.36	43	14.12

*Animals with no CL's on one or both ovaries deleted from analyses.

**Nonpregnant animals deleted from analysis.

***Analysis of variance not significant ($P > .05$).

TABLE 2. AVERAGE NUMBER OF EGGS COLLECTED, EGGS FERTILIZED, AND FERTILIZATION RATE AT 3 1/2 DAYS OF GESTATION*

Experimental Group	n	Eggs Collected	n	Eggs Collected**	n	Eggs Fertilized	n	Eggs Fertilized**	n	Fertilization Rate**
Group C	17	7.59	15	8.27	17	7.12	15	7.73	15	93.60%
Group S	38	6.89	30	8.67	38	6.32	30	7.97	30	91.17%
Group CS	38	9.32	38	9.32	38	8.32	38	8.32	38	84.65%
Group SS	59	6.86	51	7.92	59	6.34	51	7.31	51	90.48%

*Analysis of variance not significant ($P > .05$).

**Animals with no CL's on one or both ovaries deleted form analysis.

of eggs than control lines. Control lines were also higher than lines selected for low six-week body weight.

Fertilization Rate and Stage of Development of Fertilized Eggs

Fertilization rate is dependent upon both maternal and paternal factors. The number of eggs shed can be considered a maternal factor and is related to the circulating levels of follicle-stimulating hormone (FSH) (McLaren, 1962) and leutinizing hormone (LH) (Ladmun and Runner, 1953). The number of eggs fertilized is determined by both male and female. The female times the release of and releases the egg (Fugo and Butcher, 1971) into the oviduct. Finn (1964) stated that the male effect, although small, is definitely a factor in the number of eggs fertilized. The male effect is probably mediated through sperm density and sperm quality (Finn, 1964).

The number of eggs shed were not significantly different among the experimental groups (Table 2). The number of fertilized eggs and fertilization rate were also nonsignificant (Table 2). These results disagree with those of Elliot, et. al., (1968) who found that lines selected for large six-week weight had a significantly higher fertilization rate. It should be pointed out that Elliot was selecting for six-week body weight, rather than 3 to 6 week weight gain as in this study. Fowler and Edwards (1960) found, however, that lines selected for large six-week body weight had a lowered fertility. Fowler and Edwards attributed the lowered fertility they observed to a decrease in male libido. The gain lines used by Fowler and Edwards showed no significant differ-

ences in fertility which is in agreement with the results presented here.

The stage of development of the embryos collected was also examined. The percentage of blastocysts, calculated as a ratio of the number of blastocysts to the number of fertilized eggs, gives an indication of the stage of development of the eggs. No significant differences were found for the number or percentage of blastocysts (Table 3). Although these differences were nonsignificant, control lines averaged 96.1% blastocysts compared to 80.3% for selected lines, a 16% difference. This may indicate that there is a delay in ovulation or a delay in follicular growth.

The results obtained from both group CS and group SS closely followed those of the selected lines. The percentage of blastocysts were 79.6% and 81.1% for group CS and Group SS, respectively (Table 3). In comparing the F_1's to the selected lines, it seems as though the delay in ovulation also occurs in these F_1 groups. If delayed ovulation did not occur, the percentage of blastocysts for groups CS and SS would be expected to approximate the control lines. This result was not found.

Pre-implantation Mortality

The loss of young through embryonic mortality has been well documented (Bowman and Roberts, 1958; Falconer and Roberts, 1960; White, et. al., 1968). Embryonic death can occur at any stage of gestation and the stage where losses occur must be identified before corrective

TABLE 3. AVERAGE NUMBER OF BLASTOCYSTS* AND AVERAGE PERCENT BLASTOCYSTS* AT 3 1/2 DAYS OF GESTATION

Experimental Group	n	Number of Blastocysts	n	Number of Blastocysts**	n	Percent Blastocysts**
Group C	17	6.82	15	7.40	15	96.07%
Group S	38	5.58	30	7.07	30	80.32%
Group CS	38	6.76	38	6.76	38	79.64%
Group SS	59	5.41	51	6.25	51	81.13%

*Analysis of variance not significant ($P > .05$).

**Animals with no CL's on one or both ovaries deleted from analysis.

measures can be applied.

Boshier (1968) found that pre-implantation losses were the greatest contributors to total embryonic mortality. This loss may occur very soon after fertilization (Bowman and Roberts, 1958) and may be due to an endocrine factor of the female (Falconer and Roberts, 1960).

Pre-implantation mortality does not appear to be an important factor in this study. Nonsignificant differences among experimental groups were found for the number of CL's (Table 1) and the number of implants (Table 4) found at 7 days of gestation. Pre-implantation mortality, determined as a ratio of the number of implants to the number of CL's was also nonsignificant (Table 4) between these groups.

Little visual difference was found between group C and group S for pre-implantation mortality. These results are in agreement with those of Fowler and Edwards (1960) and Elliot, et. al., (1968). Both found little difference between control and large lines, each maintaining pregnancies to the implantation stage with little difficulty.

Pre-implantation mortality for group CS did not differ appreciably when compared to either parent population (Table 4). This was expected since there was not a significant difference between the parent strains. Pre-implantation losses were not appreciably different between group SS and the selected lines. This was expected since there was little difference in the number of implants (Table 4) and the number of CL's (Table 1) between these two groups.

TABLE 4. AVERAGE NUMBER OF IMPLANTS,* NUMBER OF LIVE FETUSES,* AND PERCENT SURVIVAL* AT 7 DAYS OF GESTATION

Experimental Group	n	Number Implants	n	Number Implants**	n	Number Live Fetuses	n	Number Live Fetuses**	n	Percent Survival***
Group C	17	11.41	13	14.92	17	11.35	13	14.85	13	104.09%
Group S	29	11.28	20	16.35	29	11.10	20	16.10	20	104.89%
Group CS	36	9.69	21	15.00	36	9.47	21	14.63	21	100.28%
Group SS	55	13.35	43	15.91	55	12.45	43	14.84	43	110.03%

*Analysis of variance not significant ($P > .05$).

**Nonpregnant animals deleted from analysis.

***Determined as a ratio of the number of implants to the number of CL's, nonpregnant animals deleted from analysis.

Post-implantation Mortality

The number of post-implantation losses were determined at two stages of gestation, early, at 7 days of gestation, and late, at 17 days of pregnancy. In both cases, percent survival was calculated as a ratio of the number of viable implants to the total number of implants.

No significant differences were found between experimental groups at 7 days of gestation for the number of implants or the number of live implants (Table 4). Percent survival was also nonsignificant although the control lines were slightly higher than the selected lines (Table 5). These results are in agreement with those of Elliot, et. al., (1968) who found no significant differences between control and large lines. However, Fowler and Edwards (1960) found that large mice had significantly more post-implantation losses than did controls.

Results obtained from Group CS also differed little when compared to group S and group C (Table 5). No appreciable difference was noted when comparing group SS to the selected lines, group S. This contrasts the results of Bateman (1966) and Boshier (1968) who found that hybrids had lower losses than either parent.

Late post-implantation mortality, determined at 17 days of gestation was not significant among the four experimental groups (Table 6). Little difference was found between the control and selected lines. This was expected since the number of implants and number of live fetuses differed little (Table 6). These results contrast those of both Elliot, et. al., (1968) and Fowler and Edwards (1960). Both groups

TABLE 5. AVERAGE EARLY POST-IMPLANTATION SURVIVAL AT 7 DAYS OF GESTATION.*

Experimental Group	Early Post-implantation Survival**
Group C	99.55%
Group S	98.82%
Group CS	99.80%
Group SS	93.55%

*Determined as a ratio of the number of live fetuses to the number of implants, animals with no implants deleted from analysis.

**Not significant ($P > .05$).

TABLE 6. AVERAGE NUMBER OF IMPLANTS,* NUMBER OF LIVE FETUSES,* AND PERCENT SURVIVAL* AT 17 DAYS OF GESTATION

Experimental Group	n	Number Implants	n	Number Implants**	n	Number Live Fetuses	n	Number Live Fetuses**	n	Percent Survival**
Group C	13	11.62	11	13.73	13	11.31	11	13.36	11	97.32%
Group S	14	12.86	12	15.00	14	12.29	12	14.33	12	93.92%
Group CS	27	10.74	20	14.50	27	10.44	20	14.10	20	97.50%
Group SS	51	13.22	42	16.05	51	12.63	42	15.33	42	95.80%

*Analysis of variance not significant ($P > .05$).

**Nonpregnant females deleted from analysis.

found that lines selected for large six-week body weight lost significantly more fetuses in the later stages of gestation than did control lines.

Elliot, et. al., (1968) attributed the loss to intra-uterine crowding and noted that the embryos had a tendency to be clumped together at certain sites in the uterus. No tendency towards clumping was noted in this study and may account for the contrasting results.

Fowler and Edwards (1960) also found an increase in mortality in their gain lines. Perhaps the gene(s) affecting these losses were not affected by selection in this present study whereas they did respond to selection in Fowler and Edwards' study.

There was little difference in late post-implantation mortality for group CS compared to both group S and group C (Table 6). However, group CS had a lower loss than did group S. This agrees with Bateman (1966) and Boshier (1968) who found that hybrids had lower losses at this stage of gestation. Boshier (1968) attributed the lower loss to heterosis. Since the difference was nonsignificant, it is not possible to determine if this is a heterotic effect or a chance occurence.

There was a slight visual difference in post-implantation losses of group SS when compared to group S (Table 6). Group SS sustained lower losses than did the selected lines. Negation of inbreeding depression does not seem to be the cause as Falconer and Roberts (1960) found that inbreeding did not affect post-implantation losses. The trend noted is probably due to heterosis.

Although the results were nonsignificant, there was a trend to-

wards increased post-implantation mortality in group S. Considering the improvement in both group CS and group SS over the selected lines, it might be that selection has increased mortality at this stage of gestation.

Plugging Rate

The presence of a copulation plug (CP) is a reliable indication that coitus did occur during the 24 hours preceding the finding of a plug (Fainstat, 1951). The presence of a CP was used as an indication of mating in this study.

Highly significant differences ($P < .01$) were found between experimental groups for the number of CP's found during the 17 day mating period (Table 7). Control lines plugged an average of 86.7% compared to 71.7% for the selected lines. This contrasts the results of Elliot, et. al., (1968) who found that large lines were not significantly different from controls. The conflicting results can be attributed to different strains and different selection programs where different genes may have responded to selection.

Group CS had a higher plugging rate than the selected lines (Table 7). Group CS plugged at a rate of 91%, an increase of about 20% over the selected lines. Group SS also plugged at a higher rate than did the selected lines, 85.5% and 71.7%, respectively. The improvement of both groups CS and SS can be attributed to heterosis. That this improvement is due to heterosis seems especially likely since control lines had a higher plugging rate than did selected lines. This also indicates

TABLE 7. AVERAGE PLUGGING RATE*

Experimental Group	Plugging Rate**
Group C	86.67%
Group S	71.67%
Group CS	91.67%
Group SS	85.45%

*Determined as a rate of the number of females plugging to the number of females paired.

**Significant ($P < .01$).

that this trait, or the physiological factors influencing this trait, are under genetic control. It would also seem that selection has altered this trait and it is a contributing factor to the lowered reproductive efficiency found in the selected lines.

Litter Rate

The number of females littering is a function of two processes. First, a female must accept the male and permit coitus to occur. Second, a female must maintain the pregnancy to parturition.

Litter rate was calculated as a ratio of the number of females littering to the number of females paired. Highly significant differences ($P < .01$) were found among experimental groups for litter rate (Table 8). Control lines averaged 88.3% females littering whereas the selected lines averaged 64.2%. The results indicate that the number of females littering has been decreased as a response to selection.

Group CS also had fewer females littering than did the controls, 58.3% and 88.3%, respectively (Table 8). Group CS approximated the results of the selected lines. That is, group CS and group S did not differ appreciably. Group SS also followed the trend set by the selected lines and were lower than the controls (Table 8). Group SS had only 67.3% of the females litter. The influence of the selected lines is shown and also supports the idea that this trait has responded to selection.

An interesting observation that should be pointed out is the relationship between the number of plugs found and the number of litters

TABLE 8. AVERAGE LITTER RATE*

Experimental Group	Litter Rate**
Group C	88.33%
Group S	64.17%
Group CS	58.33%
Group SS	67.27%

*Determined as a ratio of the number of females littering to the number of females paired.

**Significant ($P < .01$).

recorded. Both groups of crossbreds improved over the selected lines in the number of plugs found. However, they did not improve over the selected lines and had lower litter rates than the control lines.

Estrous Cycle

Whitten (1956) indicated that the estrous cycle in mice is normally 4 to 5 days in length and is divided into four stages. These are proestrus, estrus, metestrus, and diestrus. Normally, proestrus lasts for less than one day, 1 day is spent in estrus, 1 to 2 days are spent in metestrus, and the remaining day is diestrus (Collins, 1967).

Both cycle length and the number of days spent in each stage were analyzed. Cycle length was determined by adding the mean values for the number of days in each stage.

The number of days spent in proestrus differed significantly ($P < .01$) between the experimental groups, group S spending a longer time in proestrus than the control lines (Table 9). Mean values were 0.80 days for the controls and 1.47 days for selected lines. Group CS also spent more time in proestrus than group C, an average of 1.17 days. In contrast, group SS spent about the same amount of time in proestrus as did group C, a mean value of 0.76 days.

Selection seems to have increased the time spent in proestrus. However, no definite conclusion can be made because of the conflicting results of the group CS and group SS. The results of group CS might be expected as a result of selection, but not those of group SS.

TABLE 9. AVERAGE NUMBER OF DAYS SPENT IN EACH OF THE FOUR ESTROUS CYCLE STAGES

Experimental Group	Proestrus*	Estrus*	Metestrus*	Diestrus**
Group C	0.80	1.10	0.60	1.27
Group S	1.42	2.27	1.90	1.68
Group CS	1.17	1.31	1.28	1.56
Group SS	0.76	1.09	1.27	1.69

*Significant ($P < .01$).

**Not significant ($P > .05$).

Group SS would be expected to approximate the selected lines if group CS did; however, this was not found.

The number of days spent in estrus were significantly different ($P < .01$) for the experimental groups (Table 9). Selected lines spent almost twice as much time in estrus than did controls, 2.27 days and 1.10 days, for the selected and control lines, respectively. The selected lines also spent a longer time in estrus than either group CS or group SS. Group CS averaged 1.31 days in estrus and group SS spent 1.09 days.

Differences in the number of days spent in metestrus were significant ($P < .01$) between experimental groups. The mean for group S was 1.90 days, whereas group C averaged only 0.60 days. Values for groups CS and SS were intermediate between the selected and control lines. The means were 1.28 days for group CS and 1.27 days for group SS.

No significant differences were found for the amount of time spent in diestrus (Table 9). There was a trend, however, for the selected lines to spend more time in diestrus than the control lines, 1.68 days compared to 1.27 days. As in metestrus, the values for the crossbreds were intermediate between the selected lines and control lines. The mean for group CS was 1.56 days and 1.69 days for group SS.

Another observation should be mentioned here. Not only did the selected lines increase the amount of time spent in each of the cycle stages, they increased over the values quoted by Collins (1967) which will be discussed later.

In looking at cycle length, it is obvious that selected lines had shifted to a longer cycle than the control lines. Where cycle length was 3.77 days on the average for control lines, it increased to 7.27 days for the selected lines. This is not suprising since the selected lines spent more time in each of the cycle stages than controls. It does seem that cycle length of the selected lines has been increased as a response to selection. Fowler and Edwards (1960) noted an increase in the cycle length of large lines (6 to 7 days) compared to controls (4 to 5 days).

The cycle lengths of the F_1 groups were between those of the selected and control lines. Mean values were 5.22 days for group CS and 4.81 days for group SS. Although these cycle lengths were longer than that of the controls, they still fell within the normal 4 to 5 day cycle length. However, part of the increase found may be due to the influence of the selected lines.

The number of days from pairing until females plugged was used as an indication of cycle length. Whitten (1956) found that females were usually in diestrus until paired with a male and then began cycling. Whitten (1958) indicated that the greatest proportion of females plugged on the third day after pairing, when females were cycling normally.

The control lines followed the results of Whitten's (1958) study. It took an average of 3.39 days for females to plug. Significant differences were found ($P < .01$) between experimental groups for this trait. Selected females increased the number of days from pairing until plugging over the females of the control lines (Table 10). The average

TABLE 10. AVERAGE NUMBER OF DAYS FROM PAIRING UNTIL PLUGGING

Experimental Group	Number of Days*
Group C	3.39
Group S	5.50
Group CS	4.72
Group SS	5.03

*Significant ($P < .01$).

number of days from pairing until plugging was 5.50 days, indicating an increase in cycle length. This supports the data previously discussed.

Group CS and SS also took a longer time to plug than controls. The time required was an average of 4.72 days for group CS and 5.03 days for group SS. These crosses again fell between the averages of the selected and control lines. The increase of the crosses over the controls may be due to the effects of selection.

Litter Size

Two methods of determining litter size were used. The first determines litter size as a ratio of the number of young born to the number of females paired with males. This type of calculation is a measure of efficiency since it takes into account females that do not breed, become pregnant or maintain pregnancies to term. Litter size differences approached statistical significance ($P = .068$).

By looking at litter size in this manner, control lines were more efficient than selected lines (Table 11). Control lines averaged 10.75 pups per female paired, whereas selected lines averaged a less efficient 8.82 pups. Both group CS and SS followed the less efficient trend of the selected lines with litter sizes of 7.42 and 8.47, respectively. These results are somewhat expected since groups S, CS, and SS had fewer females littering than group C. It should be pointed out, however, that there was a significant ($P < .05$) variation between the lines comprising the groups.

Although the previous determination of litter size is useful as a

TABLE 11. AVERAGE LITTER SIZE*

Experimental Group	Litter Size**
Group C	10.75
Group S	8.82
Group CS	7.42
Group SS	8.47

*Determined as a ratio of the number of young born to the number of females paired.

**Approached significance (P = .068).

measure of efficiency, it does not give a complete picture. Litter size was also determined as a ratio of the number of young born to the number of females littering. This can be used as a measure of the capability of the female to produce young once pregnant.

Differences in litter size by this determination were found to be significant ($P < .05$). However, the trend in these results was just opposite to those calculated as a measure of efficiency. Selected lines had a larger mean litter size than the control lines (Table 12). Means were 13.74 pups for selected lines and 12.17 pups for control lines. These results agree with those of Moore, et. al., (1970) and White, et. al., (1968) who found that litters born to large line females were significantly larger than those of control lines. It would seem that females from the selected lines are superior to those from control lines in producing young, once they are pregnant.

Groups CS and SS had larger litters than the controls (Table 12). The means were 12.71 pups and 12.59 pups for group CS and group SS, respectively. The increase of the crossbreds over the controls also seems to indicate that selection has increased litter size in the large females, once they are pregnant.

TABLE 12. AVERAGE LITTER SIZE*

Experimental Group	Litter Size**
Group C	12.17
Group S	13.74
Group CS	12.71
Group SS	12.59

*Determined as a ratio of the number of young born to the number of females paired.

**Significant ($P < .05$).

DISCUSSION

Ovulation Rate

The number of eggs shed is dependent upon the level of FSH or ovarian sensitivity to this hormone (McLaren, 1962). Above a certain level, LH does not affect ovulation rate. Thus, selection could affect ovulation rate either through the levels of FSH and LH or by changing ovarian sensitivity to these hormones.

The results presented for this study found nonsignificant differences between experimental groups for the number of CL's. Both Fowler and Edwards (1960) and Elliot, et. al., (1968) found that selected large line mice had significantly greater ovulation rates than controls. However, both workers were selecting for increased body weight at six-weeks of age, rather than weight gain from three to six weeks of age as in this study. Although the traits are similar, the genetic mechanisms may differ greatly.

It should be mentioned that there was a vast difference in the amount of selection practiced between this study and those mentioned above. Both Fowler and Edwards (1960) and Elliot, et. al., (1968) were using animals from the 35th to 40th generations of selection. This study used animals from generation 12. Since there is a vast difference in generation number, it may account for the conflicting results presented here.

Ovulation rate does not appear to be a major factor in the lowered reproductive efficiency associated with the selected lines.

Fertilization Rate and Stage of Development of Fertilized Eggs

Fertilization rate and the number of fertilized eggs were found to be nonsignificant. No trends were established for either the control, selected, or F_1 lines to be superior.

Elliot, et. al., (1968) noted a significant trend for large lines to have a higher fertilization rate than control lines. One explanation for the differences encountered between this and Elliot's study is that two different populations were studied, each under a separate selection program and originating from a separate genetic base. This is supported by reports of strain differences in body composition (Robinson and Bradford, 1969), pituitary activity (Edwards, 1962), and hormone levels (McLaren, 1962). These traits have an influence on fertilization rate and may have responded differently to selection in each of the two populations.

The females role in fertilization does not appear to have been altered as a response to selection. No significant differences were found and no trends established. This does not appear to be a major factor in the lowered reproductive efficiency of the selected lines.

There was a trend established in the stage of development of the fertilized eggs between groups. Although the percentage of eggs in the blastocyst stage at the 3 1/2 day examination was nonsignificant between groups, control lines had 16% more blastocysts than the selected lines. In all cases, the majority of eggs found that were not blastocysts were in the morula stage. The morula stage is an earlier stage

of embryonic development than the blastocyst and indicates a delay in follicular growth, ovulation, fertilization, or embryonic development. Fugo and Butcher (1971) attributed a similar ovulation delay to a delay in ova growth. Differential delay in egg growth could cause an increase in the amount of time from the first to the last ovulation. Differences in embryonic growth indicate a trend for the selected lines to have delayed ovulation.

Pre-implantation Mortality

Boshier (1968) found that pre-implantation losses were the greatest contributors to total embryonic mortality. This period was believed to be one of critical importance in embryonic survival. Bowman and Roberts (1958) thought that the loss might occur very soon after fertilization.

Pre-implantation losses in this study were not significantly different among the experimental groups. Visual differences between group C and group S were small. Fowler and Edwards (1960) found little difference between large and control females in the amount of pre-implantation losses. Bateman (1966), however, found that females from a less fertile strain were particularly prone to pre-implantation losses of eggs. Elliot, et. al., (1968) found that both control and large line females were able to maintain their pregnancies to the implantation stage with little difficulty.

Post-implantation Mortality

Post-implantation mortality is dependent upon several factors.

Boshier (1968) and Moore, et. al., (1970) reported that the genotype of the embryo was a factor in its development in the uterus. Mortality may also be affected by uterine capacity since Bowman and Roberts (1958) found the number of losses to be related to the number of implants.

No significant differences were found at 7 days of gestation for the number of implants or live fetuses between the experimental groups. Percent survival was also not significant, although control lines were slightly superior to the selected lines. These results agree with those of Elliot, et. al., (1968) who found no significant differences in fetal mortality between large and control lines.

Fowler and Edwards (1960) and Bowman and Roberts (1958) attributed an increase in post-implantation losses to an increase in ovulation rate in the large lines. In this study, there was little difference in ovulation rate between the control and selected females. The pattern reported by Fowler and Edward's (1960) was not followed by the selected lines in this study.

One could also assume from these results that selection had not increased early post-implantation losses. Since no significant differences were found between the selected and control lines, this does not appear to be a major factor in the lowered efficiency of the selected lines.

Nonsignificant differences were also found between experimental groups for late post-implantation losses. There was a trend, however, for selected lines to sustain slightly more losses than control lines. This agrees with both Elliot, et. al., (1968) and Fowler and Edwards

(1960). Elliot, et. al., (1968) attributed this loss to a tendency towards intra-uterine crowding. However, this does not seem to be the case in this study. The spacing and location of implantation sites were examined. No tendency towards overcrowding as described by Hafez (1964) was noted.

It seems then that selection may have slightly increased late post-implantation losses. This is probably due to an alteration in gonadotrophin secretions. The change in secretory levels of gonadotrophin influences the maintence of pregnancy by affecting progesterone concentrations. An alternate hypothesis is that the change in secretory level of gonadotrophin has increased embryonic mortality by causing an increase in abnormal embryos.

Although the results were nonsignificant, there was a trend towards increased late post-implantation mortality in the selected lines. The effect of selection may be mediated through a change in hormone levels. However, even though a trend was found, it does not seem to be a factor in the lowered reproductive efficiency associated with the selected lines.

Plugging Rate

The presence of a copulation plug (CP) is a reliable indication of coitus occurring during the 24 hours preceeding the finding of a plug (Fainstat, 1951). The presence of a CP was considered as a reliable indication of mating in this study since very few females were found to litter that did not exhibit a CP.

Highly significant ($P < .01$) differences were found among the experimental groups for the number of CP's found during the 17 day mating period. Eighty-six point seven percent of the control females plugged compared to 71.7% for the selected lines.

Both Fowler and Edwards (1960) and Elliot, et. al., (1968) reported no significant differences between large and small females for the number of matings performed. Fainstat (1951) indicated definite strain differences for the number of females plugging.

The number of females plugging is a combination of several factors. Fowler and Edwards (1960) attributed part of the lowered fertility of large lines to a decreased libido on the part of the male. The lowered libido was attributed to a considerable increase in fat deposition (Fowler, 1958).

Male libido may have been a factor in this study since more group S males had to be replaced with proven males than the other groups. No definite conclusions can be made since experimental design did not test the male.

A contributing factor to the lowered number of selected females plugging may be female receptivity. Lane (1959), in a comparison of obese and non-obese mice, found that obese mice secreted a lower amount of pituitary gonadotrophins than non-obese females. The lowered gonadotrophin secretion was thought to be caused by a hypofunction of the pituitary gland.

Estrogen and progesterone must be properly balanced for normal female receptivity to occur. Since these two hormones are controlled

by the levels of the gonadotrophins, FSH and LH, a lowered gonadotrophin level may have altered their production. An imbalance in their interaction may have resulted, lowering female receptivity.

Group CS showed a definite improvement over both the control and selected lines. This improvement may be due to heterosis since significantly more group CS females plugged than either parent. This increase is probably due to an increase in female receptivity over the selected lines, not to a shortening of the estrous cycle.

Group SS also had a higher plugging rate than the selected lines. The improvement over the selected lines was probably due to hybrid vigor. Different, although similar, genes may have responded to selection in the individual selected lines. By crossing the selected lines, the detrimental genes may have been covered up, resulting in the improvement.

Selection for body weight gain in this study has altered the number of females plugging. The effect of selection may have resulted in a lowering of female receptivity, caused by an imbalance of estrogen and progesterone. In view of the significantly lower number of females plugging in the selected lines, this seems to be a major factor in the associated lower reproductive performance.

Litter Rate

The number of females littering is derived from two factors. First is the number of females mating and becoming pregnant. The second is the ability of the female to maintain a pregnancy to parturition.

Litter rate was found significantly different ($P < .01$) among the experimental groups. Group C had a higher litter rate than did group S. Fowler and Edwards (1960) found similar results with large body weight females having a higher percentage of sterile matings than did the controls. In contrast, Elliot, et. al., (1968) found that large lines had significantly more females littering than did control lines.

Several explanations may account for the lowered litter rate found in the selected lines. Very few females littered that did not first show a CP. Thus, the presence of a plug was a reliable indication of mating. Significantly fewer selected females plugged than did controls, as was previously discussed. Thus, the opportunity for littering was severely decreased in the selected lines.

There were fewer females littering than plugging in the selected lines. It seems that although some females plugged, they did not become pregnant. The establishment of pregnancy may then be a factor in the lower litter rate of the selected lines.

Group CS did not improve above the selected lines and had a lower litter rate than control lines. This result is somewhat suprising since group CS females had a higher plugging rate than either the controls or selected lines.

A lower ovulation rate was found at 7 days than at 3 1/2 days. This result occurred because all females that plugged were included in the analysis. This indicates that a greater proportion of group CS females were not pregnant at the 7 days post-CP examination. It seems that the loss may be due to implantation failure as it occurred between

the 3 1/2 day and 7 day examinations.

Another possible explanation is that the uterus was not prepared for implantation due to an alteration of hormone levels. Lane (1959) reported that a hypofunction of the pituitary resulted in a lowered secretion of gonadotrophins. Thus, the level of LH and prolactin may have been lowered. In mice, these two hormones work together to stimulate the production of progesterone and estrogen by the CL (Turner, 1966). Estrogen augments the blood supply to the uterus and works with progesterone to increase the number of endometrial cells. A decrease in the amount of either hormone may then have resulted in an improper preparation of the uterus. This could possibly have caused the implantation failure noted in group CS.

Group SS had a lower litter rate than did the control lines. This result was not expected as these F_1's differed little from the controls in plugging rate. The same trend was noted in group CS, although it was not as severe. That is, a number of females plugged, but were not pregnant. Although there was a lower percentage of females littering than plugging in group SS, the reduced litter rate does not seem to be due to implantation failure as in the group CS.

Selection seems to have altered the number of females littering. The lowered litter rate of the selected females is probably due to the lowered plugging rate found in these lines. Variation in plugging rate as an explanation of the results of group CS is not clear. Perhaps, linecrossing increased gonadotrophin output to the point where receptivity was increased by a more harmonious estrogen-progesterone inter-

action. However, the increased gonadotrophin secretion may not have been enough to produce the proper amounts of estrogen and progesterone needed for implantation.

The decrease in litter rate of the selected lines as compared to control lines indicates that the selected females were less efficient in producing young. Litter rate seems to be a major factor in the lowered reproductive performance associated with the selected lines.

Estrous Cycle

The normal length of the estrous cycle in mice is four to five days (Whitten, 1956). The cycle is divided into four stages: proestrus, estrus, metestrus, diestrus. The stages also occur in the order given.

The selected lines spent a longer time in proestrus, estrus, and metestrus than did the controls. Time spent in these cycle stages were significantly ($P < .01$) different among the experimental groups. A trend was found towards an increased amount of time in diestrus for the selected lines compared to the controls. This is an indication of a lengthened estrous cycle in group S. Group S also increased the amount of time spent in each stage of the cycle over the normal values quoted by Collins (1967).

Lane (1959) found that obese mice produced less gonadotrophins than did non-obese mice. It should be noted here that the selected females in this study seemed to have an increase in fat deposition and tended towards obesity. Thus, the selected females may have had a low-

ered gonadotrophin output.

Lane (1959) also indicated that the lowered gonadotrophin output was due to a hypofunctioning pituitary. The increased fat deposition noted may be related to a lowered secretion rate of thyroid-stimulating hormone (TSH). TSH acts primarily on the production of thyroxine which, in turn, also exerts an effect on reproduction.

Thyroid deficiencies in the female may cause the cycles to cease or become irregular (Turner, 1966). There was a tendency for the selected lines to have more irregular cycles than did the controls, which may have been caused by a lowered thyroxine production mediated through a decreased TSH output from a hypofunctioning pituitary gland.

A hypofunctioning pituitary may have decreased the output of FSH and LH. Normally during estrus, estrogen secretion, influenced by FSH level, is increased. The higher estrogen level causes an increase in cellular activity and growth in the uterus (Turner, 1966). Since FSH level seems to have been lowered in group S, estrogen secretion may also have been lowered. This could result in slower cell growth in the selected lines and may account for the increased estrus period of the selected lines.

Metestrus is characterized by the disappearance of cornified cells and the gradual increase in appearance of leucocytes. This period occurs immediately after estrus and ovulation. The selected lines had a definite increase over the controls in the amount of time spent in this stage. This increase may be due to slower activity of the uterus. The time needed for the uterus to diminish in vascularity and contract-

ility has increased. The possibly lowered gonadotrophin output may have severely slowed down cellular activity in the reproductive tract.

Overall, selection seems to have affected the length and continuity of the estrous cycle. This was probably through a hypofunction of the pituitary, resulting in a lowered secretion of possibly TSH, FSH, and LH.

The increase in estrous cycle length as a response to selection seems apparent by looking at the results of the F_1 groups. By summing the average number of days spent in each stage, an indication of cycle length can be obtained. Both group CS and group SS were on 4 to 5 day cycles whereas the selected lines had 6 to 7 day cycles. The shortening of cycle length in these groups seems to have come in the amount of time spent in estrous and metestrous. Both group CS and group SS spent less time in these stages than group S.

The shortened cycle length of the F_1 groups over group S, but not over group C, seems to indicate that genes controlling cycle length are not totally dominant. The results indicate that cycle length may be determined through additive genes, rather than dominance.

The increased cycle length due to selection has resulted in lowered efficiency by delaying plugging of females. There was also a tendency toward irregular cycles which may have lowered female receptivity and increased the number of nonpregnant females.

The number of days from pairing until plugging was used as an indication of the length of the estrous cycle. Whitten (1956) found that virgin female mice usually remained in diestrus when no male was pre-

sent. After the female was exposed to the male or male urine, the female began cycling. Whitten (1958) indicated that the greatest proportion of females plugged on the third day after pairing when females were cycling normally. This would account for one day in diestrus and proestrus with estrus on the following day.

Significant differences ($P < .01$) were found between the experimental groups for the number of days from pairing until plugging. Group C plugged sooner than the other groups, an average of 3.39 days, following the results of Whitten (1958). This indicates that the control females were cycling normally. In contrast, group S females took the longer time of 5.5 days to plug.

Fowler and Edwards (1960) used the number of days from pairing until littering as an indicator. Their results showed the same trend as in this study. Litters arrived significantly sooner to control females than selected large line females. This indicates that control females plugged sooner after pairing than did the large females.

The significantly longer time that it took group S females to plug may be partially attributed to a lowered female receptivity. In viewing vaginal smears, group S females had a greater number of smears that had few or no cornified cells in the smear. The lack of cellular exfoliation may indicate that females were not strongly in estrus.

The females from group S also had a greater proportion of abnormal cycles and showed a tendency to skip certain stages of the cycle. They also tended to be in one stage, move to the next stage, and revert to the previous stage.

The finding of irregular or abnormal cycles and lowered female receptivity indicates an alteration in hormone balances. Female receptivity is controlled by the interaction of estrogen and progesterone (Chipman and Bronson, 1971). Both of these hormones are indirectly controlled by the pituitary gonadotrophins.

The selected females were significantly larger in body size than the control females (La Salle, 1972). As these females aged, there was a trend towards obesity. Lane (1959) found that the pituitaries of obese mice produced less gonadotrophins than they were competant to produce. The level produced was lower than that of non-obese females. Lane (1959) suggested that the sterility observed in the obese females was the result of a hypofunction of the pituitary associated with genetic obesity.

Since the secretion rate of gonadotrophins seems to have been lowered, this may account for the decrease in female receptivity. The lowered amount of gonadotrophin may have altered the synthesis of estrogen and progesterone, resulting in an imbalance of these two steroids and a decrease in the expression of estrus and female receptivity.

The number of days from pairing until plugging was longer for group CS than the control lines. Group CS took an average of 4.72 days to plug which is also longer than that reported by Whitten (1958). The results of group CS indicate that selection had a definite effect on this trait. Group CS was less efficient than the controls, but did improve over group S and may be attributed to heterosis.

It seems that the influence of group S may have caused the same

problems to occur in group CS. There was a delay in plugging, caused either by a lengthened cycle or lowered female receptivity. As was previously discussed, group CS had a longer estrous cycle than group C and was shorter than that of group S. Although a few were found, the tendency towards extremely irregular cycles was not noted. A delay in plugging was shown in group CS. This was probably due to the same reasons as discussed for the selected lines. However, hormone levels were probably not changed to the same degree as in group S as indicated by the improvement of this group over group S.

Group SS closely followed the pattern of group S, taking a longer time to plug than group C and having a tendency towards irregular cycles. The effect of selection was also shown in group SS since they had similar patterns to group S.

In this study, selection has increased the time from pairing until plugging. This was caused either by a lengthening of the estrous cycle or lowered female receptivity. Both are controlled through hormone secretions, balances, and interactions, which have been shown to be affected by selection. In view of the increased time needed for selected females to plug, it seems that this is a contributing factor to the lowered reproductive efficiency of the selected lines.

Litter Size

Two methods of calculating litter size were used. As a measure of efficiency, litter size can be determined as the average number of young born per female paired. Using this type of calculation, litter size

approached significance (P = .068), with group C having a larger mean litter size than group S.

These results are somewhat expected. Selected females were less efficient in that fewer of these females plugged or littered. The number of non-pregnant females was the major factor in the lower mean litter size of the selected lines.

Group CS also had a lower litter size than the controls. It should be pointed out that while more of these females plugged than control females, litter rate was lower then in controls. As previously discussed, there was a marked tendency towards implantation failure in these crossbreds. The increase in the number of nonpregnant females would account for the lower mean litter size than in the control lines.

Litter size for group SS was lower than the controls. They had approximately the same plug rate as in the controls, but a lower litter rate was not attributed to implantation failure and no definite reasons were offered. Since there seems to be no appreciable increase in the percentage of non-pregnant females, no explanation of the lowered litter size can be made at this time.

The second method of determining litter size measures the capability of the female to produce young once pregnant. This method determines litter size as a ratio of the number of young to the number of females that bear litters. Litter size was significantly ($P < 0.05$) different between the experimental groups using this method.

In this respect, selected lines had a higher mean litter size than control lines. Selected lines had a higher average number of implants

and live fetuses at 17 days post-CP than did the controls, although it was not significant. It should be pointed out that there was a trend towards increased post-implantation mortality in the selected lines. This trend was nonsignificant, and its effect on litter size was masked by the number of implants and live fetuses.

The results presented here agree with those presented by several authors. Fowler and Edwards (1960) indicated that large mice had significantly larger litters than control lines. Moore, et. al., (1960) and White, et. al., (1968) also found similar results.

Group CS also had a higher mean litter size than did the controls. As previously discussed, the main problem with these females was a tendency towards implantation failure. In view of these results, it seems that group CS had a superior ability to produce young than the controls, once past the implantation stage.

Litter size for group SS was higher than in the controls. The tendency towards implantation failure as in group CS was not noted. It would seem that, once pregnant, group SS had a superior ability to produce young than did the control lines.

The results indicate that control lines were more efficient in producing young than the selected lines. More females became pregnant and littered. However, when the selected females did litter, they produced more young. The effect of selection seems to have been a decrease in the number of pregnant females.

CONCLUSIONS

One of the primary objectives of this study was to determine which phases of reproduction had changed in response to selection. Ovulation rate and fertilization rate were not found to be limiting factors. However, fertilized eggs from group S were found to be slower developing than in control females. This was thought to be a result of delayed ovulation caused by an alteration in hormone levels.

Embryonic mortality did not seem to be a major contributing factor. No significant differences between experimental groups were found for pre- or post-implantation losses. There was, however, a tendency towards increased post-implantation mortality by 17 days of gestation in group S. The decrease in viability of these embryos may have resulted from delayed ovulation and delayed implantation.

Selection for body size did decrease reproductive efficiency. There was a decrease in the number of group S females plugging and littering. A trend was also noted for more group S females to plug and not litter than in group C. Selection seems to have caused a decrease in female receptivity, possibly mediated through an alteration in the estrogen-progesterone balance.

The decrease in female receptivity was also the probable cause of the increased number of days from pairing until plugging in the selected lines. However, a portion of the increase was attributed to a lengthened estrous cycle in the selected lines. Both receptivity and cycle length are controlled by hormone levels.

Reproductive efficiency in group S females was lowered due to the increase in the number of non-pregnant females. However, group S females that did become pregnant had larger mean litter sizes than the random bred control females.

The main effect of selection seems to have been a decrease in the number of pregnant females. This may have been caused by hypofunction of the pituitary gland, resulting in a decreased production of the trophic hormones which in turn regulate hormone production by their respective target glands.

The effect of the male on lowered reproductive efficiency due to selection was also examined. Male effects were considered negligible in this study.

The last objective of this study was to determine if the decreased reproductive efficiency was under genetic control. That is, could the efficiency be regained with a linecrossing program. In certain phases of reproduction, where detrimental responses to selection were observed, the F_1's out performed the selected lines. Female receptivity was improved as indicated by a shorter interval from pairing until plugging. The estrous cycle was shortened and there was a definite increase in the number of females plugging.

However, there was no improvement in litter rate since a definite trend was noted for F_1 females to plug and not become pregnant. This suggests that some heterosis was exhibited in the F_1's and that production of pituitary trophic hormones may have increased over the selected lines. However, the increase in hormone production may not have been

sufficient to regulate all reproductive processes properly.

SUMMARY

The causes of lowered reproductive efficiency in mice selected for high body weight gain were examined. Four experimental groups were established. Group C was composed of two control lines. Group S consisted of four lines selected for increased body weight gain from three to six weeks of age. Group CS was composed of offspring from the four control lines X selected line crosses. Group SS was composed of offspring from the six selected line X selected line crosses.

Ovulation rate, fertilization rate, percent blastocysts and embryonic mortality were not significantly different among the experimental groups. However, visual trends were observed. Group S had a lower percentage of blastocysts and a higher percentage of morulas at 3 1/2 days of gestation than group C. Group S also tended to have a higher post-implantation mortality at 17 days of gestation than group C. A decreased FSH level could have slowed follicular growth and affected the timing of ovulation as shown by the delayed embryonic development in group S. The delay in ovulation and delayed embryonic development could have influenced the late post-implantation losses of group S.

Highly significant ($P < .01$) differences were found among the experimental groups for plugging rate and litter rate. The difference between group C and group S indicated that selection had decreased the number of females plugging in group S which lowered the litter rate. The lowered litter rate of group CS was not expected because of the high plugging rate. The decreased litter rate of group CS indicated an increase in implantation failure for this group.

The estrous cycle of group S was lengthened as a result of selection. Highly significant ($P < .01$) differences were found among the experimental groups for the number of days spent in proestrus, estrus, and metestrus. Group S spent more time in each of these stages than group C. The number of days in diestrus was not significantly different between groups and group S trended to spend more time (1.68 days) in this stage than group C (1.27 days). Both groups CS and SS followed the pattern of group S and had longer cycles than group C, indicating the influence of selection on this trait.

Litter size, determined as a ratio of the number of young born to the number of females paired, approached significance ($P = .068$). Group C had the largest average litter size, 10.75 pups. Group S averaged 8.82 pups, group CS, 7.42 pups, and group SS, 8.47 pups. Litter size determined as a ratio of the number of young born to the number of females littering differed significantly ($P < .05$) among the experimental groups. By this method, group C averaged 12.17 pups, group S, 13.74 pups, group CS, 12.71 pups, and group SS, 12.59 pups.

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APPENDIX

TABLE 1. ANALYSIS OF VARIANCE FOR PLUGGING RATE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	1.748	0.583	3.903*
Line (Group)	12	2.811	0.234	1.569
Residual	255	38.075	0.149	
Corrected Total	270	42.634	0.158	

*Significant ($P < .01$).

TABLE 2. ANALYSIS OF VARIANCE FOR LITTER RATE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	2.945	0.982	5.085*
Line (Group)	12	5.404	0.450	2.332*
Residual	255	49.231	0.193	
Corrected Total	270	57.579	0.213	

*Significant ($P < .01$).

TABLE 3. ANALYSIS OF VARIANCE FOR LITTER SIZE*

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	293.2910	97.7637	2.3893**
Line (Group)	12	1057.7258	88.1438	2.1542***
Residual	255	1043.9500	40.9175	
Corrected Total	270	1178.9668	43.6480	

*Determined as a ratio of the number of young born to the number of females paired.

**Approached significance (P = .068).

***Significant (P < .05).

TABLE 4. ANALYSIS OF VARIANCE FOR LITTER SIZE*

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	86.1781	28.7260	2.8348**
Line (Group)	12	288.5510	24.0459	2.3730***
Residual	172	1742.9305	10.1333	
Corrected Total	187	2117.6596	11.3244	

*Determined as a ratio of the number of young born to the number of females littering.

**Significant ($P < .05$).

***Significant ($P < .01$).

TABLE 5. ANALYSIS OF VARIANCE FOR THE NUMBER OF DAYS SPENT IN PROESTRUS

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	14.894	4.965	5.257*
Line (Group)	12	14.480	1.207	1.278
Residual	165	155.830	0.944	
Corrected Total	180	185.204	1.029	

*Significant ($P < .01$).

TABLE 6. ANALYSIS OF VARIANCE FOR THE NUMBER OF DAYS SPENT IN ESTROUS

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	50.520	16.840	5.969*
Line (Group)	12	35.162	2.930	1.039
Residual	165	465.456	2.821	
Corrected Total	180	551.138	3.062	

*Significant ($P < .01$).

TABLE 7. ANALYSIS OF VARIANCE FOR THE NUMBER OF DAYS SPENT IN METESTRUS

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	35.468	11.823	4.084*
Line (Group)	12	45.056	3.755	1.297
Residual	165	477.675	2.895	
Corrected Total	180	558.199	3.101	

*Significant ($P < .01$).

TABLE 8. ANALYSIS OF VARIANCE FOR THE NUMBER OF DAYS SPENT IN DIESTRUS

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	4.262	1.421	0.506
Line (Group)	12	50.187	4.182	1.489
Residual	165	463.297	2.808	
Corrected Total	180	517.746	2.876	

TABLE 9. ANALYSIS OF VARIANCE FOR THE NUMBER OF DAYS FROM PAIRING UNTIL PLUGGING

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value
Group	3	290.048	96.683	8.616*
Line (Group)	12	182.842	15.337	1.358
Residual	596	6688.095	11.222	
Corrected Total	611	7160.985	11.720	

*Significant ($P < .01$).

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LOWERED REPRODUCTIVE EFFICIENCY AS A CORRELATED RESPONSE TO SELECTION FOR INCREASED POST-WEANING GAIN IN LABORATORY MICE

by

Suzanne Melissa Morris

(ABSTRACT)

This study was initiated to determine the causes of lowered reproductive efficiency in mice selected for increased post-weaning gain. Four experimental groups were established: (1) Group C, the two control lines; (2) Group S, the four selected lines; (3) Group CS, the F_1 progeny from the 4 control line X selected line crosses; (4) Group SS, the F_1 progeny from 6 selected line X selected line crosses. Observations were as follows: (1) Estrous cycles were charted by vaginal smears and plugging rate, litter rate, and litter sizes recorded; (2) Ovulation rate was obtained by counting CL's at 3 1/2 and 7 days after mating; (3) Fertilization rate was obtained at 3 1/2 days after mating; (4) Pre- and early post-implantation losses were obtained at 7 days after mating; (5) Late post-implantation losses were obtained 17 days after mating.

Results were analyzed by analysis of variance. No significant differences between experimental groups were found for ovulation rate, fertilization rate, or embryonic mortality. Highly significant ($P < .01$) group differences were found for the number of days from introducing the male until a CP was observed. Group C plugged in an average of 3.39

days, whereas group S averaged 5.50 days. The number of days spent in proestrus, estrus, and metestrus were each significant ($P < .01$) for group differences, whereas diestrus was not significant. Plugging rate and litter rate, differed significantly ($P < .01$) as did litter size ($P < .05$) among the experimental groups.