REGULATION OF THE GESTATION PERIOD IN MICE

J. D. BIGGERS, R. N. CURNOW, C. A. FINN
AND ANNE MCLAREN

King Ranch Laboratory of Reproductive Physiology, University of Pennsylvania,
Philadelphia, U.S.A.; Unit of Biometry, University of Reading;
Division of Zoology, Wye College, Kent;
A.R.C. Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh

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Summary. Unilateral ovariectomy in the mouse produces little if any
reduction in the total number of eggs shed. Almost all the resulting
embryos implant in the uterine horn of the intact side, which is therefore
almost twice as crowded as normal. However, the operation does not alter
the very significant inverse regression of gestation length on litter size.
The duration of gestation in the mouse must therefore be influenced
by the total number of conceptuses in the female, rather than by the
number of conceptuses in the more crowded uterine horn.

Occluding one Fallopian tube, which reduces the number of foetuses
to about half but does not affect the number of corpora lutea, is also
without effect on the inverse regression of gestation length on litter size.
The duration of gestation in the mouse must therefore be related to the
number of conceptuses directly, rather than through the number of
corpora lutea.

The results are consistent with the view that some humoral factor
from the placentae and/or foetuses regulates the duration of pregnancy.
An analysis of the literature on the endocrinology of pregnancy in the
mouse strongly implicates oestrogens. This suggestion must remain
speculative until the presence and nature of placental oestrogens in the
mouse have been studied.

The inverse relation between duration of gestation and litter size is
well documented in many species. The sow, however, is a notable
exception, since not one of several studies has demonstrated the
phenomenon. The reasons for this are still obscure.

INTRODUCTION

The control of the duration of gestation is a complex process, not well un¬
derstood (see Marshall & Moir, 1952; Clegg, 1959; Zarrow, 1961, for reviews of
the literature). One well-known fact, however, is the inverse relation which
exists in many species, including the mouse, between the gestation period and
the number of young born.

Two types of mechanism may be invoked to explain this relation: (1) some
local effect of crowding, originating and acting in the uterus; or (2) a systemic effect, which may or may not originate in the uterus, but which must nevertheless be related in some way to the number of young carried. In the mouse, the young are distributed between two uterine horns, a situation which should enable one to discriminate between these two alternatives. If gestation period is found to be dependent on the total number of young in the litter, irrespective of their distribution between uterine horns, a systemic crowding effect is indicated. If this is not so, and it turns out to be the number in, say, the more crowded horn which determines when parturition begins, it suggests the operation of a local factor such as the degree of distension of the wall of the uterus.

A particularly effective way of producing local uterine crowding in a mouse is to remove one ovary. The remaining ovary then undergoes compensatory hypertrophy, and sheds up to twice as many eggs as normal; and virtually all of these eggs, if fertilized, develop in the ipsilateral uterine horn, since trans-uterine migration is very rare in this species. Hence, for a given litter size, the degree of local uterine crowding may be twice as great in one-ovary as in control two-ovary mice. The reproductive performance of one-ovary and two-ovary mice was investigated by Biggers, Finn & McLaren (1962a, b). Preliminary analysis of the results indicated that gestation was longer in one-ovary females (mode 20 days) than in two-ovary females (mode 19 days), which would suggest that local crowding of the uterus does not accelerate the onset of parturition. However, differences in mean litter size were not taken into account. We have now subjected this data to a more searching analysis, the results of which are presented in the first part of this paper.

The conclusion from this analysis will be that the duration of gestation depends on the total number of foetuses in a female, irrespective of whether they are distributed between both horns or confined to one. Since in a normal pregnancy there are approximately the same number of corpora lutea in the ovaries as there are foetuses in the uterus, it is possible that the control of gestation resides in the ovaries rather than the uterus. This possibility has been investigated by comparing the duration of gestation in mice where one Fallopian tube has been tied, with that in intact control animals. The number of corpora lutea in the two ovaries will be the same in both groups. The total number of embryos in the uterus of the tube-tied females will be approximately half the number in the intact females, while the degree of crowding in the functional uterine horn of the tube-tied females will be similar to that in either horn of the intact females. The results of this study are given in the second part of this paper.

**MATERIALS AND METHODS**

The observations were collected in the course of three experiments on mice belonging to Theiler’s original strain. This strain is randomly bred and of high fecundity. In Experiment 1, females were paired with males throughout their reproductive lives, and were inspected every morning for births. The number in each litter was recorded, and the young were then killed. Data were available for analysis from eighteen control (two-ovary) females, and twenty-two
experimental (one-ovary) females from which one ovary had been surgically removed before sexual maturity. Full details of the experimental procedure are given by Biggers et al. (1962a).

Data were also available from two experiments (denoted Experiments 2 and 3) in which one Fallopian tube of the experimental females had been tied off. In Experiment 2 there were twenty-four control and thirty-four experimental pregnancies, and in Experiment 3 there were 241 control and thirty experimental pregnancies.

Although we refer throughout to ‘gestation period’, what we are in fact analysing is the interval between successive litters, counting only those intervals where the female can be presumed to have mated successfully at the post-partum oestrus (Biggers et al., 1962b). Oestrus in the mouse occurs 12 to 18 hr after the birth of a litter; hence the values we give over-estimate by 12 to 18 hr the real duration of pregnancy.

RESULTS

RELATION BETWEEN GESTATION PERIOD AND LITTER SIZE
IN TWO-OVARY AND ONE-OVARY FEMALES

Systemic crowding hypothesis

If the effect of crowding is systemic, the expected gestation period for a given number of young will be the same in a one-ovary as in a normal female. The mean gestation period has been calculated for each litter size for the two-ovary and one-ovary mice in Experiment 1. The results are shown in Text-fig. 1. There is a marked negative regression of gestation period on litter size in both groups. The analysis of variance (Table 1) shows that the regression lines in the two groups do not differ significantly in slope or position. The data are therefore consistent with the systemic crowding hypothesis.

Local crowding hypothesis

Having shown that the data from Experiment 1 are consistent with the systemic crowding hypothesis, we need to see whether they refute the local crowding hypothesis.

If a local crowding hypothesis is true we would predict that the gestation period in a two-ovary female, containing \( n \) conceptuses, would be regulated by the number of conceptuses in the more crowded horn \( (z) \). Further, we assume that the gestation period would be the same in a one-ovary female containing \( z \) conceptuses in her one functional uterine horn. It follows that the expected gestation period for a given number of young should be longer in a two-ovary than in a one-ovary female, since the more crowded horn will contain fewer conceptuses.

The appropriate statistical analysis of this problem depends on knowledge of the distribution of the number of embryos in the more crowded horn \( (z) \) of mice carrying a total of \( n \) conceptuses. The distribution of \( z \), given for litter sizes twelve and thirteen in the Appendix, is far from normal, and necessitates the use of a non-parametric test of significance. This test utilizes knowledge of the median, denoted \( \tilde{z} (n) \), rather than the mean of the distribution.
Consider now, by way of illustration, a group of two-ovary females, each containing twelve conceptuses. From the definition of the median of a distribution, we know that the number of conceptuses in the more crowded uterine horn of any one mouse will be equally likely to be above or below $\xi(12) = 8$

![Text-fig. 1. Effect of litter size on the duration of gestation in two-ovary and one-ovary mice. O = one-ovary mice; • = two-ovary mice.](image)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Two-ovary</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Regression</td>
<td>1</td>
<td>32.178</td>
<td>46.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>216</td>
<td>0.699</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>1</td>
<td>14.294</td>
<td>18.52</td>
<td>&lt;0.001</td>
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<tr>
<td>Residual</td>
<td>183</td>
<td>0.772</td>
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<tr>
<td>Difference between slopes</td>
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<td>0.401</td>
<td>0.55</td>
<td>&gt;0.2</td>
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<td>Distance between lines</td>
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<td>0.128</td>
<td>0.17</td>
<td>&gt;0.2</td>
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<tr>
<td>Overall regression</td>
<td>1</td>
<td>52.878</td>
<td>72.14</td>
<td>&lt;0.001</td>
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<tr>
<td>Combined residual</td>
<td>399</td>
<td>0.733</td>
<td></td>
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</tbody>
</table>

The equation of the overall regression line is $G = 21.23 - 0.12 n$.

(see Appendix). The observed regression line of gestation period on litter size in one-ovary females (Text-fig. 2) is

$$G = 21.16 - 0.101 n$$

where $G$ is the gestation period and $n$ the litter size. Thus the expected mean gestation period of a one-ovary female containing eight conceptuses is 20.35
days. If a local crowding hypothesis is true, therefore, we expect that the gestation periods of two-ovary mice, containing twelve conceptuses, will be equally likely to be less than or greater than 20-35 days. (The median gestation periods expected on this model are shown in Text-fig. 2.) On the other hand, if a systemic crowding hypothesis is true, we expect the gestation periods to be less than 20-35 days. In fact we find from our data that out of fifteen two-ovary females with a litter size of twelve only one has a gestation period longer than 20-35 days.

**Text-fig. 2.** Experimental test of the local crowding hypothesis. The regression line has been calculated from data on one-ovary mice shown in Text-fig. 1. Solid points (●) represent gestation periods expected in two-ovary mice on the local crowding hypothesis; i.e. those corresponding to the median values, \( \bar{x} \) (n), of the numbers of embryos in the more crowded horn at each litter size n. Open points (○) represent the observed gestation periods in two-ovary mice for litter sizes twelve, thirteen and fourteen; numbers in parentheses indicate numbers of mice. For further explanation, see text.

<table>
<thead>
<tr>
<th>Litter size</th>
<th>No. pregnancies</th>
<th>Median value of gestation period expected on local crowding hypothesis</th>
<th>No. exceeding this value</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>15</td>
<td>20-35</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>6</td>
<td>20-25</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>20-25</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

Examination of the data from two-ovary females for values of n other than twelve shows that we can only test the uterine-crowding hypothesis for litter sizes of thirteen and fourteen. This is because we recorded gestation periods in whole days; so a gestation period of just over 20 days, for example, would have been recorded as 21 days. Since the regression line for one-ovary mice shown in Text-fig. 2 only spans a gestation period of 1 day for litter sizes one to eleven inclusive, the grouping arising from our method of observation precludes the use of the non-parametric test of significance. Table 2 summarizes the data for
litter sizes twelve, thirteen and fourteen. The results show that only two out of twenty-four two-ovary females had gestation periods longer than the time predicted from a local crowding hypothesis. The alternative systemic hypothesis implies shorter gestation periods for the two-ovary females: we can therefore use a one-tailed test. The probability of only 0, 1 or 2 from a binomial distribution with \( N = 24 \) and \( p = 0.5 \) is less than 0.00002. Thus the data strongly contradict a local crowding hypothesis, and favour a systemic crowding hypothesis.

The two main assumptions involved in our test of the local crowding hypothesis are that the one-ovary line is well determined and that the distribution of the number in the more crowded horn is not too asymmetric about its median, \( z \). The approximations introduced by these assumptions have been investigated. Neither affects the significance of the results appreciably. At the very worst, the asymmetry of the \( z \) distribution might reduce the significance from 0.00002 to 0.0007. Recording the gestation periods in whole days reduces the sensitivity of the experiment but is unlikely to have biased the conclusions in any way.

Account should perhaps also be taken of a possible correlation between the successive gestation periods of the same female. An analysis based on female averages rather than on gestation periods for individual litters was therefore carried out. It also showed the evidence against the local crowding hypothesis to be highly significant.

**RELATION BETWEEN GESTATION PERIOD AND LITTER SIZE**

**IN MICE WITH ONE FALLOPIAN TUBE LIGATED**

As we have pointed out earlier, the effect of number of young upon gestation period, if systemic, could depend either on the total number of conceptuses in the uterus (embryo hypothesis), or on the total number of corpora lutea in the ovaries (corpora lutea hypothesis). In intact females the number of corpora lutea will be similar to the number of conceptuses; but in females with one Fallopian tube tied, although the mean number of corpora lutea in both ovaries will remain the same, the number of conceptuses in the single functional uterine horn will be little more than half the control number.

*Embryo hypothesis*

If gestation period is regulated by the number of conceptuses, the regressions of gestation period on litter size in the two groups of females should be identical. Text-fig. 3 shows the relation between the gestation period and number of young born in intact and tube-tied females. The statistical analyses, summarized in Table 3, show clearly that there is no significant difference between the regressions for the two groups of females. These results therefore support the hypothesis that the gestation period is regulated by the number of conceptuses.

*Corpora lutea hypothesis*

If the duration of pregnancy is regulated by the number of corpora lutea, the two groups of females will have the same expected mean gestation period.
Regulation of the gestation period in mice

**Text-fig. 3.** Effect of litter size on the duration of gestation in intact and tube-tied mice. The results for Experiments 2 and 3 have been combined. ○ = tube-tied mice; ● = control mice.

**Table 3**

Comparison of the regression of gestation period on litter size in tube-tied and control mice (Experiments 2 and 3)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tube-tied</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regression</td>
<td>1</td>
<td>2.571</td>
<td>2.36</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Residual</td>
<td>32</td>
<td>1.090</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Regression</td>
<td>1</td>
<td>7.039</td>
<td>8.17</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Residual</td>
<td>22</td>
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<tr>
<td>Comparison</td>
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<tr>
<td>Difference between slopes</td>
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<td>0.421</td>
<td>0.42</td>
<td>&gt;0.2</td>
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<tr>
<td>Distance between lines</td>
<td>1</td>
<td>0.848</td>
<td>0.85</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Overall regression</td>
<td>1</td>
<td>9.814</td>
<td>9.84</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Combined residual</td>
<td>54</td>
<td>0.997</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 3</strong></td>
<td></td>
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<tr>
<td>Tube-tied</td>
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<tr>
<td>Regression</td>
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<td>2.159</td>
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<tr>
<td>Residual</td>
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<td>Control</td>
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<tr>
<td>Regression</td>
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<td>19.275</td>
<td>31.30</td>
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<tr>
<td>Residual</td>
<td>239</td>
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<td>Comparison</td>
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<tr>
<td>Difference between slopes</td>
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<td>0.096</td>
<td>0.16</td>
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<td>Distance between lines</td>
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<td>0.070</td>
<td>0.12</td>
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<tr>
<td>Overall regression</td>
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<td>23.238</td>
<td>38.65</td>
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</tr>
<tr>
<td>Combined residual</td>
<td>267</td>
<td>0.601</td>
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</table>

The equations of the overall regression lines are: \( G = 21.14 - 0.16 \) for Experiment 2 and \( G = 21.06 - 0.10 \) for Experiment 3.
Conversely if the number of conceptuses regulates the duration of pregnancy, the mean gestation period will be longer in the tube-tied females, on account of their smaller mean litter size.

Table 4 shows the mean gestation periods of the intact and tube-tied females in the two experiments. The significance of the observed difference between the two means is examined by a t-test. Since the alternative to the null hypothesis is that the mean gestation period should be greater in the tube-tied females, the probability of observing a larger positive difference is determined using a one-sided test of significance. The analyses show that the probabilities are \( P = 0.130 \) and \( P = 0.046 \) for the two experiments, respectively. Thus Experiment 3 on its own provides significant evidence that the mean gestation period in tube-tied females is longer than in intact females. Experiment 2, although not significant at the 0.05 level, also supports this conclusion. The weighted mean of the two mean differences is 0.28, s.e. 0.14, d.f. 325: a one-sided test of significance shows that this difference is significant at about the \( P = 0.02 \) level. Our data therefore contradict the hypothesis that gestation period is regulated by the number of corpora lutea.

**DISCUSSION**

As we have stated earlier, the tendency for the duration of pregnancy to be inversely related to the number of young in the litter is widespread among polytocous mammals. For example, Goy, Hoar & Young (1957) showed that in a pure-bred strain of guinea-pigs the mean gestation length was 69.9 days for a litter size of one, whereas it was 65.3 days for a litter size of six. The literature on this phenomenon in domestic animals is reviewed by Clegg (1959), who cites evidence for its occurrence in cattle, sheep, goats and rabbits. The data of McKeown & Record (1952) on the duration of pregnancy in women bearing singletons, twins, triplets and quadruplets clearly establish that the phenomenon also occurs in women.

Although Hammond (1925) is often quoted as stating that there is no inverse relation between gestation length and litter size in the rabbit, the data he gives
show a highly significant negative regression of gestation length on litter size (0·01 > P > 0·001). In addition, Manresa (1933) has shown a significant negative correlation between gestation length and litter size in the rabbit. The most recent and comprehensive study on this question is that by Wilson & Dudley (1952), who examined data from seventeen breeds and varieties of rabbit, and seventeen crosses. In twenty-two out of the thirty-four groups no significant relationship was found between gestation length and litter size; in the remaining twelve groups, however, significant inverse relationships were found.

Several investigators have studied the factors influencing duration of pregnancy in the sow (Sabatini, 1908; Carmichael & Rice, 1920; Johansson, 1929; Křiženecky, 1935; Joubert & Bonsma, 1952) and none has found any relation between gestation length and litter size. In addition McKenzie (1928) provides one set of data, and Burger (1952) provides two sets of data, from which the regression of gestation length and litter size can be calculated. We have calculated these regressions and in no case did we find a significant relationship. Thus there is an overwhelming body of evidence collected at several times in several parts of the world that in the sow the number of young in the litter has no effect on the length of gestation.

In the mouse, the data analysed in the first part of this paper suggest that the inverse relation which exists between the duration of gestation and litter size must be explained in terms of the total number of conceptuses in the mother, and not on the degree of crowding in the uterine horns. Thus, in the mouse, the stretching of the uterine wall, which increases particularly rapidly in the later stages of pregnancy, does not appear to induce parturition by increasing uterine irritability. The data analysed in the second part show, in addition, that the inverse relation must be explained directly in terms of influences arising from the conceptuses, and not in terms of the numbers of corpora lutea, although these are very highly correlated with the numbers of conceptuses in normal pregnancies.

This is fully consistent with several studies on mice, rats, rabbits, hamsters, guinea-pigs and rhesus monkeys (Mayer & Klein, 1955), which suggest that it is the placenta and not the ovary which determines the date of parturition. For example, Newton (1935) killed all the foetuses in pregnant mice between the 12th and 15th days of gestation, leaving the placentae intact. The placentae were retained until about the normal time of parturition, after which post-partum oestrus followed within 2 days. If both foetuses and placentae were removed together, oestrus followed in 3 to 4 days. Kirsch (1938) did similar experiments with pregnant rats and obtained essentially the same results. Embryos were removed between the 14th and 21st day of pregnancy leaving the placentae intact; in all cases the placentae were delivered at about the normal time of parturition. If both the foetuses and placentae were removed, and replaced with paraffin pellets of the same size and shape, the pellets were delivered within 2 days. If the placentae were left in only one horn, and paraffin pellets placed in the contralateral horn, the placentae and pellets were delivered at the expected time of parturition. Unfortunately no information is available as to whether the time of parturition was affected by the number of placentae left in the uterus.
Since there are very large differences between species in the details of the endocrine regulation of pregnancy, our results will be discussed primarily in terms of known facts about the mouse.

Harris (1927) showed that bilateral ovariectomy at any stage up to the 17th day of pregnancy in the mouse terminates pregnancy within 2 days of the operation. Ovariectomy on either the 18th or 19th day was too close to the expected time of normal parturition to decide whether or not the operation terminated pregnancy. Soon after, Parkes (1928) found that in mice, as in rabbits (Fraenkel & Cohn, 1901) and guinea-pigs (Loeb, 1923), the maintenance of pregnancy depended on the presence of functional corpora lutea in the ovaries. If these corpora lutea were removed at any time up to the 17th day, pregnancy ceased, but after this time they could be removed without effect. The 17th day is also the time when the corpora lutea commence to regress. This work showed that the ovaries of the mouse elaborate a hormone throughout most of pregnancy which is essential for the maintenance of gestation. There is good evidence that the hormone involved is progesterone, since the administration of progesterone can maintain pregnancy in adult and prepubertal ovariectomized mice, and in the obese mutant (obob) which fails to form corpora lutea (Smithberg & Runner, 1956, 1957; Steinetz, Beach & Kroc, 1957, 1959; Kroc, Steinetz & Beach, 1959). Parkes's experiments also indicate that in the mouse the placenta produces little or no progesterone at any stage of pregnancy. This conclusion is in agreement with more recent indirect evidence, though no direct attempts to detect progesterone chemically in the placenta of the mouse have yet been reported. However, there is an endocrinological basis for the placental control of the corpus luteum in the mouse, since Cerruti & Lyons (1960) have shown potent luteotrophic activity in mid-term mouse placentae. This control is direct, and not by way of the adenohypophysis (Newton & Beck, 1939).

The fact that the administration of progesterone to a mouse late in pregnancy delays parturition (Kroc et al., 1959) suggests that a decrease in progesterone level, either absolutely or relative to the level of other hormones, is a necessary process if normal parturition is to take place. Let us now consider the implications of our experimental results if we assume that withdrawal of progesterone is also a sufficient reason for parturition to occur. Since parturition occurs earlier in females bearing larger numbers of embryos, the secretion of progesterone by the ovaries would need to cease earlier in such animals. The only known direct mechanism which could produce this effect is a reduction in the secretion of the total luteotrophic activity of all placentae in the more crowded uteri. Such a theory would have intuitive appeal only if the gestation length was regulated by local overcrowding of the uterus causing disturbance of placental function. Since our analyses clearly refute the local crowding theory, there is little to support the view that the dependence of gestation length on number of conceptuses depends on the secretion of progesterone and placental luteotrophic hormone.

Relaxin is a hormone whose extreme importance in pregnancy and parturition has only just become recognized (Steinetz et al., 1959; Hall, 1960). However it appears to be produced only in the ovaries, and not the placenta, in the
mouse. Thus our results cannot be explained in terms of greater secretion of relaxin in females containing the larger numbers of conceptuses.

It has been known for many years that oxytocin from the posterior lobe of the pituitary gland plays an important role at the time of parturition. Although the uterus of the mouse will respond to oxytocin at any stage of pregnancy, maximum sensitivity to oxytocin is attained only at parturition (Robson, 1934). Robson (1936) showed that removal of the ovaries on the 14th, 15th or 16th day of pregnancy in mice caused abortion, but did not alter the sensitivity of the uterus to oxytocin. However, ovariectomy on the 17th day did not cause abortion, but led to a very marked increase in sensitivity of the uterus to oxytocin. He also demonstrated that this increase in sensitivity was greater if the placenta and foetus were left in the uterus, thereby showing some local effect which controlled the sensitivity of the uterus to oxytocin. Many investigators have shown in the ovariectomized and pregnant mouse (Parkes, 1930; Robson, 1935; Marrian & Newton, 1935), and in other species, that the administration of oestrogens increases the sensitivity of the uterus to oxytocin. That the injection of oestrogens into pregnant mice causes abortion was shown by Parkes & Bellerby (1927), first using crude extracts, and later using highly purified oestrin (Parkes, 1930).

It is well established that oestrogens increase in the later stages of pregnancy in the mouse. For example, oestrogens are secreted in sufficient quantity before parturition to initiate the changes which culminate in the post-partum oestrus that occurs less than 24 hr after delivery. Also, mitotic activity in the vagina, typical of an oestrogenic effect, increases in the last 2 to 3 days of pregnancy (J. D. Biggers, unpublished observations). The experimental results which we have reported may be accounted for on two assumptions: (a) that the responsiveness of the uterus to oxytocin, and hence the time of onset of parturition, depends on a threshold level of oestrogen acting on the uterus, a level adequate to overcome the previous progesterone dominance; (b) that the oestrogens concerned are produced by the placenta. As the number of young, and therefore of placentae, increased, the threshold level of oestrogen in the blood would be reached earlier; hence the inverse relation between number of young and duration of pregnancy would be expected. But we stress that this explanation must remain speculative until the source of oestrogens in pregnant mice has been identified.

Appendix

DISTRIBUTION OF EMBRYOS IN ANIMALS WITH BICORNUATE UTERI

In many species possessing a bicornuate uterus, embryos cannot readily pass from one uterine horn to the other. This may be because there is no anatomical connexion between the two horns, or because some functional phenomenon bars the passage of the embryos.

The mouse is an animal with a bicornuate uterus, in which transuterine migration is an exceedingly rare occurrence (McLaren & Michie, 1954). The distribution of embryos between uterine horns in a normal pregnancy has been found to be random, in the sense that it approximates to a binomial distribution with \( p = 0.5 \) (Falconer, Edwards, Fowler & Roberts, 1961; McLaren, 1963).
Consider a two-ovary mouse with a litter of size \( n \) (\( n = 1, 2, \ldots \)), and let \( z \) be the number of embryos in the more crowded horn. We are interested in the distribution of \( z \). Let the probability of \( z \) be denoted \( P_z \). Two cases must be considered, where \( n \) is odd and \( n \) is even, respectively.

**Case 1** (\( n \) odd). Let \( x = (n+1)/2 \). Then \( z \) will take the values \( x, (x+1), \ldots, (x+i), \ldots, n \), and the distribution of \( z \) is given by

\[
P_z = \binom{n}{x+i} / 2^{n-1}, \quad [i = 0, 1, \ldots, (n-x); z > x]
\]

**Case 2** (\( n \) even). Let \( x = n/2 \). Then \( z \) will take the values \( x, (x+1), \ldots, (x+i), \ldots, n \), and the distribution of \( z \) is given by

\[
P_z = \binom{n}{x} / 2^n, \quad [i = 0; z = x],
\]

\[
P_z = \binom{n}{x+i} / 2^{n-1}, \quad [i = 1, 2, \ldots, (n-x); z > x].
\]

In this case we must include the possibility that there is an equal number of embryos in each horn, since it is impossible to exclude such instances from our data.

To illustrate, the distributions for \( n = 12 \) and \( n = 13 \) are shown in Text-fig. 4.

![Text-fig. 4. Expected distribution of embryos in the more crowded horn (z) of female mice for litter sizes of twelve and thirteen, respectively. (a) n = 12; median = 8; (b) n = 13; median = 9.](image)

It is readily seen that the distributions are far from normal. In the analysis of our data the median of the distribution \([\hat{z}(n)]\) is required. Since the distribution of \( z \) is discrete, the median has been calculated as that value of \( z \) which does not include any instances in the lower 50% of the distribution. This prevents any overestimation of the significance of departures from the local crowding hypothesis. Thus \( \hat{z}(12) = 8 \), and \( \hat{z}(13) = 9 \).

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