Increased ovulation rate in virgin mice induced by males

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Introduction

The pheromonal regulation of the ovarian cycle in many species including mice has recently been extensively reviewed (*Vandenbergh* 1983).

It is generally known that if a male mice is introduced into a cage with female mice, which have previously lived in a group of females thereby syncronizing their oestrus cycles, the majority of the females will be in oestrus three days later (*Whitten* 1958). The male has a luteolytic effect which relases the gonadotrophin surges and eventually results in oestrus and ovulation (*Bronson* 1975, *Ryan & Schwartz* 1977). Male induced oestrus is similar to spontancous oestrus with regard to reproductive organ weights and pituitary luteinizing hormone content (*Bingel & Schwartz* 1969). The present study was undertaken in order to determine whether contact with males affected the number of ova shed by female mice at oestrus, and if the number varied as a function of the time spent with the males prior to ovulation.

Materials and methods

Virgin female mice of the outbred Quackenbush (QS) stock aged 8–10 weeks, weighing 25–30 gm and raised in isolation from males were used in all experiments. The female mice were allocated two per cage among either a colony of intact male mice or a colony of vasectomized males. The mice were maintained in rooms with controlled temperature (23°–27°C) and humidity (55 %–60 %). Artificial lighting was provided by fluorescent tubes which were switched on and off automatically at 06.00 h and 18.00 h respectively.

The female mice were killed 24 hours after the detection of a copulatory plug, the oviducts were removed and flushed with 0.9 % NaCl solution and the number of ova recovered from each oviduct were recorded.

This experiment was repeated four times to obtain a minimum of 60 mice mated with intact males and 60 mated with vasectomized males on each of days 1–4 after setting up with the males.

Table 1. Number of ova recorded in females (group size 30) which mated on days 1, 2, 3 or 4 after housing with intact or vasectomized males.

Day of mating after placing with males	Day 1	Day 2	Day 3	Day 4
No. of ova $\bar{x}$ ± S.E. mated with intact ♂	13.2 ± 0.42	14.3 ± 0.44	15.4 ± 0.35	14.9 ± 0.49
No. of ova $\bar{x}$ ± S.E. mated with vasectomized ♂	13.4 ± 0.38	14.7 ± 0.39	15.6 ± 0.33	14.2 ± 0.45

Results and discussion

There was a significant increase in the number of eggs found in females which mated on days 2, 3 or 4 after housing with intact or vasectomized males, when mated on day 1 ($p < 0.05$, $p < 0.01$ and $p < 0.01$ respectively) (Table 1).

This experiment was repeated using females which were housed 20 per cage and placed in the same room as the males for three days prior to distributing the females among the males. The result did not differ from those found in the previous experiment.

To evaluate whether the increased ovulatory response was mediated via male induced increase in pituitary release of follicle stimulating hormone (FSH), females were injected immediately prior to contact with the males with either 0.1 ml of normal rabbit serum (NRS) or 0.1 ml of an anti rat FSH (ARFSH) raised in a rabbit.

Immunological cross-reaction between rat FSH and mouse FSH was indicated since a crude mouse pituitary extract gave positive reaction in a double antibody radioimmunoassay employing the ARFSH.

In accordance with the results of the previous experiments a significantly larger number of eggs ($p < 0.02$) were ovulated on day 3 compared with day 1 after introduction of the males in the group of females treated with NHS (Table 2). By contrast, no significant increase from day 1 to day 3 was found in the group of females which prior to the male contact had been treated with ARFSH.

Table 2. Number of ova recorded in females which were injected immediately prior to contact with normal male mice with either 0.1 ml of normal rabbit serum (NRS) or 0.1 ml of a rabbit anti rat FSH serum (ARFSH) (Group size 30).

Day of mating after placing with intact ♂	Day 1	Day 3
No. of ova $\bar{x} \pm$ S.E. 0.1 ml NRS	13.3 ± 0.51	14.9 ± 0.4
No. of ova $\bar{x} \pm$ S.E. 0.1 ml AR FSH	12.8 ± 056	13.3 ± 0.5

This experiment suggests that FSH may be an important factor in the effect of males on the number of ova shed by females, and consequently it was tried out whether it was possible to enhance the effect of the males by administering FSH to the females.

The female mice were injected with either 10 µg or 50 µg of rat FSH (NIH B-1) in 0.1 ml of gelatine vehicle or vehicle alone just prior to their housing with males.

Table 3 shows the comparison between number of ovulated eggs in the FSH treated groups and the control group which mated an day 1 or day 3 following contact with the males.

Table 3. Number of ova recorded in females which were injected with 10 µg or 50 µg rat FSH in 0.1 ml of a 15 % gelatine vehicle or vehicle alone just prior to housing with normal male mice (Group size 30).

Day after placing with intact ♂	Day 1	Day 3
No. of ova $\bar{x} \pm$ S.E. 0.1 ml 15 % gel	12.6 ± 0.62	14.8 ± 0.41
No. of ova $\bar{x} \pm$ S.E. 10 µg rat FSH	13.0 ± 0.52	17.6 ± 0.87
No. of ova $\bar{x} \pm$ S.E. 50 µg rat FSH	13.1 ± 0.6	25.1 ± 2.41

There was no significant difference in number of eggs between animals in the three groups which mated on day 1, but a significant difference was seen in all three groups when comparing number of eggs in the animals mating on day 1 compared with those mating on day 3.

The administration of 10 and 50 µg FSH had a significant positive effect on the number of eggs ovulated on day 3 ($p < 0.001$).

The conclusion drawn from this work is that virgin mice ovulate a significantly greater number of eggs if they are in contact with males for a period of time prior to ovulation, and that this response appears to involve an increased release of FSH.

These findings have important implications, because they indicate that there is a linear relationship between the number of eggs ovulated by female mice and the length of time spent with the males prior to mating. This suggests that, when using either number of eggs ovulated or litter size as a measure of an effect of any experimental treatment one should be cautions not to mix females mated on different days after the intraduction of the males. All experiments should be standardized, comparing only females mated after similar periods of time spent with the males prior to mating, in order to avoid the variation in number of eggs ovulated to influence the experimental results.

References

Bingel, A. S. & N. B. Schwartz (1969): Timing of LH release and ovulation in the cyclic mouse. J. Reprod. Fert. *19*, 223—229.

Bronson, F. H. (1975): Male induced precocial puberty in female mice: confirmation of the role of estrogen. Endocrinology *96*, 511—514.

Ryan, K. K. & N. B. Schwartz (1977): Grouped female mice: demonstration of pseudopregnancy. Biol. Reprod. *17*, 578.

Vandenbergh, J. G. (ed.) (1983): Pheromones and Reproduction in Mammals, Academic Press.

Whiten, W. K. (1958): Modification of the oestrus cycle of the mouse by external stimuli associated with the male. Changes in the oestrus cycle determined by vaginal smears. J. Endocr. *17*, 307.

Sammendrag

Det er velkendt, at når en hanmus anbringes i et bur med hunmus med østrussynkroni, kommer størstedelen af hunnerne i østrus tre dage senere.

Denne undersøgelse viser, at hunmus, der parres på dag 3, efter hannen er blevet introduceret, ovulerer signifikant flere æg end de hunner, der parres dag 1 og dag 2.

Dette respons syntes at involvere en øget udskillelse af follikel stimulerende hormon (FSH).

Resultaterne indicerer, at eksperimenter med drægtige mus bør standardiseres, således at kun hunner, parret efter samme antal dage tilbragt med hannen, sammenlignes. I modsat fald kan den systematiske forskel i antallet af ovulerede æg afhængig af parringsdag føre til forkert tolkning af forsøgsresultater.

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