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## The Role of Olfaction in Mediation of Pregnancy Block in White-Footed Mice, *Peromyscus leucopus noveboracencis*

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THE ROLE OF OLFACTION IN MEDIATION OF PREGNANCY  
BLOCK IN WHITE-FOOTED MICE, PEROMYSCUS  
LEUCOPUS NOVEBORACENSIS

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A Thesis

Presented to

The Faculty of the Department of Biology  
The College of William and Mary in Virginia

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In Partial Fulfillment

Of the Requirements for the Degree of  
Master of Arts

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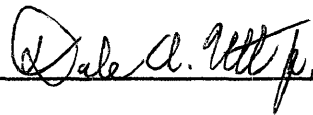
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Dale Alan Utt, Jr.

1984

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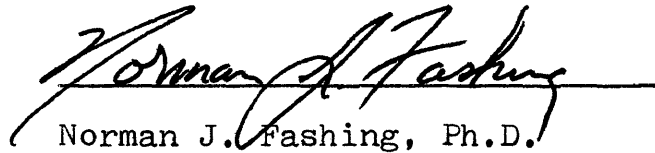


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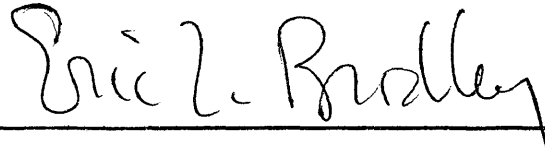
Approved, August 1984



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## ABSTRACT

Disruption of pregnancy in recently inseminated female mice is known as pregnancy block. Recent evidence indicates that both the presence and odor of a stranger male may cause pregnancy block in females. The purpose of this study was to determine if pregnancy block occurs in Peromyscus leucopus noveboracensis, and if so, identify the mechanisms by which a stranger male causes pregnancy failure.

Recently inseminated P. leucopus females were placed in contact with a stud and/or stranger male. Females caged with a stranger male were found to have fewer pregnancies than those caged with a stud alone. Females were also exposed to a stud and/or stranger male on the other side of a wire barrier. Pregnancy rates were lower in females exposed to strange males than females exposed to the stud only.

Pregnancy rates did not significantly differ in females exposed to bedding soiled by a stud and/or stranger male. Exposure to stud and/or stranger male urine applied to the female's cage also showed no pregnancy blocking effect.

Females subjected to a vaginal smearing technique for determination of insemination showed no difference in pregnancy rate from undisturbed or handled females.

Pregnancy rates appear to be reduced in P. leucopus by the presence of a stranger male, though not significantly. The importance of olfactory cues as the causative mechanism of pregnancy failure in this species is doubtful.

THE ROLE OF OLFACTION IN MEDIATION OF PREGNANCY  
BLOCK IN WHITE-FOOTED MICE, PEROMYSCUS  
LEUCOPUS NOVEBORACENSIS

## INTRODUCTION

Bruce (1959, 1960) first demonstrated that pregnancy can be disrupted in a recently inseminated house mouse, Mus musculus, if it is exposed to a male other than the one with which it was mated. This blocking of pregnancy in the female following exposure to a stranger male has become known as the "Bruce effect". Since Bruce's first experiments, pregnancy block has been shown to also occur in a number of other species: prairie deer mouse, Peromyscus maniculatus bairdi (Eleftheriou, Bronson, & Zarrow, 1962; Bronson & Eleftheriou, 1963; Bronson, Eleftheriou, & Garick, 1964; Terman, 1969), field vole, Microtus agrestis (Clulow & Clark, 1968), meadow vole, Microtus pennsylvanicus, (Clulow & Langford, 1971; Watson, Clulow, & Mariotti, 1983), prairie vole, Microtus ochrogaster, (Stehn & Richmond, 1975), and the red-backed vole, Clethrionomys gapperi, (Clulow, Franchetto, and Langford, 1982); as well as wild house mice, Mus musculus (Chipman & Fox, 1966a).

Early evidence suggested that pregnancy block might be caused by the odor of the stranger male, and subsequent experiments indicated that olfactory stimuli received by the female were triggering mechanisms preventing implantation.



Postimplantation pregnancy disruption has also been observed in some species (Kenney, Evans, and Dewsbury, 1977). Females made anosmic by surgical removal of the olfactory lobes no longer have their pregnancies blocked by the presence of a stranger male (Bruce & Parrott, 1960). In addition, recently inseminated females housed in cages containing shavings soiled by a stranger male also have a high rate of pregnancy failure (Parkes & Bruce, 1962; Watson, Clulow, and Mariotti, 1983). Urine from a stranger male was identified as the pheromonal source for olfactory stimuli affecting female pregnancy in house mice (Dominic, 1964, 1966 ). Prior to the present study nothing was known about pregnancy blocking abilities in the white-footed mouse, Peromyscus leucopus noveboracensis.

The purpose of the following study was:

- 1) to determine if pregnancy block occurs in the white-footed mouse,
- 2) to identify the mechanism by which a stranger male is able to cause pregnancy failure in a female.

#### EXPERIMENTAL PROCEDURES

Animals in all of the following experiments were white-footed mice whose ancestors were field caught and the progeny maintained in the Laboratory of Endocrinology and Population Ecology at the College of William and Mary for approximately four years. Wild-trapped animals were added to the colony at intervals to prevent inbreeding and

sibling matings were not permitted. All young were weaned at 21 days after birth and placed with siblings of the same sex in double chambered opaque plastic laboratory cages measuring 28cm X 12.5cm X 15cm. The cages were covered by a wire mesh top. Each cage contained wood shavings for bedding, and water and food were supplied ad libitum. A light cycle of 14L/10D (0700h-2100h) and an approximate temperature of 24°C were maintained in both the colony and experimental rooms.

### Experiment I (Initial Smearing)

The purpose of the initial experiment was to expose recently inseminated females to direct physical contact with a stud male and/or a stranger male. The rates of pregnancy in each group could then be used to determine if pregnancy block does occur in females exposed to a stranger male shortly after insemination.

### MATERIALS AND METHODS

Animals used in this experiment ranged in age from 43 to 158 days. Pairing of females with males was random with special attention to avoid sibling matings, and was done between 1600h and 2000h. Pairs were placed in similar cages as described above, one pair to a chamber, and the cages moved to a different experimental room. Approximately 24 hours later, between 1730h and 2030h, vaginal smears were taken of all females with open vaginae. The smearing technique consisted of rinsing of the vagina with a small amount of deionized water, and the water sample placed

on a microscope slide. A criterion of 20 sperm per 100X magnification field was used for determination of insemination (Terman, 1969). Females were smeared for five days or until determined to have been inseminated. After smearing on day five, all males of non-inseminated pairs were removed from the female. Females that revealed no insemination by smearing remained alone in their cages for twenty-five days to check if any successful inseminations had been missed by the smearing technique.

Twenty-four hours after a female was determined to have been inseminated, she was randomly placed into one of four treatments:

- A) Stud removed and female remained alone.
- B) Stud remained with female.
- C) Stud remained and stranger male added.
- D) Stud removed, stranger male added.

Animals were maintained in the experimental treatments for 48 hours, after which all males were removed. Females remained alone in the cages and the nests were checked for young beginning 20 days after insemination. All females were sacrificed between 25 to 30 days after insemination and examined for evidence of pregnancy. Embryos and uterine scars were noted to determine if infanticide by the mother or premature abortion of the litter had occurred. The weight of each female and perforation of the vagina were also noted.

## RESULTS

Only 50% of the females, 36 of 72, were found by the smear technique to have been successfully inseminated within five days of pairing with a stud male (Table 1). Of the 36 inseminated females, 31 were inseminated within the first 72 hours following pairing (86%). A total of 18 females were placed into treatments with no exposure to a stranger male (A and B). Only one pregnancy was recorded in each treatment (Table 2). Females in Treatment C were in contact with both the stud and stranger male simultaneously, and only one pregnancy occurred out of seven females. No pregnancies were observed in 11 females who had contact with a stranger male without the stud male present.

Two of the stranger males in Treatment C were killed in fighting between 24 and 48 hours after introduction into the cage containing the stud male and a female. Two females became pregnant which were not shown to be inseminated by the vaginal smear technique. No embryos or uterine scars were found in any females that failed to produce a litter.

## DISCUSSION

Thirty-one of 36 inseminations occurred within 72 hours of pairing. These data are consistent with a previous study on P. leucopus which under the same conditions of pairing showed 89%, 25 of 28, of inseminations during a five day period to occur within 72 hours of pairing (Wolfe, 1978 unpublished). The high frequency of inseminations within 72 hours of pairing in both studies is characteristic

TABLE 1  
 Number of days after pairing in which  
 insemination occurs.

	N	Days after pairing					Total Inseminations
		1	2	3	4	5	
Experiment I	72	13	8	10	2	3	36
Experiment VI	20	2	1	2	1	3	9

Experiment I - 31/36, (86%), inseminations occur by day 3.

Experiment VI - 5/9, (55%), inseminations occur by day 3.

TABLE 2

Experiment I. - Pregnancy rate for females treated  
following inseminations noted  
by vaginal smearing.

Treatment*	N	Pregnant	
		#	%
A-Female alone.	9	1	11
B-Female + stud male.	9	1	11
C-Female + stud male + stranger male.	7	1	14
D-Female + stranger male.	11	0	0

\*24-72 hours post insemination.

of a "Whitten effect". Whitten (1956) has shown that the estrus cycle of the female can be accelerated by the introduction of a male. The presence of the male can cause disruption of the normal estrus cycle and bring the female into a receptive condition. The disruption and acceleration can result in the estrus synchronization seen within 72 hours of pairing in both P. leucopus studies.

Results from the initial smearing experiment indicated that factors other than the presence of a stranger male may be causing pregnancy to be blocked in all treatments. Females which remained with the stud or had the stud removed and remained alone exhibited the same low pregnancy rate as females exposed to a stranger male. Reproduction of parous females in the P. leucopus colony at the time of the experiment was over 60%. Reproduction in the colony can not be compared directly with the experimental data because females in the colony had varying degrees of reproductive experience; whereas experimental females were virgins when paired and allowed contact with a stud for only five days. The colony pregnancy rate does indicate young being produced during the experimental time period at rates higher than the one pregnancy out of nine females (11%) observed in the controls. The cause of the low pregnancy rates through all treatments is unclear.

#### Experiment II (Male Contact)

Because of the low pregnancy rates found in all treatments of the first experiment, it was necessary to design a procedure which tested the effect of the presence

of a male on female pregnancy, but reduced the amount of handling. Trauma caused by handling of the animal in the smearing technique was believed to be blocking pregnancy in all treatments before the influence of the male could be tested. To circumvent the difficulties encountered in the smearing procedure, an experiment was done based on the "Whitten effect" previously described.

#### MATERIALS AND METHODS

Pairing of mice and establishment of environmental conditions were carried out in the same way as for the preceding experiments, however animals used were between 90 and 230 days old. Insemination was assumed to occur in 43% of the pairs by day three following pairing, based on the high frequency of inseminations observed within the first 72 hours after pairing in the first smearing experiment (86%, Table 1). Therefore experimental treatments began 72 hours after pairing. Each female remained in her home cage and was randomly chosen to be placed into one of three treatments. Treatments here are labeled B, C, and D for their similarity to corresponding treatments in the first smearing experiment.

- B) Stud remains with female.
- C) Stud remains and stranger male added.
- D) Stud removed, stranger male added.

All males were removed 48 hours after initiation of the experimental treatments. Females then remained alone in the home cage and the cages were checked for young



beginning 20 days after initial pairing. Twenty-five to 30 days following pairing, all females were killed and the uteri examined for scars and embryos.

### RESULTS

Frequency of pregnancy was found to be dependent on male contact conditions (P<sub>0.05</sub> Table 3). Females permitted direct physical contact with a stranger male had fewer pregnancies than females which only had contact with the stud male (Table 4). Fifty percent of the females who remained in contact with only the stud male produced litters. In both Treatment C and Treatment D, in the presence of a stranger male, pregnancy rates were low. Even with the combined influence of the stud and a stranger male in Treatment C, the pregnancy rate was lower than that found in Treatment B. Five stranger males and one stud male died from wounds received in fighting during the 48 hours of cohabitation in Treatment C. No females received any visible injury. No embryos or uterine scars were found in females that failed to produce a litter.

### DISCUSSION

The pregnancy rate of female Peromyscus leucopus is reduced, though not significantly, when a stranger male is permitted physical contact with her between 72 and 120 hours after pairing with her stud male. The pregnancy rate of females in the presence of the stud male at the time of exposure to a stranger male is approximately the same as

TABLE 3

Experiment II. - Frequencies of pregnancy in females  
allowed physical contact with males.

Treatment	Pregnant	Not pregnant	Totals
B-Stud remains with female.	10 (5.7) ((3.31))	10 (14.3) ((1.31))	20
C-Stud remains and stranger male added.	3 (5.7) ((1.25))	17 (14.3) ((0.50))	20
D-Stud removed, stranger male added.	4 (5.7) ((0.49))	16 (14.3) ((0.19))	20
Totals	17	43	60

Total chi-square = 7.06\*

\*Significant at  $P \leq .05$  using R X C test of independence.

() indicate expected frequencies.

(( )) indicate individual contribution toward total chi-square.

TABLE 4  
 Experiment II. - Effect of male contact  
 on pregnancy.

Treatment	N	Pregnant		Comparisons	$\chi^2$
		#	%		
B-Stud remains with female.	20	10	50	B vs. C	4.1025
C-Stud remains and stranger male added.	20	3	15	B vs. D	2.7472
D-Stud removed, stranger male added.	20	4	20	C vs. D B vs. (CD)	0.0000 5.4274

All comparisons NS using a posteriori chi-square test of independence.

females that were exposed to the stranger male alone. These results differ from Terman's (1969) work with Peromyscus maniculatus bairdi which demonstrated that the presence of the stud with the female when she is exposed to the stranger male is able to reduce the pregnancy blocking effect.

### Experiment III (No Contact-Wire Cage)

The third experiment was designed to isolate the female, but allow visual, olfactory, and auditory stimuli to be received from the males through a wire barrier. This barrier prevented direct physical contact between the female and the stud male or stranger male during the 48 hour experimental exposure period.

### MATERIALS AND METHODS

Pairs of mice were established randomly as in the previous experiments and were between 90 and 120 days old. Both the stud and the female were placed on the same side of a wire mesh divider which split a galvanized steel cage 24cm X 24cm X 14cm into two equal areas. Wire mesh also covered the bottom and top of the cage. Each cage was placed on a stainless steel tray covered with wood shavings. Light, food, water, and temperature were regulated as in the previous experiments. After 72 hours each pair was placed into one of four treatments:

- A) Stud and female remained undisturbed on the same side of the divider, the other side was left empty.

- B) Stud placed on other side of divider, female remained on original side.
- C) Stud and stranger placed on other side of divider, female remained on original side of divider.
- D) Stud removed, stranger placed on other side of divider, female remained on original side.

All males were removed 48 hours after initiation of the experimental treatments. Females remained on the original side of the divider and were checked for young beginning 20 days after pairing. All females were sacrificed and examined for embryos and uterine scars.

#### RESULTS

Frequency of pregnancy was found to be related to male exposure conditions in each treatment ( $P \leq .025$  Table 5). Females which remained undisturbed with the stud male on the same side of the divider showed a pregnancy rate of 30%. Females which had the stud male placed on the other side of the wire mesh divider after 72 hours produced only three litters out of 20 females tested. This was only half the rate of success of the undisturbed pairs. When the female was exposed to a stranger male, but separated by the divider, pregnancy appeared to be inhibited. There were no litters produced in 20 females when the female was exposed to the stranger male alone. Even with the presence of both the stud and a stranger male, only one female produced a litter. There was no significant difference between any of the

TABLE 5

Experiment III. - Frequencies of pregnancy in females  
exposed to males on opposite side of wire  
barrier from female.

Treatment	Pregnant	Not pregnant	Totals
A-Stud and female on same side.	6 (2.5) ((4.90))	14 (17.5) ((0.70))	20
B-Stud placed on opposite side.	3 (2.5) ((0.10))	17 (17.5) ((0.01))	20
C-Stud and stranger placed on opposite side from female.	1 (2.5) ((0.90))	19 (17.5) ((0.13))	20
D-Stud removed, stranger placed on opposite side.	0 (2.5) ((2.50))	20 (17.5) ((0.36))	20
Totals	10	70	80

Total chi-square = 9.60\*\*

\*\*Significant at  $P \leq .025$  using R X C test of independence.

() indicate expected frequencies.

(( )) indicate individual contribution toward total chi-square.

TABLE 6

Experiment II. - Pregnancy success of females  
exposed to males on opposite side of wire  
barrier from female.

Treatment	N	Pregnant		Comparisons	$\chi^2$
		#	%		
A-Stud and female on same side.	20	6	30	A vs. B	0.5734
B-Stud placed on opposite side.	20	3	15	A vs. C A vs. D	2.7705 2.7705
C-Stud and stranger placed on opposite side from female.	20	1	5	B vs. C B vs. D	0.2775 0.2775
D-Stud removed, stranger placed on opposite side.	20	0	0	C vs. D (AB)vs.(CD)	0.5263 5.6000

All comparisons NS using a posteriori chi-square test of independence.

individual treatments (Table 5), but the data did indicate a trend which suggested a pregnancy blocking effect caused by exposure to a stranger male. Females which were exposed to a stranger male (C & D) had fewer pregnancies than females exposed to a stud only (A & B), though the difference was not found to be significant (Table 6). The data were similar to the pregnancy rates of the previous experiment in which direct physical contact was allowed, but the pregnancy rates of undisturbed females and females exposed only to the stud male were below what was seen under conditions allowing physical contact. No embryos or uterine scars were found in females that failed to produce a litter.

#### DISCUSSION

Results of the wire cage treatments (Table 5) suggest that the stranger male may cause pregnancy block when physical contact with the female is prevented by a wire mesh barrier. Olfactory, visual, and auditory cues remain as possible means by which the pregnancy block response is elicited in the female. Previous studies with other species have indicated olfaction as a primary means of receiving stimuli which result in pregnancy block (Watson, Clulow, & Mariotti, 1983).

#### Experiment IV (Soiled Shavings)

Work on house mice (Parkes & Bruce, 1962; Chipman & Fox, 1966b) and the meadow vole (Watson, Clulow, and Mariotti, 1983) has shown that shavings soiled by a stranger male are able to induce pregnancy block in recently inseminated females. This experiment was designed to test the hypothesis



that olfactory stimuli found in shavings soiled by a stranger male are able to induce pregnancy block in recently inseminated P. leucopus.

#### MATERIALS AND METHODS

Pairs were randomly established and placed in the standard double chambered opaque plastic cages used for the colony. One pair was placed in each chamber. The mice ranged in age from 100 to 135 days old. The volume of shavings contained in each cage was measured and equal amounts were added to each cage, approximately one pint by volume per cage. Light, temperature, food, and water were all maintained in accordance with previous experimental conditions.

In addition to the pairs, a number of males were placed singly in the same type of cages, one male to a chamber. Each cage contained the same volume of shavings as was placed with the pairs.

After 72 hours all males were removed from the home cage. The females were also removed and placed into new cages under one of three experimental treatments:

- B) Female placed in new cage with original soiled shavings.
- C) Female placed in new cage with original soiled shavings and shavings of a stranger male.
- D) Female placed in new cage with soiled shavings of a stranger male.

Females were checked daily for young beginning 20 days after pairing. Twenty-five days after pairing the mice were killed and examined for embryos and uterine scars.

### RESULTS

Females exposed to the soiled shavings of males had low pregnancy rates irrespective of the treatment (Table 7). The highest rate of pregnancy was found in females which were exposed to shavings soiled by the stranger male only. Thirty-two percent of the females in this treatment produced litters (Table 8). Females exposed to a combination of shavings soiled by the stud male and stranger male showed a pregnancy rate of only 24%. The fewest litters were produced by females that were placed in new cages containing soiled shavings from their original cage. None of the treatment results were determined to be significant. No embryos or uterine scars were found in females that had failed to produce a litter.

### DISCUSSION

The effects of soiled shavings on pregnancy as seen in this experiment remain unclear. Experiments by Parkes & Bruce (1962) and by Dominic (1966) using soiled shavings to induce pregnancy block in house mice strongly indicate that the freshness of the male urine deposited in the shavings can influence the rate of pregnancy block in females. Fresh urine and shavings soiled frequently by stranger males cause higher rates of pregnancy block than if shavings are treated with urine only once (Parkes &

TABLE 7

Experiment IV. - Frequencies of pregnancy in females  
exposed to shavings soiled by males.

Treatment	Pregnant	Not pregnant	Totals
B-Original soiled shavings.	5 (6.3) ((0.28))	20 (18.7) ((0.10))	25
C-Original shavings + shavings of stranger male.	6 (6.3) ((0.02))	19 (18.7) ((0.01))	25
D-Shavings of stranger male.	8 (6.3) <u>((0.44))</u>	17 (18.7) <u>((0.15))</u>	25
Totals	19	56	75

Total chi-square = 0.99 NS

( ) indicate expected frequencies.

(( )) indicate individual contribution toward total chi-square.

TABLE 8  
 Experiment IV. - Effect of soiled shavings  
 on pregnancy.

Treatment	N	Pregnant		Comparisons	$\chi^2$
		#	%		
B-Original soiled shavings.	25	5	20	B vs. C	0.0000
C-Original shavings + shavings of stranger male.	25	6	24	B vs. D	0.4158
D-Shavings of stranger male.	25	8	32	C vs. D	0.9920

All comparisons NS using a posteriori chi-square test of independence.

Bruce, 1962). The volatile nature of the pregnancy blocking pheromone in the urine appears to decrease its potency over time (Dominic, 1966). The inconclusive results of the previous experiment may have been caused by a weakening of the pregnancy blocking pheromone in the soiled shavings, or the total absence of a pregnancy blocking pheromone in P. leucopus urine.

#### Experiment V (Urine)

Dominic's (1964, 1966) work with house mice has identified stranger male urine as the agent responsible for pregnancy block in females. In spite of ambiguous results using soiled shavings in Experiment IV, the following procedure tested the effects of urine collected from stud and stranger males on pregnancy in female white-footed mice.

#### MATERIALS AND METHODS

Pairs were randomly established and placed in the standard plastic cages, one pair in each chamber. Animals ranged in age from 110 to 170 days. After 72 hours the males of each pair were placed in a urine collecting device. Individuals were placed in a funnel apparatus consisting of a 200 ml glass jar with a screen top inverted within a polypropylene funnel through which urine was collected in a 12 X 75 mm plastic tube. Urine was collected for 15 hours (1730h-0830h) overnight and used for treatment the next morning. Application of urine was by three strokes of a #2 artist's brush, freshly dipped in the appropriate urine

or distilled water, to the inside wall of the cage 2 cm above the level of the shavings. Separate brushes were used for the urine of each animal. Cages were painted between 0900h and 1000h on days four and five following pairing. Treatments were as follows:

- A) Water only.
- B) Stud urine.
- C) Stud and stranger urine.
- D) Stranger urine.

Males were killed following urine collection and the urine was refrigerated between treatments. Stud urine from group B was used as stranger urine for group C, and stud urine in group C was used as stranger urine in group D. Urine from group A males was used as stranger urine when group B or group C males only had enough urine for their own stud treatment.

Females were checked for litters beginning 20 days after initial pairing. All females were killed and examined for embryos and uterine scars between 23 and 27 days following the last possible day of insemination.

## RESULTS

Differences between treatments were not found to be significant (Table 9). Pregnancy rates for all treatments ranged from 16% for females exposed to stranger male urine, to 25% for females exposed to stud male urine (Table 10). Females exposed to water as a control produced four litters out of 19 animals tested. This rate of pregnancy

TABLE 9  
 Experiment V. - Frequencies of pregnancy  
 in females exposed to male urine.

Treatment	Pregnant	Not pregnant	Totals
A-Water only.	4 (3.9) ((0.00))	15 (15.1) ((0.00))	19
B-Stud urine.	5 (4.1) ((0.20))	15 (15.9) ((0.05))	20
C-Stud and stranger urine.	4 (4.1) ((0.00))	16 (15.9) ((0.00))	20
D-Stranger urine.	3 (3.9) ((0.21))	16 (15.1) ((0.05))	19
Totals	16	62	78

Total chi-square = 0.51 NS

( ) indicate expected frequencies.

(( )) indicate individual contribution toward total chi-square.

TABLE 10  
 Experiment V. - Effect of male urine  
 on pregnancy.

Treatment	N	Pregnant		Comparisons	$\chi^2$
		#	%		
A-Water only.	19	4	21	A vs. B	0.0076
B-Stud urine.	20	5	25	A vs. C	0.0994
C-Stud and stranger urine.	20	4	20	B vs. D	0.0994
D-Stranger urine.	19	3	16	C vs. D	0.0056

All comparisons NS using a posteriori chi-square test of independence.



fell below both the rate for females exposed to stud urine alone and females exposed to a combination of stud and stranger male urine. One female escaped from Treatment A and as a result only 19 animals were able to be tested as a control. One female in Treatment D received an incorrect urine treatment and was eliminated from the data. No embryos or uterine scars were found in any females that failed to produce a litter.

#### DISCUSSION

The exposure of recently inseminated females to male urine does not appear to significantly alter pregnancy rates from those found in females exposed to water only (Table 9). Contrary to findings in house mice (Dominic, 1966) there is no evidence in this study that urine is a major factor in the mediation of pregnancy block in Peromyscus leucopus. Pregnancy rates of females treated with the urine of a stranger male do not significantly differ from those of females treated with water or stud urine only.

#### Experiment VI (Effects of Smearing)

The final experiment was designed to observe the effects of handling and the vaginal smearing technique on the pregnancy rate of female white-footed mice.

#### MATERIALS AND METHODS

Animals in this experiment were between 57 and 120 days old. Pairs were randomly established from the colony, with sibling matings avoided. Pairs were placed into the same

type of plastic cages as were used in the previous experiments. The cages were then moved to a different room. Light, food, water, and shavings were all supplied as per previously stated conditions.

Pairs were assigned randomly to one of three treatments:

- A) No handling, no smear - Male and female remained together undisturbed for five days.
- B) Handling only - Male and female remained together for five days. Each day between 1730h and 2030h the female was removed from the cage and placed in position for smearing for five seconds. Without having actually performed a smear, the female was placed back into the cage.
- C) Smearing - Male and female remained together for five days. Each day between 1730h and 2030h the female was removed and a vaginal smear taken. The smearing technique and criterion established for successful insemination were as previously described for Experiment I. The female was then placed back into the cage.

For each female shown to be inseminated by the vaginal smear in Treatment C, a female from Treatment B was no longer handled. Inseminated females in Treatment C were no longer smeared. Five days after pairing males were removed from all treatments. At 19 days after pairing all females were killed and examined for embryos and uterine scars.

## RESULTS

The effects of smearing and handling on pregnancy are shown in Table 11. Nine females were inseminated through observation of vaginal smears in Treatment C. Only four of these nine females produced litters. Five of the nine inseminations occurred within 72 hours of pairing (Table 1).

Twenty-five percent of the females handled but not smeared produced young (Table 12). This result was identical to that found for females which had remained undisturbed for five days. Of the five females in Treatment B which produced litters, only two were animals which were handled for less than the full five days of treatment.

## DISCUSSION

There was no significant difference in pregnancy rate between animals which were handled or smeared and those animals which were left undisturbed (Table 12). There appears to be no evidence that smearing as performed in this experiment, significantly influenced the pregnancy rate. Overall pregnancy rates in all treatments were below the 50% expected based on the insemination data of experiments I and VI. The majority of inseminations in the smearing treatment did again occur within the first 72 hours following pairing, as in the previous experiments. The cause of the difference between pregnancy rates of females smeared in Experiment I and those smeared in Experiment VI remains unclear. Differences in the degree of proficiency in the smearing technique between the two experiments, or seasonal effects may have influenced the pregnancy rates of both groups.

TABLE 11

Experiment VI. - Frequencies of pregnancy in females  
following smearing or handling.

Treatment	Pregnant	Not pregnant	Totals
A-No handling, no smear.	5 (4.7) ((0.02))	15 (15.3) ((0.01))	20
B-Handling only.	5 (4.7) ((0.02))	15 (15.3) ((0.01))	20
C-Vaginal smear.	4 (4.7) ((0.10))	16 (15.3) ((0.03))	20
Totals	14	46	60

Total chi-square = 0.19 NS

( ) indicate expected frequencies.

(( )) indicate individual contribution toward total chi-square.

TABLE 12

Experiment VI. - Pregnancy rate of females following smearing or handling.

Treatment	N	Pregnant		Comparisons	$\chi^2$
		#	%		
A-No handling, no smear.	20	5	25	A vs. B	0.0000
B-Handling only.	20	5	25	A vs. C	0.0000
C-Vaginal smear.	20	4	20	B vs. C	0.0000

All comparisons NS using a posteriori chi-square test of independence.

## CONCLUSIONS

Female P. leucopus placed in contact with a stranger male have been shown to have lower pregnancy rates than females which remained with the stud male only, though the difference is not significant. (Table 4). The presence of the stud with the female during her exposure to a stranger male did not increase her pregnancy rate above that found in females housed with a stranger male alone. Previous work with other species, under similar conditions allowing physical contact and interaction between the female and a stranger male, has shown the occurrence of pregnancy block (Bruce, 1959; Bronson & Eleftheriou, 1963; Chipman & Fox, 1966a; Clulow and Clarke, 1968; Clulow and Langford, 1971; Stehn and Richmond, 1975).

Attempts to isolate the mechanisms by which the blocking stimuli are transmitted have focused on olfactory communication. Female house mice made anosmic by destruction of the vomeronasal organ (Bellringer, Pratt, and Keverne, 1980) or removal of the olfactory bulbs (Bruce & Parrott, 1960) are no longer susceptible to the pregnancy blocking effect of a stranger male. The odor of a stranger male or his soiled bedding was also shown to block pregnancy in the house mouse (Bruce, 1960; Parkes & Bruce, 1962), the deer mouse (Bronson & Eleftheriou, 1963), and the meadow vole (Watson, Clulow, and Mariotti, 1983). Finally, female house mice exposed to urine collected from unfamiliar males show a significantly higher number of blocked pregnancies than females treated with water

(Dominic, 1964, 1966). Pregnancy block in mice caused by application of stranger male urine has so far only been demonstrated in house mice. The results of Watson et. al. (1983) support the role of olfaction in pregnancy block of Microtus pennsylvanicus, but urine has not been isolated as the causative agent in this species.

The data of Experiment II at least suggest the occurrence of pregnancy block in the white-footed mouse. The second objective of the present study was to determine the mechanism by which the stranger male may cause pregnancy failure in the female. Olfactory cues appeared most effective in causing pregnancy block in other species and it was believed that these cues may also be important in P. leucopus. The first attempt to isolate the females from any physical interaction with males after insemination took place in the wire cages. Animals were separated by the wire barrier, but visual, auditory, and olfactory communication were still possible across the barrier. Pregnancy rates in all treatments of this experiment were found to be low in comparison to corresponding treatments of animals allowed physical contact. Though none of the individual treatments were shown to be significantly different, females which had been exposed to a stranger male (C & D) had fewer pregnancies than females exposed to a stud only (A & B, Table 6). The stranger male may have influenced pregnancy across the wire barrier, but his effectiveness appeared to be reduced. In Microtus pennsylvanicus the high incidence of pregnancy block found in females allowed contact with

a stranger male is not significantly reduced when the stranger is separated by a metal barrier allowing air flow between chambers (Watson, Clulow, and Mariotti, 1983).

The overall low pregnancy rates in all treatments was a matter of concern. The low pregnancy rates found even when the stud and female remained undisturbed on the same side of the barrier indicated that some factor other than exposure to a male could be disrupting pregnancy in all treatments. A change in physical environment such as cage size has been shown to cause an increase in pregnancy block in the deer mouse (Eleftheriou, Bronson, and Zarrow, 1962). The size of the plastic cages in which the animals were housed in the colony is approximately that of the chamber size allowed in the wire cage experiment, but the environment of the wire cage was slightly different. Wire mesh formed the bottom and the cage was placed on a tray covered with wood shavings. Bedding was not made available in the chamber, but most of the mice were able to pull some shavings through the wire for nesting material. This lack of cover for nesting or change in cage structure may have disturbed the females enough to lower pregnancy rates in all treatments.

To further test the possible significance of olfaction in pregnancy block, without the presence of a stranger male, the female was placed in contact with shavings soiled by an unfamiliar male (Experiment IV) or with urine of a stranger male (Experiment V). The results of these experiments provide no evidence for the importance of olfactory cues



or ingested materials from the sources tested in the production of pregnancy block. Future research should re-examine the function of olfactory or ingested cues with larger sample sizes. Such research should also focus on the possible significance of social interaction during physical contact or visual and auditory communication between the sexes. The present findings further substantiate the premise that not all Cricetid or Murid rodents can be assumed to interact inter- or intraspecifically through the same behavioral and physiological mechanisms. Because of extreme diversity of animals within these groups, each species must be considered separately and care must be taken in applying assumptions to more than one member of a family.

A final observation from this study must be considered. The control females of all experiments exhibited lower pregnancy rates than expected (Range- 11% to 50%). In contrast, the monthly pregnancy rate of females in the production colony ranged from 60% in January to 73% in June. These colony birth rates can not be compared directly with the experimental treatments because most colony females had been paired with the same male for an extended period of time and had varying degrees of reproductive experience. The colony pregnancy rates represent the total number of births by all breeding females during a 30 day period. Experimental control pregnancy rates were based only on the total number of pregnancies observed within 30 days of pairing. Since experimental pairs remained

together for only 72 hours after pairing, an approximate maximum of only 45% of all control animals could be assumed to have been inseminated based on the distribution of inseminations by day in Experiment I (Table 1). Even with 100% reproductive success following insemination, this low pregnancy rate severely limited these experiments. In retrospect a much larger sample size for each treatment would have been better, but the small size of the colony supplying experimental animals made it impractical to place more than 20 animals in each treatment.

An attempt to determine the number of litters that may have been produced from inseminations within the first five days of pairing in the colony was unsuccessful. Variability allowed in recording of birth dates and the infrequency with which new pairs were established made accurate determination of probable insemination dates impossible.

Another factor causing the expected pregnancy rate of controls to fall below expected levels may have been the parity of the females used. Terman (1969) in a study of pregnancy block in P. maniculatus bairdii, found significantly more blocked pregnancies in nulliparous females than parous females regardless of the treatment. The difference between parous and nulliparous females in all treatments was essentially the same. Even under conditions in which the female remained with the stud for 48 hours following insemination, only 63% of nulliparous females produced litters compared to 97% of parous females.

All animals used in the present study were nulliparous. If the findings of the Terman study can be applied to P. leucopus, the pregnancy rates in this study may have been depressed by the lack of reproductive experience in females. Further research is needed to assess the influence parity may have on reproductive success in white-footed mice.

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