Male-pheromone effect on the efficiency of pregnancy in female mice

Anna Marchlewska-Koj, B. Jemioło, J. Woźniacka and K. Kozłowski*

*Department of Genetics and Evolution, Institute of Zoology; *Department of Animal Ecology, Institute of Environmental Biology, Jagiellonian University, 30-060 Krakow, Poland*

Summary. Outbred female mice were exposed during 3 oestrous cycles and mated to outbred or C57BL/kw males. Although ovulation number (CL and flushed eggs) was similar in all females, there were significantly fewer ($P < 0.01$) fertilized eggs and implantations in females exposed and mated to C57BL/kw males. Exposure to outbred males and mating with C57BL/kw males led to some improvement but the best results were obtained with females mated to outbred males. The activity of uterine dehydrogenases on Day 4 of pregnancy was also greatest in females mated to outbred males. It is concluded that the pheromonal stimulation leads to hormone concentration differences which affect implantation.

Introduction

The number of young born to females depends on the number of ovulated and fertilized eggs but is also influenced by hormonal changes during pregnancy. The most critical period is at the time of implantation. In mice, maximal sensitivity to decidualizing stimuli is on the 4th day of pregnancy (Finn, 1965). The uterus is prepared for implantation by oestrogen and progesterone (Finn & Martin, 1969). The changes in the pattern of mitosis between 3rd and 4th day after copulation, and the formation of decidual tissue on the 4th day of pregnancy, are related to 3 hormones. The uterus is primed by oestrogen release during pro-oestrus and then stimulated by progesterone and oestrogen after copulation (Finn & Martin, 1970, 1972).

It is well documented that pheromones exert a great influence on hormonal mechanisms in female mice. A pheromone produced by the male was found to be responsible for accelerating puberty in females (Vandenbergh, 1967), and shortening the oestrous cycle in mature female mice (Whitten, 1956). One of the most dramatic effects of olfactory stimulants is the block to pregnancy which occurs in newly mated females after exposure to the odour of an alien male (Bruce, 1959).

The present experiments were designed to determine the influence of pheromones on the course of pregnancy in female mice.

Materials and Methods

Adult, virgin outbred females and sexually experienced males from an outbred stock and the C57BL/kw strain from our laboratory were reared in polyethylene cages, and had free access to a standard pelleted food and water. The lighting schedule was 14 h light (07:00–21:00 h)/24 h. Females were placed, 5 to a cage, with an outbred or C57BL/kw male behind a wire net (the 'stimulating' male). The oestrous cycle was determined from daily vaginal smears taken between

08:00 and 09:00 h. A smear containing numerous squamous epithelial cells was regarded as indicating the oestrous phase. At the third consecutive oestrus, some females were transferred to a clean cage and mated to an outbred or C57BL/kw male. Others were placed in a cage smeared with urine from an outbred male and mated to a C57BL/kw male. The females were checked for the presence of vaginal plugs after 4 h and the day of finding a vaginal plug was considered to be Day 1 of pregnancy.

Percentage of fertilized eggs

At 30–32 h after copulation females were killed by decapitation, and both oviducts were excised and flushed with physiological saline (9 g NaCl/l). Eggs with 2–4 blastomeres were counted under a binocular microscope. The ratio of cleaved zygotes to the total number of eggs ovulated gave the percentage of fertilized eggs.

Activity of dehydrogenases in the uterus

At 82–84 h after coitus females were killed, and the uterus was excised and weighed. The activity of dehydrogenases was estimated by a colorimetric method in 25 mg fresh tissue based on reduction of 2:3:5-triphenyltetrazolium chloride (BDH, England) to formazan (for details of the method see Marchlewska-Koj, 1973).

Number of implanted eggs

Laparotomies were performed on the 14th day of pregnancy and the numbers of implanted embryos (alive and dead) were counted. The ovaries were also examined macroscopically for the presence of corpora lutea. Large, pink, vascularized structures were considered indicative of functional corpora lutea.

Statistical analyses

Data were analysed by single-factor or two-factor analysis of variance. The influences of stimulating males and copulating males were treated as one factor for single factor analysis of variance and for Duncan's multiple range test. The arcsine transformation was used for percentage data (Zar, 1974).

Results

The length of the oestrous cycle in the outbred females was measured as the period between typical oestrous phases. The cycle (mean $\pm$ s.e.m.) was shorter in females exposed to outbred males (4.40 ± 0.3 days, $n = 68$) than in those exposed to C57BL/kw males (5.2 ± 0.3 days, $n = 75$). However, the difference was not significant.

In females reared in the light schedule used in the present experiments ovulation occurred after midnight and at the time females with oestrous smears were chosen for mating at 08:00 h the eggs would be in the ampullae. Maturation of the eggs and their release from follicles took place in the presence of the 'stimulating male'. The numbers of ovulated eggs were similar in outbred females stimulated by outbred or C57BL/kw males (Table 1). However, significant differences were found in the percentages of fertilized eggs (Table 1). More zygotes were present in the oviducts after copulation of outbred females with an outbred male than with a C57BL/kw male, regardless of the genotype of the 'stimulating male'. Ovulation numbers were unaffected (Table 2), but implantation numbers in females mated to outbred males were significantly higher

Table 1. Number of ovulated and fertilized eggs counted at 30–32 h after coitus in outbred females stimulated during the oestrous cycle and mated to outbred or C57BL/kw males

Genotype of male		No. of eggs ovulated	No. of eggs fertilized	% fertilized eggs
Stimulating	Copulating			
Outbred	Outbred	10.4 ± 0.3	10.2 ± 0.4	98.3
	C57BL/kw	9.4 ± 0.4	8.0 ± 0.4	85.9
C57BL/kw	Outbred	9.6 ± 0.5	9.4 ± 0.4	97.5
	C57BL/kw	9.6 ± 0.5	8.4 ± 0.5	86.9

Analysis of variance						
Source	d.f.	F	d.f.	F	d.f.	F
Genotype of male:						
stimulating	1	0.41	1	0.41	1	0.09
copulating	1	1.63	1	14.60*	1	11.53*
stimulating × copulating	1	1.63	1	2.21	1	0.05
Error	40		40		40	

Values are mean ± s.e.m. for 11 females/group.

* $P < 0.01$.**Table 2.** Numbers of corpora lutea and embryos counted on the 14th day of pregnancy in outbred females stimulated during the oestrous cycle and mated to outbred or to C57BL/kw males

Genotype of male		No. of corpora lutea	No. of embryos	% implantation
Stimulating	Copulating			
Outbred	Outbred	10.8 ± 0.6	10.1 ± 0.8 ^a	93.1 ^a
	C57BL/kw	10.3 ± 0.6	8.4 ± 0.9 ^{ab}	81.2 ^a
C57BL/kw	Outbred	11.2 ± 0.7	10.1 ± 0.8 ^a	90.0 ^a
	C57BL/kw	9.9 ± 0.6	7.5 ± 0.5 ^{ab}	88.9 ^{ab}
	+ outbred urine			
	C57BL/kw	11.1 ± 0.9	6.3 ± 0.8 ^b	57.6 ^b

Values are mean ± s.e.m. for 10 females/group. Means with different superscripts are significantly different, $P < 0.01$.**Table 3.** Analysis of variance for data in Table 2

Source	No. of corpora lutea		No. of embryos		% implantation	
	d.f.	F	d.f.	F	d.f.	F
Genotype of male:						
stimulating	1	0.80	1	1.74	1	4.25*
copulating	1	0.20	1	11.93**	1	11.17**
stimulating × copulating	1	0.10	1	1.74	1	0.90
Error	36		36		36	

* $P < 0.05$; ** $P < 0.01$.

than in those mated with C57BL/kw males (Table 2). In females exposed during the oestrous cycle to an outbred male and mated in clean cages, the percentage implantations was significantly higher than in females 'stimulated' by C57BL/kw males (Table 3). Since there was no interaction between the 'copulating male' and the 'stimulating male', a single factor analysis of variance was used for comparison of these results with the data when copulation took place in a cage contaminated with outbred urine (Table 2). The increased implantation percentage was not significantly greater than for females mated to C57BL/kw males in clean cages, and was not significantly lower than for females mated with outbred males.

The genotypes of the 'stimulating male' and the 'copulating male' influenced the activity of dehydrogenases in the uterus on the 4th day of pregnancy (Table 4). The lowest specific activity was observed in uteri of females stimulated by and mated to C57BL/kw males. The outbred male genotype for stimulation or copulation significantly increased dehydrogenase activity in the uterus. However, in females stimulated by C57BL/kw males and mated to C57BL/kw males in the presence of outbred urine the amount of formazan was not significantly higher than after copulation with males of the same genotype in a clean cage and not significantly lower than after copulation with an outbred male.

Table 4. Formazan content of the uterus at 82–84 h after coitus in outbred females stimulated during the oestrous cycle and mated to outbred or to C57BL/kw males

Genotype of male		Wt of female (g)	Wt of uterus (mg)	Formazan content	
Stimulating	Copulating			µg/100 mg tissue	µg/uterus
Outbred	Outbred	24.7 ± 0.8	101.4 ± 6.6	64.4 ± 1.3 ^a	65.0 ± 3.8 ^a
	C57BL/kw	24.3 ± 0.5	90.5 ± 4.6	65.0 ± 1.7 ^a	58.7 ± 2.9 ^a
C57BL/kw	Outbred	24.4 ± 0.6	95.9 ± 4.9	67.9 ± 3.0 ^a	64.8 ± 3.1 ^a
	C57BL/kw + outbred urine	24.9 ± 0.4	106.3 ± 7.4	52.6 ± 3.6 ^{ab}	54.2 ± 4.9 ^{ab}
	C57BL/kw	24.1 ± 0.5	99.7 ± 9.5	44.3 ± 2.1 ^b	46.0 ± 3.9 ^b

Values are mean ± s.e.m. for 7 females/group. Means with the same superscript are significantly different, $P < 0.05$.

Discussion

During preliminary experiments we observed that outbred females reared with and mated to outbred males gave birth to significantly more young (10.9 ± 1.3) than did females from the same stock but in cages with C57BL/kw males (6.1 ± 2.5). The results presented here partly explain this phenomenon. Mating to C57BL/kw males was less effective in that fewer eggs were fertilized than in females copulating with outbred males. This may be related to the fact that a very high percentage of abnormal spermatozoa was found in the semen of C57BL/kw males (Krzanowska, 1976).

Males from the C57BL/kw strain used in the present experiments are known to produce weaker pheromones than outbred males; they are unable to block pregnancy in recently mated females (Marchlewska-Koj, 1977) and cannot induce ovulation in immature mice (Zarrow, Eleftheriou & Denenberg, 1973).

The most critical period for the development of zygotes occurs at implantation (see 'Introduction'). By direct measurement of serum hormones Bronson (1975) found that male pheromones significantly increased LH concentrations in mice and that this was followed by a 15- to 20-fold increase of circulating oestrogen levels. The lowest number of embryos was found in females stimulated during the oestrous cycle by C57BL/kw males and mated to C57BL/kw

males (Table 2). In females which had contact with outbred males, or with urine of outbred males, implantation numbers were greater, presumably because the stronger pheromones of outbred males influenced hormone release at the time of implantation since the numbers of ovulations were similar in all the females. Bronson & Desjardins (1974) observed that immature female mice exposed to a male had markedly increased levels of gonadotrophins and steroids in the blood, and uterine weight was increased although ovulation had not occurred.

Oestrogen treatment enhances the responsiveness of a target tissue to progesterone (O'Malley, McGuire & Korenman, 1967) and the oestrogen released during pro-oestrus can affect the amount of progesterone-binding receptors in the mouse uterus during pregnancy (Feil, Glasser, Toft & O'Malley, 1972). A direct effect of hormonal changes is the significant increase of uterine dehydrogenase activity on the 4th day of pregnancy (Schultze, 1964; Marchlewska-Koj, 1973). The data in Table 4 indicate that the 'stimulating males' have an influence on uterine enzyme activity in pregnant mice. The lowest values were again found for females stimulated by and mated to C57BL/kw males, while the presence of outbred male urine during copulation with a C57BL/kw male led to increased, albeit not significant dehydrogenase activity.

The influence of the male mouse on events in early pregnancy thus appears to operate by pheromonal stimulation of the brain-pituitary-ovary-uterus axis.

References

- Bronson, F.H. (1975) Male-induced precocial puberty in female mice: confirmation of the role of oestrogen. *Endocrinology* **96**, 511–514.
- Bronson, F.H. & Desjardins, C. (1974) Circulating concentration of FSH, LH, estradiol, and progesterone associated with acute, male-induced puberty in female mice. *Endocrinology* **94**, 1658–1668.
- Bruce, H.M. (1959) An exteroceptive block to pregnancy in the mouse. *Nature, Lond.* **184**, 105.
- Feil, P.D., Glasser, S.R., Toft, D.O. & O'Malley, B.W. (1972) Progesterone binding in the mouse and rat uterus. *Endocrinology* **91**, 738–746.
- Finn, C.A. (1965) Oestrogen and the decidual cell reaction of implantation in mice. *J. Endocr.* **32**, 223–229.
- Finn, C.A. & Martin, L. (1969) Hormone secretion during early pregnancy in the mouse. *J. Endocr.* **45**, 57–65.
- Finn, C.A. & Martin, L. (1970) The role of the oestrogen secreted before oestrus in the preparation of the uterus for implantation in the mouse. *J. Endocr.* **47**, 431–438.
- Finn, C.A. & Martin, L. (1972) Endocrine control of the timing of endometrial sensitivity to a decidual stimulus. *Biol. Reprod.* **7**, 82–86.
- Krzanowska, H. (1976) Inheritance of sperm head abnormality types in mice: the role of the Y chromosome. *Genet. Res.* **28**, 189–198.
- Marchlewska-Koj, A. (1973) The effect of male on the activity of dehydrogenases in the mouse uterus. *Bull. Acad. pol. Sci. Cl. II. Sér. Sci. biol.* **21**, 449–453.
- Marchlewska-Koj, A. (1977) Pregnancy block elicited by urinary proteins of male mice. *Biol. Reprod.* **17**, 729–732.
- O'Malley, B.W., McGuire, W.L. & Korenman, S.G. (1967) Estrogen stimulation of synthesis of specific proteins and RNA polymerase activity in the immature chick oviduct. *Biochim. Biophys. Acta* **145**, 204–207.
- Schultze, A.B. (1964) Triphenyltetrazolium reduction by uterine tissue of rats. *Proc. Soc. exp. Biol. Med.* **116**, 653–655.
- Vandenbergh, J.G. (1967) Effect of the presence of a male on the sexual maturation of female mice. *Endocrinology* **81**, 345–349.
- Whitten, W.K. (1956) Modification of the oestrous cycle of the mouse by external stimuli associated with the male. *J. Endocr.* **13**, 399–405.
- Zar, J.H. (1974) *Biostatistical Analysis*, pp. 190–197. Prentice-Hall, Inc., New York.
- Zarrow, M.X., Eleftheriou, B.E. & Denenberg, V.H. (1973) Sex and strain involvement in pheromonal facilitation of gonadotrophin-induced ovulation in the mouse. *J. Reprod. Fert.* **35**, 81–87.

Received 29 May 1979