REPRODUCTIVE CAPACITY AND LITTER SIZE IN MICE: EFFECT OF AGE AND ENVIRONMENT

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Summary. Various factors concerned in the regulation of litter size and total reproductive output in mice have been investigated. Length of daylight did not significantly affect litter size. The decline of litter size as the animals aged was associated with a high level of embryonic loss after implantation. Ligature of the Fallopian tube on one side or unilateral ovariectomy caused total reproductive output to be about halved. In the latter case, initial litter size was about three-quarters of control but decline of litter size started earlier and reproductive life-span was shorter, whereas in the former case, litter size was approximately half control level throughout but reproductive life-span was approximately equal to that of the controls. Reproductive performance of mothers that had been kept virgin or continuously pseudopregnant for the first 9 months and then paired with fertile males did not differ significantly from the performance over an equivalent period of mothers bred continuously from puberty.

The cause of the decline of reproductive capacity with age is discussed. It is suggested that it is due to the declining ability of the uterus to maintain pregnancy, associated primarily with chronological ageing.

INTRODUCTION

The theoretical reproductive capacity of a female mammal can be estimated from the total number of young she produces when allowed to breed continuously with a fertile male in a favourable environment. This total, in polytocous animals, is the product of the size of individual litters, the frequency of litter production and the reproductive life-span of the animal. Factors determining litter size have been fairly extensively studied in rats and mice, although most studies have been confined to the early litters. The other parameters have received less attention (for a review of the literature see Biggers, Finn & McLaren, 1962b).

Biggers, Finn & McLaren (1962a) investigated the total reproductive performance of entire mice and mice with one ovary removed. They showed that the litter size varied with parity in a fairly regular pattern. After an initial increase, the litter size remained constant for a variable period (plateau period) and then decreased linearly until breeding ceased (decline period). The removal

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of one ovary caused the plateau litter size to be somewhat smaller and the decline to set in earlier, so that breeding ceased sooner and the total output of the hemicastrates was approximately half that of the controls.

The present investigation was undertaken to obtain further information about the factors influencing the reproductive output of female mice, especially during the period when the litter size is declining.

The earlier study presented several problems requiring investigation which may be summarized briefly as follows:

**Does the environment influence litter size?**

As these experiments extended over nearly 2 years, any seasonal effect of the environment would be confounded with age. Length of daylight has been shown to affect many reproductive phenomena (see Amoroso & Marshall 1960), but its effect on litter size seems to have been little studied. A relevant experiment is that of Shukhet-Kagen & Emme (1960), although they were concerned essentially with the effect of continuous light or dark. A preliminary experiment was therefore made to determine whether the extremes of light/dark ratio experienced throughout the year influence litter size.

**Is deficiency of luteal tissue a significant factor in reproductive ageing?**

This could be investigated by increasing the ratio of corpora lutea to embryos, for instance by ligature of the Fallopian tube on one side. Examination of the literature revealed that Jones & Krohn (1960) included a tube-ligated group in their initial experiment. However, the mice were of very low prolificacy, the control females only averaging four litters. Thus the results are rather inconclusive and do not help very much in answering the present question. In a subsequent experiment, with a more prolific strain of mice, they did not include a tube-ligated group, being primarily interested in the effect of unilateral ovariectomy. Bloch (1952) studied the effect of ligature of one Fallopian tube on a single measure of litter size, and obtained, as expected, a 50% reduction. However, there is no information on the effect of the ageing process. A group with the Fallopian tube tied on one side was therefore included in the present experiment, together with a unilaterally ovariectomized group for comparison.

**Why is the litter size reduced in the aged mice?**

Theoretically it could be due to a gradual decrease in the number of oocytes produced or to failure of an increasing percentage of eggs to result in living offspring. In this study laparotomies were performed on some of the females during the declining phase, and the incidence of normal and abnormal embryos was compared with that in young virgin mice of the same strain. The results of this section of the experiment have already been published (Finn, 1962) and it need only be stated here that the incidence of embryonic loss after implantation in aged mice was much higher than in young virgins.

**How is the decline period affected by previous breeding history?**

Evidence from the section above supports the hypothesis that the decline in litter size is due primarily to ageing of the uterus. However, it would be
interesting to know whether this reduced efficiency is caused by simple chronological ageing, by ageing due to work imposed on the uterus by carrying the foetuses or by excessive stimulation by the reproductive hormones of pregnancy. Information on this point was sought by subjecting the females to three different treatments during their early breeding life.

These were (a) mice bred continuously so that the natural maximum amount of work was done by the uterus, (b) mice kept virgin for the first 9 months, so that the uterus was reproductively inactive, (c) mice kept with a vasectomized male for 9 months, so that the uterus was almost continuously stimulated by progesterone.

MATERIALS AND METHODS

The mice were from a random bred strain of high fecundity (Theiler's original). Males and females were placed together soon after weaning so that breeding started as soon as the animals were sufficiently mature. Operative procedures were carried out at approximately 5 weeks. Daily inspection was made for litters, which were counted, sexed and then removed, thus the young were never nursed for more than a few hours. Occasionally litters were found which had been mutilated at birth. These, of course, could not be counted and therefore could not contribute to the total output or litter size. However, they were included in the computation of the number of litters. The investigation consisted of three separate experiments started at different times and not strictly comparable.

EXPERIMENT 1

This was a preliminary experiment to determine the effect of daylight. Under natural conditions in this laboratory, animals are subjected to approximately 16 hr light/day in mid-summer and 8 hr/day in mid-winter. These two extremes of light period were therefore used to determine whether the litter size varied in mice housed under such regimes.

The mice were housed in a small room, artificially ventilated, heated and lighted. The same room was used for both light regimes, the experiments being done consecutively. At the start of each trial pregnant females were placed in the environment room, and the young born to these dams used for the experiment, so that they spent their entire life under that experimental light regime. Records of first and second litters were collected. Seven days after the birth of the first litter, a laparotomy was performed on the female and the number of implantation sites was counted. These data were collected in order that any difference in litter size under the two regimes could be broken down into a pre- or post-implantation effect.

The experiment was started with thirty pairs of mice in each group. Seven of the females died during or after the laparotomy, five failed to mate at post-partum oestrus and two failed to breed at all. Fortunately, these throw-outs were evenly divided between the two groups, leaving a final group size of twenty-three.
This was performed in the same room as Experiment 1, with a light/dark ratio of 16:8 during the entire experiment. Seventy pairs of mice were used, being about 4 weeks old at pairing. The treatments received by the groups were as follows: (1) Control—untreated. (2) One ovary removed (or group). (3) Fallopian tube ligatured on one side (or group). (4) Laparotomy performed during decline period to count normal and regressing implantations. (5) Kept virgin for 9 months, and then allowed to breed. The mice were left until the female died or had to be killed due to extensive tumour development especially of the mammary glands, a condition which was quite common in the aged females.

Eight pairs of mice were rejected from the final analysis for the following reasons: Three female mice in the control group died or had to be killed whilst very young. Two mice in the control group and one in the or group stopped breeding after two or three litters within the first 4 months, and two in the or group did not breed at all. As these mice were abnormal in their breeding record, it was considered better to omit them from the analysis.

EXPERIMENT 3

Twenty female mice were divided into two groups. The mice in Group 1 were placed in cages with a vasectomized male, those in the other group were left virgin for 9 months. The females in Group 1 were inspected daily for vaginal plugs and a record was kept. After 9 months the females were placed in individual cages with a fertile male and a record kept of litters born.

RESULTS

EXPERIMENT 1

Effect of length of daylight

It will be seen from Table 1 that there was very little difference between the mean size of litters in the two groups, and the difference was not statistically significant.

EXPERIMENT 2

Total reproductive performance

Table 2 shows the mean figures for total young per animal, number of litters produced and litter size. In order to follow variations of litter size we need to
construct an average curve of litter size against parity. Due to the variability in the length of reproductive life this presents some difficulty, which is discussed by Biggers et al. (1962b). They have shown a method by which a curve can be obtained representing the reproductive performance of the average female in the group. To do this the reproductive life-span of each female in the control group is divided into elevenths (this being the nearest whole figure to the average number of litters per female in this group) and its litters allotted to the corresponding ‘standard litter’ of the ‘standard female’. The other groups are treated in a similar manner. The resulting curves are shown in Text-fig. 1. The shape of the curve of the control group follows the established pattern with an increase after the first litter followed by a period of reasonably constant litter size, and then a period of declining litter size.

Both the ovary-removed and the oviduct-ligated groups produced approximately half as many offspring as the controls. However, the behaviour of the litter size curve differed in the two groups. The or group had a significantly shorter reproductive life span than the controls, during which the size of their litters tended to be significantly more than half that of the controls (level of plateau is about three-quarters that of the controls), whereas the or group had almost as many litters as the controls, but with a much lower plateau size.

**Table 2**

**breeding record groups 1, 2 and 3**

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Mean output/female</th>
<th>Mean No. litters/female</th>
<th>Mean litter size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23</td>
<td>87·22 ± 4·57</td>
<td>11·52 ± 0·58</td>
<td>7·97 ± 0·21</td>
</tr>
<tr>
<td>Oviduct tied</td>
<td>7</td>
<td>45·43 ± 8·01</td>
<td>10·14 ± 1·44</td>
<td>4·81 ± 0·27</td>
</tr>
<tr>
<td>Ovary removed</td>
<td>10</td>
<td>42·90 ± 6·15</td>
<td>7·40 ± 0·82</td>
<td>6·22 ± 0·36</td>
</tr>
</tbody>
</table>

**Text-fig. 1.** Variation of litter size with parity. ◊, or group; □, or group; ×, Control group.
One unexpected feature of these results was the high first-litter size of the
or group. A possible explanation of this is that the first litter would be conceived
soon after the Fallopian tube was ligatured. This operation must tend to inter-
fere temporarily with the blood supply to the ovary on that side, so that whilst
the circulation to this ovary is being re-established, the gonadotrophins perhaps
reach the opposite ovary with greater facility, thus causing more ovulations
than usual on that side.

Table 3

| Breeding Record from Time of First Possible Litter after Virgin Females Paired with Males (Experiment 2) |
|-----------------|----------------|----------------|----------------|
|                  |     No.     | Output/female | Litters/female | Mean litter size |
| Kept virgin 9 months Bred continuously | 10 | 16.9 ± 5.10 | 4.0 ± 1.20 | 4.97 ± 0.49 |
|                  | 23 | 12.9 ± 2.32 | 3.0 ± 0.47 | 4.95 ± 0.26 |

Incidence of litters which were mutilated at birth

In the computation of the control curve there were altogether 216 litters
contributing to Litters 1 to 9, of these only four were found mutilated at the
time of inspection. However, for litters 10 and 11 there were ten mutilated
litters in a total of fifty. The occurrence of mutilated litters thus increases mark-
edly towards the end of reproductive life.

Effect of previous breeding history

To compare the performance of the mice kept virgin for 9 months with
those bred from maturity, we must compare total output, litter size and number
of litters separately. An average curve of performance would be of little value as
it is apparent from inspection of the figures that after 9 months the mice are at
different stages of their reproductive life; some still being in the plateau period,
whilst others have reached the end of the decline period. However, we can
compare the total output of the virgin group, and the number of litters they
produce, with the data from the control group—taking only those litters born
21 days or more after the day on which the virgin group were put with the
males. Some error will arise here due to the fact that whereas all the virgin mice
will be in a condition to mate from the time they are put with the males,
some of the control mice will be pregnant at this time, so that their first effective
mating cannot occur until after parturition of the litter conceived before this
date. This will give a slight bias in favour of the virgin group.

The results shown in Table 3 show that mice kept virgin for the first 9 months
produced slightly more offspring from the 9th month onwards, but this differ-
ence is not statistically significant and, in view of the above bias, we can safely
say that there is little or no difference between the performance of the two groups,
indicating that the virgins have aged reproductively to the same extent as
those bred continuously. Roman & Strong (1960) taking a single measure of
litter size in aged virgin and aged parous mice, similarly concluded that ageing
prior to mating did not significantly affect litter size.
EXPERIMENT 3

Effect of keeping females continually pseudopregnant

As this experiment was performed separately from the main experiment above, the results are not directly comparable. However, they do give a comparison between the effect of keeping animals virgin and that of keeping them continuously pseudopregnant.

Table 4 shows that there was no significant difference, so that in combination with the results above, it appears that previous breeding history had little or no effect on subsequent output of young.

### Table 4

| Breeding Record of Females Kept Virgin or Pseudopregnant for First 9 Months (Experiment 3) |
|---|---|---|---|
| | No. | Output/female | Litters/female | Mean litter size |
| Kept virgin | 10 | 24.7 ± 7.13 | 4.6 ± 1.07 | 6.18 ± 0.44 |
| Kept pseudopregnant | 10 | 29.4 ± 8.56 | 5.0 ± 1.24 | 6.53 ± 0.36 |

DISCUSSION

The preliminary experiment demonstrated that length of ‘daylight’, within the limits normally found in this country, had no significant effect on litter size. Similarly Shukhet-Kagen & Emme (1960) were unable to show any difference of litter size in mice subjected to continuous darkness, continuous light or 9 hr light per day.

However, several workers have shown a seasonal effect on litter size of rats kept under laboratory conditions (Murray, 1941; McKinlay, 1951) and mink (Hronopulo, 1955). Possibly the mouse is less sensitive to the seasonal effect of light. However, the field mouse (*Apodemus sylvaticus*) has been shown to breed under natural conditions only during the summer and autumn (Raynaud, 1950). Possibly there is an interaction between light and nutrition as demonstrated for the rat by Alexander & Frazer (1952) who showed that the effects of light and food on mating behaviour are interchangeable. As our mice are on ad-libitum feeding of standard cubes they are presumably getting a high level of nutrition which might mask any effect of light ratio on breeding behaviour. However, the experiment demonstrates that under these conditions one is unlikely to confound variations of litter size with parity with variations due to seasonal lighting conditions.

The control females in the main experiment showed a breeding pattern similar to that found in earlier work, both in mice (Bittner, 1936; Biggers et al., 1962b) and rats (King, 1916; Asdell, Bogart & Sperling, 1941; Ingram, Mandl & Zuckermann, 1958).

Biggers et al. (1962b) postulated that the decline of litter size with parity is due primarily to embryonic loss after implantation, probably due to ageing processes in the uterus. This conclusion was based on the fact that at post-mortem examination of old mice, well after the end of their reproductive life, large
numbers of corpora lutea were found in the ovaries and in several cases remnants of implanted conceptuses in the uteri which had failed to survive. Similarly, Perry (1954) and McDowell & Lord (1925) noted the disparity between the number of corpora lutea in the ovaries and the size of litters in old pigs and mice and concluded that there must be considerable loss after ovulation. The high incidence of moles found in the present experiment during the phase of declining litter size provides further direct evidence for this hypothesis.

The number of implants in the aged females was not very different from the plateau litter size for these mice (Finn, 1962). McLaren & Michie (1959), working with this strain of mice, found very little embryonic loss after implantation in young adult mice so that the plateau litter size probably gives a reasonable index of the implantation rate in young mature animals. Therefore it seems reasonable to assume that the implantation rate in the old mice has not dropped much and the principal loss during the decline period occurs after implantation. This embryonic loss could be due to one or more of several possible causes, for example (a) deficiency of luteal tissue in the ovary, (b) changes in the uterus which might interfere with vascularity or placenta formation, (c) lethal factors in the embryos causing their death. Very little is known about this latter factor, although one may mention the higher incidence of mongolism in children from older mothers. Changes in the uterus seems the most likely explanation although again no direct evidence is available. Boot & Muhlbook (1954) have shown that ova from young mothers transferred to old uterus fail to develop and they concluded that the loss of fertility in old females is not caused exclusively by anomalies in the ova. However, from their paper it is difficult to judge the significance of their results. Loeb, Suntzeff & Burns (1939) demonstrated an increase in the collagen content of the uteri of old mice and this was confirmed histologically by Biggers et al. (1962a). Possibly this interferes with uterine function; however, Harkness & Harkness (1956) have shown that during pregnancy the collagen content of the uterus increases considerably and then decreases rapidly at parturition, indicating that synthesis of collagen is possibly essential during pregnancy. It is therefore interesting that Kao, Chen Lu, Hitt & McGavack (1962) have demonstrated that slices of uterine tissue can synthesize collagen in vitro and that the capacity for this synthesis is an inverse function of the age of the donor. One could speculate that the gradual loss of this synthetic ability might interfere with the maintenance of pregnancy.

The performance of the group with one oviduct ligated indicates that the possession of an excess of luteal tissue has not halted the ageing process in these mice. Thus it seems unlikely that deficiency of luteal tissue plays a significant part in causing the increased embryonic loss in aged mice.

Unilateral ovariectomy, as in earlier experiments (Hunter, 1787; Jones & Krohn, 1960; Biggers et al., 1962a), caused premature reproductive ageing, both in terms of the earlier onset of the period of declining litter size and shorter reproductive life-span. This is presumably due to the increased work the remaining single side of the reproductive tract is called upon to perform, there being no transcornual migration of blastocysts in the mouse (McLaren & Michie, 1954). In view of this it was surprising that the early breeding history
of entire mice in this experiment had little or no effect on the subsequent reproductive performance. It seems that at the normal level of reproductive output the decline in fertility is a manifestation of simple chronological ageing and normal maximal breeding does not increase this. But, under conditions of overloading, ageing occurs earlier than usual.

ACKNOWLEDGMENTS

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