

Correlation Between Duration of Courtship and Litter Size in Outbred NMRI Mice

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Abstract. When group-housed female mice are exposed to male mice, male pheromones induce female ovarian cyclicity resulting in a majority of the females entering estrus and spontaneous ovulation on the third night following introduction of the male – the so-called Whitten effect. We previously demonstrated that females mating on the third night after being housed with a male mouse ovulated significantly more eggs than did females mated on the first, second and fourth night, and that this effect was mediated by follicle stimulating hormone (FSH). The present study demonstrated that female mice, which were mated early after being housed with a male mouse (1-2 days), delivered significantly smaller litters than did females mated after spending 3-4 days with the male prior to mating. The implications both from practical animal production perspective, as well as from a fundamental biological perspective may be significant. In addition, the findings may have a practical research value making scientists in reproductive research aware of the natural variation in litter size, which may be an important confounding variable in all research in which litter size is a variable or a parameter used as a measure of the impact of an experimental treatment.

Ovarian cycles of females living and interacting together without the presence of males have been shown to synchronise in a number of species including man, and pheromones of women have been demonstrated to affect the duration of the menstrual cycles of other women (1). Recent studies demonstrated that pheromone-activated hormonal systems extend widely within the brain and that the main olfactory pathway can mediate responses to pheromones, as well as to

common odours (2). When a male mouse is housed with female mice that have previously lived in an all-female group and thereby synchronised their oestrus cycles (3), there is an immediate effect on the females' cycle. A new oestrus cycle is initiated and a majority of the females will be in oestrus on the third night (4). This is known as the Whitten effect.

A study by Gidley-Baird and co-workers in 1986 (5) demonstrated that contact with males affected the number of ova ovulated by female mice at oestrus and that the number varied as a function of the time spent with the males prior to mating. The conclusion of this study was that virgin mice ovulated a significantly greater number of eggs if they were mated on the third night after being housed with a male than if they were mated on the first, second or fourth night. This effect was mediated by follicle stimulating hormone (FSH).

The hypothesis tested in the present study was whether females mated after three or four days' housing with a male exhibit a higher rate of mating, conception and implantation, and subsequently produce larger litters than those mated after spending just one or two days in the presence of the male.

Materials and Methods

Twelve male and 36 female mice of the outbred NMRI stock (Charles River, Wiga, Germany) aged 7 weeks were used in the study. The mice were of Specified Pathogen Free (SPF) quality and were maintained in a facility equipped with individually ventilated cage racks (Techniplast, Varese, Italy). On arrival at the Department of Experimental Medicine, University of Copenhagen, the mice were caged in ventilated polycarbonate type III cages (Scanbur, Koge, Denmark), three females together with one male. They were subjected to standard animal house conditions: the light regime was 12/12 h dark/artificial light cycle, temperature was maintained at $21 \pm 1^\circ\text{C}$ and the relative humidity was 30-60%. Aspen chips (Tapvei, Oy, Korttinen, Finland) were used as bedding and shredded cardboard and cardboard hides used as environmental enrichment. Water and standard rodent pellet diet (Brogården, Gentofte, Denmark) were available at all times.

The female mice were examined every morning for five days between 8 am and 9 am for the presence of vaginal plugs. When having confirmed the presence of a vaginal plug on the specific day,

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the mated females were placed together ($n < 5$) in separate cage(s) under similar housing conditions.

After 17 days, the female mice were placed in individual cages. On the morning, when the females delivered their pups (on days 19-20 of pregnancy), the pups were counted. The study was terminated when all females had delivered their pups.

The relevant Danish authorities did not consider the present study subject to licensing.

Results

Vaginal plugs were observed in 56% of the females (20 of 36). Two females were mated on the first night, 3 on the second night, 12 on the third night, 3 on the fourth night, and 0 on the fifth night after being housed with the male mice.

The females mated on days 1 and 2 after introduction of the male mouse to the cage (Group A) had a significantly ($p < 0.05$, Student's *t*-test) smaller average litter size compared with females mated after 3 and 4 days' housing with a male mouse (Group B) (Table I).

Discussion

The present study was carried out to mimic a typical breeding scheme for laboratory mice using type III Macrolon cages in which three females and one male mouse were introduced at the same time. On each of the following five mornings the females were examined for the presence of vaginal plugs. Females with vaginal plugs were transferred to new cages. Vaginal plugs were observed in 56% of the females. This is a somewhat lower proportion than what has been reported in the literature (6). The majority of the twenty females (60%) were mated on the third night, confirming information in the literature (4, 5). Gidley-Baird and colleagues (5) assumed that the increased number of ova shed by females mated on the third night would eventually result in larger litter sizes but this was not investigated. Important biological events between an egg is ovulated and a live pup is born include successful fertilisation, implantation, and intra uterine growth and development. Any of these events might be impaired in mice ovulating more eggs than other mice resulting in a loss of fertilised eggs/embryos and a subsequent litter size similar to the females who had ovulated fewer eggs. However, the present study demonstrates, for the first time, that the increased number of ova shed by the females mating on the third (and fourth) night resulted in larger litter sizes demonstrating that fertilisation, implantation and growth *in utero* in these females was equally successful as compared with females ovulating fewer eggs.

This is a potentially important finding. Firstly, the fact that the actual litter size in mice (and perhaps also in other mammals) can vary significantly depending on olfactory stimuli from the male is a new finding and the implications

Table I. Mean number of pups born, range and standard deviation in groups A and B.

	Group A (days 1 and 2)	Group B (days 3 and 4)
No. of females	5	15
Mean No. of pups born	10.00	13.13
Range	6-13	9-17
Standard deviation	2.65	1.77

both from a practical animal production perspective as well as from a fundamental biological perspective may be significant. Secondly, the findings may have a practical research value making scientists in reproductive research aware of the natural variation in litter size, which may be an important confounding variable in all research in which litter size is a variable or parameter used as a measure of the impact of an experimental treatment *e.g.* a drug's impact on numbers of young born.

In terms of further realisation of the three Rs (replacement, reduction, refinement) concept of Russell and Burch (7), characterisation of this variation in litter size may lead to a reduction in animal use by taking this variation into account when designing experiments. This could be done, *e.g.* by dividing experiments into blocks of females mated on the same day or by preventing physical access of the males to females except for *e.g.* day 3, thereby reducing the between animal variation in litter size due to varying time spans spent with the male prior to mating. These strategies ought to result in a reduction in the number of animals needed in experiments where litter size is a measure of an effect of an experimental treatment.

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