PARTURITION AND INCREASED LITTER SIZE IN MICE AFTER SUPEROVULATION

EVERETT D. WILSON* AND R. G. EDWARDS

National Institute for Medical Research, Mill Hill, London, N.W.7

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Summary. The length of gestation and the duration of parturition were similar in mice that had mated during natural oestrus and in those with large numbers of full-term foetuses following superovulation. After superovulation many offspring die at parturition, but by giving every possible assistance to the offspring at birth, mean litter size was raised from 6-8 to 10-7 living young (controls that mated during natural oestrus gave birth to 6-2 living young). The mortality rate after birth was high in large litters, and offspring weighing less than 0.7 g at birth survived less than 24 hr. When one-half of the large litters which included offspring weighing 0.7 g or more were fostered on to control females, mortality was reduced to control levels and the offspring grew at the same rate whether a superovulated or control mother was suckling. The overall fecundity of superovulated mice was lower than controls because (a) 11 out of 137 of them died, (b) more than one-half of them failed to implant any embryos.

INTRODUCTION

Numerous attempts have been made to increase litter size in laboratory and domestic animals by inducing superovulation (Hammond, 1952; Gordon, 1958). The results have been far from encouraging. Even though the number of young born alive has been increased, the early death of many offspring often reduces litter size to normal levels or lower. Offspring in larger litters are smaller than those in smaller litters. In mice, parturition is especially disastrous for foetuses in overcrowded uteri, and almost none of the offspring survive (Edwards & Fowler, 1959). The present paper records data on the length of gestation and the duration of parturition, and describes attempts to assist mothers and offspring through parturition in an effort to reduce the mortality rate of the offspring.

MATERIALS AND METHODS

The mice used were of the outbred Strain TO maintained at the National Institute for Medical Research, Mill Hill, London. They were fed ad libitum on standard diet 41B supplemented with a ground maize preparation.

*Present address: Department of Biology, Sam Houston State Teachers College, Huntsville, Texas, U.S.A.
Nulliparous females ranging in weight from 16 to 20 g were injected with 3 or 6 i.u. pregnant mares' serum,* followed 40 hr later by 3 or 6 i.u. human chorionic gonadotrophin* (Fowler & Edwards, 1957). Five hours later the mice were paired overnight with males of the same strain. Mating was judged by the presence of a vaginal plug on the following morning. Females which had mated were placed three to a cage for the first 12 days of pregnancy and then put in individual cages, except for one group which was caged with a non-pregnant female until after parturition.

The pregnant mice which were caged individually were divided into two groups just before parturition. Females in one group were given no assistance and went through parturition unaided. Those in the other group were watched very closely and the offspring were recovered as they were born. The maximum possible assistance was given to these offspring, namely, the removal of the foetal membranes, attempts to induce breathing, and maintenance of body temperature. The time at which parturition began and ended, an assessment of the amount of maternal care, and birth weights of offspring were recorded for all three groups of females. Control mice of the same strain, age and body weight, but which had mated during natural oestrus, were used in similar studies to compare with treated mice (Table 1).

Some mice were killed just after parturition for an examination of the uterine contents. The remainder were allowed to raise their own litters except that one-half of the offspring from five litters, thirteen or fourteen in number, were fostered to lactating control females whose young had been removed. Body weights of all offspring were recorded at birth, and many were weighed at 3 and 6 weeks of age.

**RESULTS**

DURATION OF THE GESTATION PERIOD AND OF PARTURITION

The duration of gestation and parturition were studied in relation to the number of implantation sites (i.e. where a fully-developed placenta had been implanted), and to the number of young born. Both living foetuses and foetuses which had died late in gestation would be included in the numbers of implantation sites. Difficulties arise in counting the number of young born,

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*The pregnant mares' serum and human chorionic gonadotrophin used were similar to the International Standard preparations.
especially in large litters, because some young can be eaten in the intervals between examination of the mice. We have therefore based our data primarily on the numbers of implantation sites.

Thirty-seven control mice were killed immediately after parturition and examined for implantation sites and moles. Between three and eleven implantation sites were found in these mice, and the duration of pregnancy was not correlated with the number of sites (Text-fig. 1). A correlation did exist

![Text-fig. 1. Effect of the number of implantation sites on the length of gestation in control females. The regression coefficient did not differ from zero ($t_{35} = 0.50, P > 0.05$).](image1)

![Text-fig. 2. Effect of the number of implantation sites on the length of gestation in treated females. The regression coefficient differed significantly from zero ($t_{47} = 2.51, P < 0.05$).](image2)
between the number of implantation sites and the duration of pregnancy after superovulation (Text-fig. 2), although this appeared to be due primarily to the prolonged duration in the three mice with four sites (Text-fig. 2). The difference between the regression coefficients in control and treated mice was not significant ($t_{82} = 1.33, P > 0.05$). In the forty-nine induced pregnancies, the average duration was 19.3 days. Results were similar when the duration of pregnancy was compared with the number of young born (control mice, $b = -0.011$, $t_{35} = 0.95$, $P > 0.05$; treated mice, $b = -0.078$, $t_{40} = 2.40$, $P < 0.05$).

The duration of parturition was estimated as the interval between the birth of the first and last offspring. Two treated mice, which had fifteen and seventeen foetuses, respectively, died during parturition and have been excluded from these observations. The duration of parturition in forty-three treated and nine control mice was not influenced by the number of young born (Text-fig. 3). No correlation was found between the amount of foetal mortality at parturition and the duration of parturition.

**LITTER SIZE**

The control pregnancies consisted of two groups; those that went through parturition alone and those pregnant females that were each housed with a non-pregnant female. The mean number of living young produced by thirty-one females which were caged separately $(6.2 \pm 0.4)$ was similar to that produced by fourteen females caged with another female $(6.2 \pm 0.1)$. 

![Text-fig. 3. Effect of the number of young born on the duration of parturition in treated and control females. ○ = Controls; ● = Treated females.](image-url)
The superovulated mice were divided into three groups. In the group that went through parturition unassisted, the average was 6·8 ± 0·4 living young (twenty-nine litters). The forty-nine females with a non-pregnant partner delivered an average of 7·0 ± 0·5 living young. Many of the offspring in these litters were dead; others had been eaten. The difference between litter size in these groups and in controls was not significant ($P < 0·2$). In the last group, i.e. those given assistance, mean litter size was 10·7 ± 0·6 living young (twenty-seven litters) (Text-fig. 4). Litter size in this group was significantly higher than in the other superovulated groups ($t_{103} = 4·94, P < 0·01$) or in controls ($t_{70} = 7·65, P < 0·01$). In these twenty-seven litters, a further twenty-six offspring were dead when first seen or were eaten by their mothers after being born alive.

![Text-fig. 4. Histogram showing the distribution of litter sizes in control mice and in treated mice given assistance at parturition.](image)

Despite the increased size of litters after superovulation, the overall fecundity of the treated mice was lower than that of the controls. In the treated group, 290 mice mated, 137 possessed implanted embryos, and eleven died late in pregnancy or at parturition. These eleven had between nine and nineteen foetuses (mean 14·9 ± 0·7), which is above the mean litter size (see above). If it is assumed that mean litter size is 10·7, overall fecundity is equivalent to 4·6 living offspring per treated female. A similar calculation for controls gives 4·7 per mated female. Assistance to control mice at parturition would presumably have raised this figure; if calculated on the number of eggs shed the value becomes 5·1. Two factors were mainly responsible for the decreased overall fecundity after superovulation: many females had no implanted embryos and, secondly, more of the treated mice died, presumably because of the greater demand on the mothers' physiological resources (Healy, McLaren & Michie, 1960).
As would be expected, birth weight was negatively correlated with the number of implantation sites (Text-figs. 5 and 6). Results were similar when birth weight was compared with the number of young born (controls, $b=0.027$, $P<0.05$; treated mice, $b=0.046$, $P<0.01$). Among the living offspring were forty-four that weighed between 0.5 and 0.7 g at birth; none of them were alive 24 hr after parturition. Several animals weighing between 0.7 and 0.8 g were weaned at 21 days of age.

Table 2 gives the mean 3- and 6-week weights of offspring from (a) control mice (litter size five, six and seven) and (b) six superovulated mice from which one-half of the offspring were fostered. Birth weights of offspring from control mice were between 1.1 and 1.5 g, those of treated mice weighing

\[ \text{Mean birth weight of offspring (g)} \]

\[ \text{No. implantation sites} \]

\[ b = -0.039 \pm 0.015 \]

\[ (t_{28} = 2.79, P<0.01) \]

\[ \text{Text-fig. 5. Effect of the number of implantation sites on the average birth weight in control females.} \]

\[ \text{The regression coefficient differed significantly from zero (t}_{28} = 2.79, P<0.01).} \]

\[ \text{No differences were found between the regression coefficients for control and treated females (t}_{58} = 0.65, P>0.05).} \]

\[ \text{Text-fig. 6. Effect of the number of implantation sites on average birth weight of young from treated females.} \]

\[ \text{The regression coefficient differed significantly from zero (t}_{30} = 8.78, P<0.01).} \]
Litter size after superovulation between 0.7 and 1.2 g. The offspring weighing between 0.7 and 0.8 g were alive at 6 weeks of age. By 21 days of age, mean weights were similar in the three groups of offspring.

Of 126 young born alive to control mice, 107 (84.9%) were alive at 6 weeks of age. Nine treated mice gave birth to eighty-six living young (litter size six to eleven), and fifty-six (65.1%) were alive at 6 weeks. In those litters from treated mice where one-half of the litter was fostered, sixty-two were born alive and fifty-three (85.5%) were alive at 6 weeks of age.

**Table 2**

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<th>Type of litter</th>
<th>Foster mother</th>
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<td>Birth</td>
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<td>1.28±0.02</td>
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<td>Treated</td>
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<td>Treated</td>
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**DISCUSSION**

The considerable mortality at parturition in large litters after superovulation appears to be due largely to the small size of the young at birth. These small offspring are either eaten by the mother during parturition, or die at birth, or are trapped under the mass of young and prevented from suckling. None of the offspring weighing less than 0.7 g survived for more than 1 day. If the large litters are reduced to one-half, the mothers are able to care for all their young, and the growth rate and incidence of mortality of their offspring is then quite comparable to that of control offspring up to 6 weeks of age. Provided that they weigh 0.7 g or more at birth, offspring from treated mice are clearly as viable as those from control mice, and the treated mother is as capable of caring for her young as are controls.

But two factors detracted from the usefulness of the superovulation procedure. First, many of the treated females (11 out of 126) died during late gestation or at parturition, a proportion similar to that found in an earlier experiment (2 out of 41; Edwards & Fowler, 1959). Four of these died after having delivered some or all of their foetuses, and another was found with one foetus in the cervical canal. The second factor was the failure of many females to carry any foetuses to later stages of gestation. The proportion of females without foetuses at full term varies greatly; it is partly dependent on the number of implanted embryos (McLaren & Michie, 1959), and on the dose of hormone, the strain of mouse, and the oestrous cycle (Edwards, Wilson & Fowler, in preparation). This type of infertility is not alleviated by injecting progesterone (Fowler & Edwards, 1960). In Strain TO this factor was the major one reducing the effectiveness of superovulation in the overall fecundity of the mice, for fewer than one-half of the treated mice had implanted embryos after mating as
compared with almost two-thirds in controls. Had we been able to use a strain such as JB or 3HI, in which more than 85% of females have implanted embryos after superovulation, the increased rate of survival at parturition would have led to an increase in fecundity of approximately 50% above controls.

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REFERENCES


