LONG-TERM REPRODUCTIVE PERFORMANCE OF FEMALE MICE

I. EFFECT OF REMOVING ONE OVARY

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Summary. The breeding performance of normal intact female mice (two-ovary females) was compared with that of female mice from which one or other ovary had been surgically removed before sexual maturity (one-ovary females). The comparison was continued throughout the reproductive lives of the females. The one-ovary mice produced fewer litters, and stopped breeding at an earlier age, than did the intact controls. The total number of young produced per female in the one-ovary group was 56% of the total number produced per female in the two-ovary group. Each female was killed when it had failed to produce a litter for 16 weeks, since its reproductive life was then judged to have ended. However, nearly half the females were or had recently been pregnant when killed. The weight at autopsy of the surviving ovary in the one-ovary females was not significantly less than the combined weight of both ovaries in the two-ovary females. Histological observations of the ovaries and uteri of the aged females are described.

INTRODUCTION

The factors determining litter size in polytocous mammals, and in particular the relative roles of ovulation rate and prenatal mortality, have been extensively studied. On the other hand, the factors which regulate the total number of young born to a female throughout her lifetime, a reproductive index of central importance both for evolution and for animal breeding, remain almost wholly obscure. In the hope of throwing some light on this problem, we undertook an investigation of the pattern of reproductive performance throughout life in normal female mice, and also in females in which one ovary had been surgically removed before sexual maturity.

This approach had been suggested to us by the work of John Hunter, who in 1787 described the results of an experiment in which he compared the long-term reproductive performance of two sows, one of which had two ovaries, the other only one. The two sows, and the boar to which they were mated, were from a single litter. The sow with one ovary produced both fewer and

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smaller litters than the control sow. The ratio between the total number of young produced by the two animals was almost exactly 0.5: from this, Hunter concluded 'It seems most probable that the ovaria are from the beginning destined to produce a fixed number, beyond which they cannot go.' Hunter’s experimental findings are in general confirmed by the results of the present experiment, and also by the recent work of Jones & Krohn (1960); however, his interpretation will need re-examination in view of the fact that the number of oocytes initially present in the ovary is now known to exceed by many thousands the number of young born.

The general results of our experiment are reported in the present paper. In a second paper (Biggers, Finn & McLaren, 1962) we deal with the pattern of variation of litter size throughout reproductive life in the two groups of mice, and discuss, in the light of these results, possible limiting factors involved in the regulation of total reproductive performance. A preliminary account of this work has been published elsewhere (Biggers, Finn & McLaren, 1961).

**MATERIALS AND METHODS**

Forty-seven female mice of Theiler’s Original (to) strain were used. This is a random-bred strain, and unusually prolific. The mice were born between 14th January and 3rd February, 1958. At the age of 5 to 7 weeks they were divided, using a table of random numbers, into three groups. One group of twenty-three animals was left intact (referred to as two-ovary mice); a second group of twelve had the left ovary removed, and a third group of twelve had the right ovary removed (one-ovary mice). To ensure complete ablation of the ovary, the oviduct and tip of the uterine horn were also removed. In none of the operated females were any signs of ovarian tissue observed at autopsy. One week after the operation each female was placed in a box with a to strain male of approximately the same age.

The cages were examined each morning for births, and a record kept of the date on which litters were born, and the number of live and dead mice in each. In three litters from one-ovary females, young which were considered as belonging to a single litter were in fact born in two batches, at intervals of 2, 5 and 5 days, respectively. The occurrence of ‘split parturition’ has also been reported in women. Accepted theories of the mechanism of parturition have been critically discussed in the light of this phenomenon by Csápo (1959).

Each litter was removed immediately, so that throughout the experiment none of the females nursed their young. This was done for two reasons: (1) In lactating female mice, delayed implantation of blastocysts causes prolongation of pregnancy, to a degree dependent on the number of young being suckled. (2) Foetal mortality is increased in lactating mice (Bruce & East, 1956) by an amount which again varies with the number of young nursed. For both these reasons the number of young born during a lifetime might be decreased to a degree which varied from female to female if the mice were allowed to lactate.

When no further litters were born during a period of 10 weeks the male was replaced. When a further 6-week period had elapsed without the birth of a litter, the female was judged to have reached the end of her reproductive life,
and was killed. An autopsy was done, the ovaries were weighed and the ovaries and uterus were fixed in Zenker's fluid containing 3% acetic acid.

Certain mice were omitted from the calculations for the following reasons:
(1) One female in the two-ovary group had not produced a litter within 9 weeks of being placed with a male, and the mating was therefore assumed to be sterile.
(2) Five females died (three in the two-ovary group and two in the one-ovary group) before reaching the end of their reproductive life-span as judged by the criteria described above. Since we were primarily interested in comparing the pattern of reproductive performance in one-ovary and two-ovary females, these five females, where breeding was interrupted by relatively early death, were omitted.

(3) As will be shown in the following paper, litter size remains high during the early part of reproductive life (the plateau period), and later declines. A single female in the two-ovary group proved an exception to this pattern, being still in the plateau period when breeding ceased, with a final litter containing thirteen young. Comparison with Table 1, which shows the frequency distribution of the number of young in the final litter for the rest of the control group, and also for the one-ovary females, makes it clear that this female was an outlier in the sense of Dixon (1953), and it has therefore been omitted.

Thus in analysing the data we made use of results from eighteen two-ovary females, eleven females with the right ovary remaining, and eleven with the left ovary remaining. Sections of both uterine horns were prepared from thirty-six of the mice, and stained with haematoxylin and eosin, Van Gieson's picrofuchsin and Cason's modification of Mallory's connective tissue stain (Cason, 1950). Sections of ovaries were prepared from sixteen two-ovary and nineteen one-ovary females, and were stained with haematoxylin and eosin.

### RESULTS

#### TOTAL NUMBER OF YOUNG BORN PER FEMALE
The total numbers of young (live and dead) born per female in each group are shown in Table 2. There is no significant difference between the number born in the two one-ovary groups, and the data from both have therefore been pooled. This procedure has been adopted in the analysis of other data described later, since the results from these two groups were found to be homogeneous in
all respects. The overall estimate of the total number of young born per female in the one-ovary group is $64.1 \pm 3.0$.

The ratio between the total young produced per female by the one-ovary mice and by the two-ovary mice is 0.56. This value is not significantly different from a hypothetical ratio of 0.5 ($\chi^2_{[n]} = 2.725$, $0.1 > P > 0.05$). Since the observations within the groups are heterogeneous ($\chi^2_{[8]} = 130.7$, $P < 0.001$), the fiducial limits of error have been calculated allowing for this heterogeneity. These limits ($P = 0.05$) are 0.49 to 0.63. Thus the ratio is only just consistent with a value of 0.5.

### Table 2

**Total Number of Mice Born Per Female**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. mice</th>
<th>Mean No. young</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-ovary</td>
<td>18</td>
<td>115.3 ± 5.0</td>
</tr>
<tr>
<td>One-ovary:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>left ovary removed</td>
<td>11</td>
<td>64.3 ± 5.1</td>
</tr>
<tr>
<td>right ovary removed</td>
<td>22</td>
<td>63.9 ± 3.3</td>
</tr>
</tbody>
</table>

Standard errors follow the ± sign.

### Table 3

**Number of Litters Per Female and the Mean Age of the Females When the Last Litter Was Born**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. mice</th>
<th>Mean No. litters</th>
<th>Mean age at birth of last litter (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-ovary</td>
<td>18</td>
<td>15.5 ± 0.67</td>
<td>435.4 ± 18.4</td>
</tr>
<tr>
<td>One-ovary</td>
<td>22</td>
<td>11.2 ± 0.44</td>
<td>332.7 ± 13.4</td>
</tr>
</tbody>
</table>

Standard errors follow the ± sign.

**Number of Litters Born Per Female**

The mean number of litters born per female in each group is shown in Table 3. The two-ovary mice produced more litters than the one-ovary mice, the difference between the two groups being highly significant ($t_{[38]} = 5.44$, $P < 0.001$).

**Mean Age at the Birth of the Final Litter**

The mean age at the birth of the final litter in each group is also shown in Table 3. The mean age in the two-ovary mice is greater than in the one-ovary mice, the difference being highly significant.
Reproductive performance of mice. I

BODY WEIGHT

The mean body weights of the two-ovary and one-ovary mice at autopsy were 35.7 g (s.e. 1.29, d.f. 16) and 34.5 g (s.e. 0.83, d.f. 21), respectively. It is evident that removal of one ovary had no influence on body weight.

Table 4

<table>
<thead>
<tr>
<th>Ovary</th>
<th>Mean ovarian weight (mg)</th>
<th>Variance-covariance matrix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>7.26</td>
<td>[11.92 8.93]</td>
</tr>
<tr>
<td>Right</td>
<td>7.93</td>
<td>[8.93 8.07]</td>
</tr>
</tbody>
</table>

The two means are calculated from fourteen pairs of highly correlated observations.

Table 5

Wet weight of ovarian tissue in the two-ovary and one-ovary mice

<table>
<thead>
<tr>
<th></th>
<th>Two-ovary mice (total of both ovaries)</th>
<th>One-ovary mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight (mg)</td>
<td>15.19*</td>
<td>13.61</td>
</tr>
<tr>
<td>s.e.</td>
<td>2.70</td>
<td>0.936</td>
</tr>
<tr>
<td>d.f.</td>
<td>13</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Left</th>
<th>Right</th>
</tr>
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<tbody>
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<td>13.61</td>
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</tr>
<tr>
<td>s.e.</td>
<td>0.936</td>
<td>2.07</td>
</tr>
<tr>
<td>d.f.</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

* The standard error of the sum of two means, x̄ and ȳ, each based on n paired observations, is given by

\[
\sqrt{\frac{1}{n} \left\{ V(x) + V(y) + 2 \, \text{Cov}(x,y) \right\}}.
\]

In this case, from Table 4, \((x+y) = (7.26+7.93) = 15.19\), and s.e. \((x+y) = \sqrt{\frac{1}{14} (11.92 + 8.07 + 2 \times 8.93)} = 2.70.\)

OVARIAN WEIGHT

The wet weights of the left and right ovaries were obtained in fourteen two-ovary mice (Table 4). There is a suggestion that the left ovary is slightly lighter than the right ovary, but this is not statistically significant \(t _ {13} = 1.703, \ 0.2 > P > 0.1\).

The total weight of ovarian tissue per two-ovary female, and also per female in the two one-ovary groups, is shown in Table 5. There is obviously no
difference between the ovarian weights of left and right one-ovary mice. The single ovary of the one-ovary animals is considerably heavier than each ovary of the two-ovary animals. In fact, the total weight of ovarian tissue in the two-ovary mice does not significantly exceed the weight of the single ovary in the one-ovary group ($t_{1321} = 0.786, 0.5 > P > 0.4$).

**MORPHOLOGY OF THE UTERUS AT AUTOPSY**

Uteri from thirty-six females were available for examination. In three, the uteri bore swellings which were diagnosed histologically as myofibromas. Of the remainder, in nineteen the uteri were empty, but in fourteen evidence of present or recently terminated pregnancy was obtained, in the form of live or dead embryos, placental remains, or decidual tissue. Table 6 lists the

<table>
<thead>
<tr>
<th>Group</th>
<th>Mouse No.</th>
<th>No. swellings noted at autopsy</th>
<th>No. swellings examined histologically</th>
<th>Nature of uterine contents</th>
<th>Estimated age of embryo (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-ovary</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>2RE; 3D</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>6</td>
<td>6</td>
<td>4LE; 2D</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0</td>
<td>-</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>0</td>
<td>-</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>0</td>
<td>-</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td>One-ovary</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>1LE; 1RE; 1D</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>12</td>
<td>4</td>
<td>2LE; 4D</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>0</td>
<td>-</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0</td>
<td>-</td>
<td>D</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>0</td>
<td>-</td>
<td>1LE</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>Several</td>
<td>5</td>
<td>1LE; 4P</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>43</td>
<td>4</td>
<td>1</td>
<td>1LE</td>
<td>7 to 8</td>
</tr>
</tbody>
</table>

Since serial sections of the uteri were not made, some decidua and early embryos may have been missed. LE = live embryo; RE = regressing embryo; D = decidua; P = placental remains.

uterine contents of these mice in detail. Thus if decidual tissue is included as a sign of pregnancy, and if the three mice which developed tumours are omitted, fourteen out of thirty-three (42%) of the females which appeared to have reached the end of their reproductive lives were, or had recently been, pregnant at the time of autopsy.

There was a large difference in the size of the two uterine horns in the one-ovary mice. The horn on the side of the missing ovary was relatively thin, while the horn on the intact side was large and thickened (Pl. 1, Fig. 1). In contrast, the uterine horns in the two-ovary mice were usually more or less equal in size. Table 7 shows the condition in nine two-ovary and nine one-ovary mice (all non-pregnant and devoid of myofibromas). The hypertrophy of the functional uterine horn in one-ovary mice is highly significant ($P \sim 0.001$).
Fig. 1. The genital tract in situ of an approximately 390-day-old female mouse which had produced nine litters after removal of the right ovary.

Fig. 2. Transverse section of the non-functional uterine horn of an approximately 430-day-old mouse which had produced thirteen litters after removal of the left ovary. (Haematoxylin and eosin. \( \times 15 \).)

Fig. 3. Transverse section of the functional uterine horn of the same mouse used in Fig. 2. (Haematoxylin and eosin. \( \times 15 \).)
The difference in size between the uterine horns in the one-ovary mice is caused by hypertrophy of the endometrium, myometrium and the large blood vessels running between the inner and outer muscle coats (Pl. 1, Fig. 2). In some specimens a considerable amount of collagen fibres had infiltrated the inner muscle tissue, particularly in the enlarged horn. The two uterine horns in the two-ovary mice resembled one another closely in histological structure.

**Table 7**

**Size discrepancy between the two uterine horns in two-ovary and one-ovary mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. mice in which the two uterine horns were classified as</th>
<th>Total No. classified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equal in size</td>
<td>Unequal in size</td>
</tr>
<tr>
<td>Two-ovary</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>One-ovary</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

The uteri were examined and classified by an observer who did not know to which group the animals belonged.

The tissues in almost all specimens contained brown pigment deposits, which gave a strong positive reaction for ferric iron. This pigment is probably similar to the lipoidal-haemosiderin complexes which have been described in the uterus of the rat (Warbrick, 1956). These deposits were found in all layers of the uterine wall. In the two-ovary mice they were present in both uterine horns, but in the one-ovary mice they were much more densely distributed in the hypertrophied horn. The pigment thus occurs mainly in uterine horns which have borne foetuses.

**Histology of the ovaries at autopsy**

The ovaries from sixteen two-ovary and nineteen one-ovary females were examined histologically. The ages of these females at autopsy ranged from 53 to 93 weeks for the two-ovary group, 49 to 82 weeks for the one-ovary group. Within each group, the appearance of the ovaries did not seem to be correlated with the age of the females. The general structure resembled that described by Loeb (1948) and others for the ovaries of moderately senile mice. Corpora lutea and follicles in all stages of development were present, and ovulation was still occurring. Some connective tissue infiltration into the ovarian stroma was seen, but the cystic follicles characteristic of aged rat ovaries (Wolfe, 1943) were not found.

The most striking feature in which the aged ovaries differed from the ovaries of young mice lay in the presence in the interstitial tissue of groups of very large yellow cells, sometimes multinucleate. Such cells could be found in all the ovaries, forming a few small scattered clumps in some, but in others
occupying large areas of the ovary. They have been termed 'lipochrome cells' by Fekete (1946). According to Green (1957), lipochrome cells appear in the ovaries of C57BL mice at 4 to 5 months of age, and become progressively more numerous with age. The histochemical characteristics of the lipochrome pigment have been described by Deane & Fawcett (1952). Loeb (1948) implies that these cells develop from 'interstitial gland cells'; but our material suggests rather that they represent the remains of former corpora lutea, as a series of intermediate stages between normal luteal tissue and lipochrome tissue was seen in some of the ovaries. The relation between the lipochrome cells of the mouse and the persisting theca lutein cells of the pig ovary (Corner, 1921) is not clear.

In three females, the ovaries contained large highly luteinized corpora lutea of the type normally associated with advanced pregnancy (> 10 days) in the mouse. Two of these (Nos. 14 and 42) had shown living foetuses in the uterus (Table 6). (Mouse 41 in Table 6 also had a late foetus in utero; its ovaries were not available for analysis.) The majority of the remaining females showed several sets of corpora lutea in the ovaries, less highly luteinized than the fully developed corpora lutea of pregnancy, but larger than the typical corpora lutea of ovulation of the young mouse. These would not necessarily indicate a state of pseudopregnancy or early pregnancy, however, since Mandl (1959) reports that in senile rats the corpora of ovulation are abnormally large, corresponding to those of pseudopregnancy or mid-pregnancy in young rats. In a few of our females, the ovaries contained small corpora lutea; in others again, more or less luteinized tissue was distributed diffusely about the ovary, either not organized into discrete corpora lutea or else forming corpora that merged one into the other, with no distinct boundaries.

DISCUSSION

Migration of embryos from one uterine horn to the other is uncommon in the mouse (McLaren & Michie, 1954). In one-ovary females, therefore, the great majority of embryos will implant and develop in the uterine horn on the side of the surviving ovary. The striking hypertrophy, illustrated in Plate 1, of this horn relative to the unused contralateral horn was found in all the one-ovary females. This suggests that the uterine hypertrophy which accompanies breeding in normal females is a local rather than a systematic effect. It could be associated either with the implantation reaction or with the actual accommodation of the foetuses and consequent distension of the horn.

Histological studies on the uteri of mice and rats at different ages have shown that there is a gradual increase in the amount of collagen as the animals grow older, and that the collagen fibres become particularly prominent in aged animals (Loeb, Suntzeff & Burns, 1939; Burack, Wolfe, Lansing & Wright, 1941). Recent work by Harkness & Harkness (1956) and Harkness & Moralee (1956) on the collagen content of pregnant rat uteri and uteri during post-partum involution respectively show, however, that in primiparous animals the collagen content of the uterus is very much more labile. These workers demonstrated that during the first 10 days of pregnancy there is a hormonally-
induced increase in collagen. Thereafter, mechanical distension of the uterus by the foetuses promotes the local synthesis of collagen. They also showed that in a uterine horn without foetuses no synthesis of collagen occurred in the second half of pregnancy, although it continued to be formed in the horn containing foetuses. During the involution that follows a first pregnancy, about 90% of the collagen was lost, following an exponential curve with a half-life of 1 day. It is interesting to speculate on the kinetics of the synthesis and dissolution of collagen in the mice described in the present paper. Since they usually became pregnant at each post-partum oestrus, pregnancy was almost continuous (Biggers et al., 1962). After each pregnancy involution would commence, but when pregnancy was re-established at the post-partum oestrus, the hormones secreted during early pregnancy would halt the dissolution of collagen and initiate a new phase of synthesis. Thus residual collagen would remain in the uterus to be added to that synthesized in subsequent pregnancies. Since there is a local effect due to mechanical distension, more collagen would be expected to accumulate in the functional uterine horn than in the non-functional horn in one-ovary mice.

At the time when the females in our experiment were killed, the mean weight of the single surviving ovary in the one-ovary females did not differ significantly from the combined weight of both ovaries together in the two-ovary mice. In other words, complete compensatory hypertrophy in the surviving ovary had taken place. This phenomenon, presumably due at least in part to the stimulating effect of the additional amount of follicle-stimulating hormone which becomes available to the remaining ovary after unilateral ovariectomy, has been reported by many authors (Bond, 1906; Hatai, 1913, 1915; Lipschütz, Wagner & Tamm, 1922). Indeed, in the opossum Hartman (1925) has shown over-compensation; removal of one ovary leads to a threefold increase in weight of the surviving ovary, which ovulates on average three times as many eggs as would each ovary of a normal opossum. Arai (1929) showed that the greater part of the twofold increase in weight in the ovaries of semi-spayed rats is made up of excess corpora lutea and well developed follicles.

Most previous work on compensatory hypertrophy has concerned animals killed within a few weeks of unilateral ovariectomy. Our mice showed the same phenomenon at a much later stage of their lives, namely after reproduction had ceased. A discordant result comes from King (1911), who found that the ovaries of semi-spayed rats, removed 'several months' after ovariectomy, showed little or no compensatory hypertrophy. However, the numbers involved were small.

We have shown in the present paper that one-ovary mice produced fewer litters, and stopped breeding at an earlier age, than did intact controls. The ratio between the total number of young produced per female in the one-ovary and in the two-ovary groups is greater than, though just consistent with, the value of 50% which might be predicted on theoretical grounds. In order to interpret these findings, it will be necessary to consider the relation of litter size to litter order in the two groups: this forms the subject of the following paper.
For acknowledgments, see the end of the following paper.

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