EFFECT OF AGE ON OVULATION RATE IN MICE

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Introduction

Together with productive traits, reproductive traits are of prime importance in animal breeding. Particularly for multiparous animals, productivity largely depends on efficient use of the dam's reproductive lifetime.

Litter size in mammals may be limited and controlled by ovulation rate, fertilization rate, and pre- and post-implantation embryo mortality. The complex relationships among body weight, litter size, and fittness in mice have been the subject of many genetical and reproductive studies.

It is well known that selection for body weight or weight gain leads to correlated changes in reproduction. An increase in litter size has been noted in many-but not all-experiments with mice.[11,19] Although a positive genetic and phenotypic relationship of body weight with ovulation and prenatal embryo survival rate has been reported,[2,10,16] ELLIOTT *et al.*[12] reports an increase of post-implantation embryo loss during body weight selection experiments and LASALLE *et al.*[17] reports a non-significant correlation between the increased post-weaning gain and litter size.

Among the lines of mice that have been selected for juvenile body weight in this laboratory, a decrease in litter size was observed in the M strain in which selection for increased body weight has been conducted at 6 weeks of age for males alone. At the 15th generation, the average litter size was 8.8 in contrast with 14.3 in the control line (unpublished data).

we have limited the reproductive period of all strains to a period of a few weeks after the mice reach 9 weeks of age, and mating for breeding is usually performed only once in this laboratory. In general, reproductive ability of the female mammal begins during pre-pubertial sterility, reaches a maximum in the early stage of maturation and then declines.[4,13~15,20]

Due to a higher sensitivity to gonadotrophines, administration of exoge-

neous hormones to artificially induce ovulation in animals appears to be the most effective when animals are at a very early age. In research on the physiological and genetic properties of ova and embryos, very young animals are convenient for the collection of ova and fertilized eggs. The purpose of this study is to investigate the effect of age at mating on the natural ovulation rate and on pre-implantation embryo mortality, and to investigate interaction between age and line in different strains including the M line, a control strain (C line) and their crossbred groups.

Material and Methods

This study used mice from a selected line (M line) and an unselected control line (C line), that were derived from a crossbred population between ICR and dd flocks. The M line has been selected for high body weight at 6 weeks of age for males only. After the progenies of the 15th generation were weaned, the parents were diallelcrossed between two lines to obtain two interline-bred groups of MC (males of M line with females of C line) and CM (its reverse cross). Litters were weaned at 3 weeks of age and divided into two groups by sex to be raised separately. The raising room was maintained at 23 °C with relative humidity of 50-60% and 12 hr of artificial light daily. Water and pelleted diet were supplied *ad libitum.*

Eighteen males were randomly sampled from MC and CM groups and caged singly before mating. Some females from each group were randomly mated with those crossbred males on the day they turned 6, 9, or 15 weeks of age. No more than four females were placed together in one cage for mating. Inspection for vaginal plug (VP) was performed twice a day. Females exhibiting VP within 2 weeks after being placed together were treated as belonging to the same age group.

The day VP was detected was referred to as the first day of pregnancy and the pregnant females were killed by cervical dislocation between 1 : 00 and 5 : 00 pm on the third day of pregnancy. Oviducts and uterine horns were flushed with 1% gelatine physiological salt solution and eggs were recovered in a watch glass. Under a dissecting microscope, the eggs were morphologically classified as normal or abnormal. In general, normal eggs have developed into more than 8 cells by this time. Unfertilized eggs, fragmented eggs, eggs without zona pellucida, zona pellucida without blastomeres, and eggs retarded two stages behind were classified as abnormal eggs in this study.

Results

Mean litter size and birth weight for the four mating groups of mice used in this study are shown in Table 1. Though there was no significant differences in litter sizes between mating groups, the MC group seemed to have litters larger by one offspring than other groups' litters. The mean birth weight of the CM group was the highest and that of CC group was lowest of the four groups ($P<0.01$).

TABLE 1. Mean litter size and birth weight for four mating groups

	Mating groups			
	MM	MC	CM	CC
Mean litter size (offspring)	11.9±1.13 (7)*	13.0±1.96 (6)	12.2±3.36 (6)	12.2±3.35 (5)
Mean birth weight (g)	1.81±0.07$^{a\,b}$	1.75±0.12$^{a\,c}$	1.85±0.09^{b}	1.68±0.11^{c}

* The numbers in parentheses are the number of female parent mice.
a, b, c Means in same row with different letters differ significantly at $P<0.01$.

Table 2 gives the mean body weight of female mice at the time they were killed for egg collection. There were differences in the mean weight among mating groups but the differences were smaller than standerd deviations. However, within each group, body weight appears to increase with aging.

The mean ovulation rate of each group is shown in Table 3. There were no significant differences either between mating groups or between

TABLE 2. Mean body weight for female parent mice at egg collection

Mating groups	Weekly age group (nearly weekly age)		
	6	9	15
MM	27.3±7.6 (4)[a]	31.0±4.4 (8)	35.3±4.5 (5)
MC	32.0±4.6 (4)	31.9±4.4 (4)	33.8±14.6 (4)
CM	28.7±0.5 (6)	29.7±2.1 (6)	31.5±5.2 (6)
CC	29.7±4.2 (4)	33.1±5.1 (4)	35.8±11.8 (4)
Age group mean	29.5±1.5 (18)	31.2±1.7 (22)	33.9±2.9 (19)

a The numbers in parentheses are the number of female mice included in each subclass.

TABLE 3. Mean ovulation rate (eggs)

Mating groups	Weekly age groups			Mating group mean
	6	9	15	
MM	12.3±5.3	13.6±3.1	13.8±4.2	13.4±1.9 (17)[a]
MC	15.5±5.3	16.5±6.2	11.2±5.2	14.2±2.6 (13)
CM	13.2±2.3	13.5±2.2	13.8±2.3	13.5±1.0 (18)
CC	13.5±2.1	13.7±2.2	14.8±1.5	13.9±0.9 (14)
Age group mean	13.6±1.3	14.1±2.7	13.4±1.4	13.7±1.1 (62)

a The numbers in parentheses are the number of female mice.

age groups, though the ovulation rate seemed to increase gradually week by week, except for the MC group at 15 weeks of age. The correlation estimates of body weight with ovulation rate at 6 and 9 weeks of age were significant ($P<0.05$), but not at 15 weeks. This fact showed that while the ovulation rate tends to rise as body weight becomes larger for 6- and 9-week

TABLE 4. Frequency of abnormal eggs (per cent)

Mating groups	Weekly age group			Mating group mean
	6	9	15	
MM	0.00 (21, 2)*	1.83 (109, 8)	0.00 (69, 5)	1.01 (199, 15)
MC	8.86 (79, 6)[a]	1.22 (82, 6)[b]	1.43 (70, 5)[b]	3.90 (231, 17)
CM	12.20 (41, 3)[a]	0.00 (60, 4)[b]	0.00 (59, 4)[b]	3.13 (160, 11)
CC	8.06 (62, 4)	6.06 (66, 4)	0.00 (52, 4)	5.00 (180, 12)
Age group mean	8.37 (203, 15)[a]	2.21 (317, 22)[b]	0.40 (250, 18)[b]	
Classification of abnormal eggs (combined mating groups):				
Fragmented eggs	35.3 (6)**	28.6 (2)	0.0 (0)	32.0 (8)
Unfertilised eggs	35.3 (6)	14.3 (1)	0.0 (0)	28.0 (7)
Retarded eggs	5.9 (1)	0.0 (0)	100.0 (1)	8.0 (2)
Eggs without zona pellucida	11.8 (2)	42.9 (3)	0.0 (0)	20.0 (5)
Eggs zona pellucida only	11.8 (2)	14.3 (1)	0.0 (0)	12.0 (3)
Age group total	100.0 (17)	100.0 (7)	100.0 (1)	100.0 (25)

* The numbers in parentheses are the number of eggs collected and mice used, respectively.

** The numbers in parentheses are the number of abnormal eggs each.

a, b Means in same row with different letters differ significantly at $P<0.01$.

age groups, this relationship becomes negligible for 15-week age group.

The percentage of abnormal eggs to the total number of eggs collected from each group is persented in Table 4. Two mice from the MM group and one mouse from the CM group from which no normal eggs were recovered were excluded from the calculation. The difference in percentage of abnormal eggs is not statistically significant among mating groups in any age groups, but there is a statistical significance among age groups in the MC and CM groups. In these mating groups, the proportion of abnormal eggs to total ovulated eggs is significantly higher in the 6-week age group than those in 9- and 15-week age groups. This results in a significantly higher proportion of abnormal eggs in 6-week age group than in the other two age groups when data is combined for all mating groups. The distribution of each type of abnormal eggs reveals that fragmented eggs and unfertilized eggs are proportionately highest (35.3% each) amounting to 70.6% of abnormal eggs in the 6-week age group.

Table 5 shows the distribution of classes of cell stages of normally developing eggs at the time of recovery. The distribution was significantly different among weekly age group. 34.2, 10.6, and 7.6 percent of eggs had less than 8 cells, in the 6-, 9-, and 15-week age groups, respectively. As the age at collection increases from 6 to 15 weeks, the proportion of eggs delayed in development tends to decrease, though there is little difference between the 9- and 15-week age group.

TABLE 5. Distribution of normal eggs in each cleavage stage of cell (eggs, percent)

Age groups	Classification of cleavage stage					Total
	2-4 cell	5-8 cell	8 cell	8 cell-morula	Blastocyst	
6	34 (20.4%)	23 (13.8%)	65 (38.9%)	45 (26.9%)	0 (0.0%)	167 (100.0%)
9	10 (3.2%)	23 (7.4%)	91 (29.4%)	170 (54.8%)	16 (5.2%)	310 (100.0%)
15	17 (6.8%)	2 (0.8%)	184 (73.6%)	34 (13.6%)	13 (5.2%)	250 (100.0%)

Discussion

It is clear from Table 1 that selection for body weight actually had an effect on the birth weight of the M line. However, some dams of the MM mating group died during lactation and foster mothers were used, postnatal maternal influence of the M line could not be assessed. It seems that differences in 6-, 9-, and 15-week body weight between MM and CC groups are

non-significant.

Although, as described in the introduction, at the 15th generation of selection a marked reduction in litter size in the M line as compared to the C line has been observed elsewhere, in this study, there is no significant difference of mean litter sizes among mating groups. This may be due to sampling and/or due to the same factors which caused large fluctuations in litter size between consecutive generations, as observed in body weight selection experiments by BARRIA and BRADFORD.[3]

BRUMBY[7] transferred embryos and cross-fostered whole litters of two lines of mice selected for high and low 6-week body weight, finding that a major part of the maternal influences on the growth of young occurs during the prenatal period and that there is a difference in maternal influences resulting from changes in her body size. Because the birth weight of the CM group (dams were from the M line) was significantly higher than that of the MC group (dams were from C line), it can be inferred that the female of the M line has some effect on prenatal embryo growth. It can be concluded that the change in birth weight was caused by selection for large body weight in males, although whether influence of the sex-linked gene or cytoplasmic influence in the intra-uterine environment was the most important is unknown.

A positive genetic relationship between body weight and ovulation rate has been reported by several workers[3,6,10,16] in selection experiments for body weight. Ovulation rate shows a consistent genetic connection with body weight, while the genetic association of litter size with body weight disappears during the course of long-term selection for body weight.[6,16] In this study, there was no significant difference in the ovulation rate between mating groups and evidence could not be obtained for the effect of body weight selection on ovulation rate. This could be because there was little difference in body weight among mating groups. However, a positive relationship of body weight with ovulation rate was found at 6- and 9-weeks (0.59 and 0.77 respectively), but not at 15-weeks (0.06). This suggests that body weight has a positive influence on ovulation rate at early maturity but less influence at a later age, which is not in conflict with the fact that puberty is more closely associated with body weight than with age in mice.[1]

A significant difference was found in the rate of abnormal eggs among age groups, indicating that mice have a higher proportion of abnormal eggs at 6 weeks than at 9 and 15 weeks, and that mice of only 6 weeks are not always completely mature in reproductive functions. Many studies on the decline of reproductivity in older female mice have been done but few reports

have been published on the influence of pre-maturity on reproduction. CHANG *et al.*[9] reported in their study with rabbits and rats that fertility generally declined when pre-mature males were mated to pre-mature female rats. They attributed this to the lower functional ability of pre-mature females to transport and to fertilize the eggs. BLANDOU and JORDAN[5] and CHANG and FERNANDEZ-CANO[8] have shown that rat and hamster eggs not exposed to spermatozoa until several hours after ovulation often develop abnormally. In this study, fragmented eggs and unfertilized eggs together amounted to 70 per cent of the total abnormal eggs of the 6-week age group. Failure of eggs to be fertilized may be the major cause of egg abnormality, for newly-mature females. Although excluded from Table 4, two mice from the MM mating group and one mouse from the CM mating group had unfertilized and/or fragmented eggs at this age. It may be assumed that the fertilization rate is lower not only at puberty as mentioned above but also at the age of early maturity of 6 weeks. This trend is consistent among mating groups. Selection for body weight may not affect the time of sexual maturity. A possible cause for this increase in abnormal eggs at 6 weeks may be the delay in fertilization.

In this study, eggs were recovered on the third day, 2 days after the virginal plug was observed. This was assumed to be about 2 days and a half after ovulation. In mice, fertilized eggs normally develop into 8 or more cells by this time.[18] The proportion of eggs of less than 8 cells adds up to 34.2 per cent in the mice of the 6-week age group, while the 9- and 15-week groups have only 10 per cent or less (Table 5). In addition to the increase in abnormal eggs, there tends to be a delay in the development of eggs of mice of only six weeks of age. Since the males used for mating were the same age as females in this study, whether the age of males or of females has more influence on the development of eggs is unclear.

Summary

The effect of age on natural ovulation rate, and on pre-implantation embryo loss, as well as the effect of age on various strains of mice, was examined in four groups of mice from diallele mating between two strains. Eggs were recovered from the mice at 6, 9, or 15 weeks of age. The following results were obtained:

1) There is no significant difference in ovulation rate, neither among the four crossbred groups, nor among age groups.

2) Correlations between body weight and ovulation rate were significant in the 6- and 9-week age groups (0.59 and 0.77) but non-significant in the

15-week age group, indicating that body weight has more influence on ovulation rate than dose age, at an age earlier than 15 weeks.

3) A significantly higher proportion of abnormal eggs was observed in mice of 6 weeks than in those of the two age groups, owing to an increase of fragmented and unfertilized eggs. This is expected to lead to increase of pre-implantation embryo loss.

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