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Strange (unfamiliar) males affect survival of progeny of reproductive female rodents in a variety of ways. In mice, strange males block pregnancy before implantation of embryos (Bruce, 1959) but only in females that are not lactating (Bruce and Parkes, 1961). In voles these males can block pregnancy both before and after implantation (Stehn and Richmond, 1975; Marks and Schadler, 1979; Schadler, 1981). Strange males have also been shown to kill nursing pups in lemmings (Mallory and Brooks, 1978) and mice (Labov, 1980 and vom Saal and Howard, 1982).

The present study in pine voles (*Microtus pinetorum*) examines the effect of strange males on blockage of pregnancy in lactating voles and on survival and growth of nursing pups.

Pine voles used in these experiments were descendents of animals trapped near New Paltz, N.Y. in 1974 and 1975. Animals were housed in metal laboratory mouse cages 18.5x26x14.5 cm with solid bottoms and wire tops and were maintained in a photoperiod of 12L:12D at a temperature of 16-18°C. They were fed Wayne Rat Lab Blox, Wayne Guinea Pig Pellets, apple and water. Wood chips provided litter and nesting material. Cages were cleaned once a week such that disturbance was spread uniformly over all groups of experimental animals.

All mothers used in the study had successfully reared at least one litter. In order to minimize variation, females with litters of two to four neonates were used and they were assigned on Day 4 post partum to control and experimental groups in such a way as to equalize any difference in litter number and in weight of offspring. All females were housed with the stud male throughout pregnancy and after parturition until they were placed in treatment groups.

The mothers with their litters (n=74) received the following treatment: In the first group (n=34) females were housed with the stud male until infants were weaned. A second group (n=30) were housed with the stud male for 4 days post partum at which time the stud male was removed and the strange male introduced following the procedure described below. A third group (n=10) was housed with the stud male for 4 days after which he was removed and not replaced. This group was used to determine if pregnancies were missed or if survival of young was adversely affected because of the removal of the attending stud male.

Before the strange males were released into the cages with the nursing mother and her litter, they were introduced behind a small wire enclosure in the female's cage on Day 4 and released into the cage on Day 5. Unacquainted pine vole adults fight vigorously and previous attempts to place strange males directly with lactating females resulted in the killing or wounding of one of the parents and/or the young. In order to assess the effect of the presence of the male, such aggressive encounters were avoided with the 24 hour familiarization

period. After this period, previously unacquainted males and females do not fight.

During the experiment all cages were checked daily and observed for a minimum of five minutes for condition of young, behavior of all cage occupants, and for recording of deaths. Young from the two groups that were housed with males, either stud or strange, throughout the experiment were weighed at 2 days, at 10 days and at 21 days of age when they were weaned.

The criterion used for determining blockage of pregnancy was the difference in number of days post partum until delivery of a litter. Previous findings (Schadler and Butterstein, 1979) showed that 87% of lactating females delivered litters that were conceived within 3 days post partum. Since gestation is 24 days, the expected interval between litters is 24-27 days. Therefore, if a female exposed to a strange male at 5 days lost her pregnancy and was subsequently re-inseminated, her young would be born after 29 or more days.

To check the effect of strange males on infants the following criteria were used: 1) the number of litters that survived intact until weaning; 2) the number of young that survived; and 3) weight gain of infants.

Chi square and the Student's t-test were used in the statistical analyses.

RESULTS

Data on delivery of post partum litters showed that a significant number of pregnancies ($x^2=6.8$, $p<0.01$) were blocked by the strange male (Table 1). In all groups 90-91% of the females bore litters conceived during the post partum period. Of the females that were not exposed to strange males, 9 of 10 (90%) of the animals caged alone after 4 days and 30 of 34 (88%) of the animals caged with stud males delivered litters 24-27 days post partum. This is compared with 18 of 30 (60%) of the group exposed to strange males. In the latter group 9 females (30%) delivered litters that were born 29 or more days post partum.

Data on infant size and survival for all three groups appear in Table 2. A comparison of survival of litters between the group that had the stud males removed and not replaced and the ones in which the stud was not removed showed no significant difference ($x^2=.55$). Comparison of the groups caged with a male, either stud or strange, that successfully reared intact litters showed the following: The group caged with a stud male had a weaning success rate of 65% (22 of 34 litters) versus a 7% success rate (2 of 30) for the group housed with a strange male. The difference is significant at $p<0.001$ ($x^2=21.7$). The number of offspring that survived for 10 days was 95% for the first group versus 68% for the second and at 21 days was 84% versus 34%. Both differences were significant ($x^2=26.1$, $p<0.001$ and $x^2=63$, $p<0.001$ respectively).

Infants exposed to strange males not only survived less well but those that did survive had a reduced rate of growth (Table 2). Mean gain in weight of infants from the first group from 2-10 days was

Table 1. Birth of litters conceived during lactation period to females that were caged with males under varying conditions

Treatment	Number litters born (%)	Litters born 24-27 days post partum (%)	Litters born 29 or more days post partum (%)
Stud male present throughout lactation (n=34)	31(91)	30(88)	1(3)
Stud male replaced by strange male (n=30)	27(90)	18(60)*	9(30)
Stud male removed and not replaced (n=10)	9(90)	9(90)	0

*$p<0.01$ by x^2 test

4.3±0.19 and 2-21 days was 9.9±0.22 compared with infants from the second group (3.1±0.19 and 8.7±0.50). Both differences were significant ($t=4.4$, $p<0.001$; $t=2.2$, $p<0.05$ respectively).

Daily examination of living infants and carcasses revealed little sign of wounding. Of the 30 experimental animals that died before 10 days, none died within the first several hours after they were exposed to the strange male, 8 died within the next day and the rest survived two or more days. Dead animals were often found intact but in some cases dead infants in all groups were totally or partially consumed, a common occurrance in pine voles. Observance of adults showed no detectable differences in the behavior of stud males versus strange males. In both cases males hovered with females over the young in the nest.

DISCUSSION

Strange males did not block pregnancy after parturition in meadow voles, Microtus pennsylvanicus, when they were introduced before implantation on the second day after post partum coitus. (Mallory and Clulow, 1977). Kenney, Evans, and Dewsbury (1977) found that the incidence of abortion after implantation in parous female M. ochrogaster and M. pennsylvanicus that had recently lactated was low and they speculated that the females may have experienced protection from their recent lactation.

In 30% of the pine voles, lactating mothers underwent blockage of pregnancy and subsequent re-insemination by strange males in this experiment. This phenomenon is interpreted to be a true case of pregnancy blockage and not delayed implantation mediated by removal of the stud male because 88-90% of all females from both sets of controls, with or without stud males after Day 4, had litters conceived within 3 days post partum. This loss of pregnancy was less than that noted in non-lactating females in which 84% of the females aborted when they were placed with strange males at 4 days post partum (Marks and Schadler 1979) and 88% aborted at 10 days post partum (Schadler, 1981).

Reduced survival of offspring of lactating rodent females exposed to strange males has been reported by Mallory and Brooks (1978), Labov (1980), and vom Saal and Howard (1982). In lemmings, Mallory and Brooks (1978) found that a strange male placed with lactating females killed the young unless the female was successful in attacking the male and keeping him at bay. In pine voles, familiarizing strange males with their new associates allays aggressive tendencies and the female does not actively defend the nest.

Labov (1980) working with mice placed strange males with females before the litters were born. He noted that allowing males to co-habit with pregnant females for an extended period of time before parturition or letting them copulate with estrous females painted with urine containing pheromones from pregnant female cagemates appeared to repress killing of the young. vom Saal and Howard (1982) found that dominant male mice placed alone in cages with newborn infants were more likely to kill the infants than subordinate ones. In voles, since strange males did not physically attack the young, reduced survival must be ascribed to other causes.

Table 2. Effects of males on survival and mean (± SEM) gain in weight of nursing pine vole infants

Treatment	Num. Lit. born	Lit. surv. intact (%)	Num. Young born	Young surv. 10 da. (%)	Weight gain 2-10 da	Young surv. 21 da (%)	Weight gain 2-21 da
Stud male present throughout lactation	34	22 (65)	106	101 (95)	4.3±0.19	90 (84)	9.9±0.22
Stud male replaced by strange male	30	2* (7)	95	65* (68)	3.1±0.19+	33* (34)	8.7±0.50++
Stud male removed and not replaced	10	5 (50)	29	26 (90)	—	23 (80)	—

* $p<0.001$ by x^2 test

\+ $p<0.001$ by t-test

++ $p<0.05$ by t-test

Some authors have suggested that decreased secretion of prolactin which is both luteotropic and lactogenic in voles follows the introduction of a strange male. The luteotropic effect has been described in the vole M. agrestis, by Milligan and MacKinnon (1976) and Charlton, Milligan and Versi (1978). Milligan, Charlton and Versi (1979) noted that pregnant females with functional corpora lutea and elevated prolactin levels had their pregnancies blocked upon exposure to strange males. This blockage was accompanied by degeneration of corpora lutea and suppression of secretion of prolactin. The necessity for adequate levels of circulating prolactin to stimulate lactation is well known.

If prolactin levels are lowered in pregnant and lactating pine voles exposed to strange males, this could account for the noted blockage of pregnancy in these animals and for reduction in survival and growth of nursing young.

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