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Title: SEXUAL DEVELOPMENT AND REPRODUCTIVE SUCCESS OF YOUNG FEMALE VOLES MICROTUS CANICAUDUS

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Exposure to a strange male increased the sexual development of 18-day-old females to a greater extent than did exposure to either young or adult females. Reproductive tract weights, mean diameters of Graafian follicles, and incidence of vaginal estrus were all greater in the male-exposed group during either the first or second week of treatment. No consistent differences in these parameters were apparent between the female-exposed groups. By the end of three weeks large numbers of females in all experimental groups appeared to have reached first estrus. Exposure to males did not appear to have a stimulatory effect on overall growth in 18-day-old females.

Females were shown to be capable of becoming pregnant as early as 18 days of age. Young females produced healthy appearing litters of comparable weight to those of adult mothers. However, offspring survival to 18 days and mean weight of surviving young were much greater for litters born to adult females. Adult females also showed a much greater incidence of successful post-partum mating than did young females.

Corpora lutea in both young and adult females increased in size throughout pregnancy. There was no increase in number of corpora lutea in either group. However, adult females tended to have a greater number of corpora lutea than embryos. The mean diameter of Graafian follicles was greater in adult females throughout pregnancy. By the end of pregnancy though, both young and adult females had large, well-developed Graafian follicles.

Pseudopregnancy lasts for between one and two weeks in young females mated to vasectomized males. Corpora lutea in these females were indistinguishable from those of pregnancy at the end of one week. By the end of the second week these corpora lutea had degenerated. In some cases new sets of corpora lutea were present after two weeks.

SEXUAL DEVELOPMENT AND REPRODUCTIVE SUCCESS OF YOUNG FEMALE VOLES *MICROTUS CANICAUDUS*

by

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SEXUAL DEVELOPMENT AND REPRODUCTIVE SUCCESS OF YOUNG FEMALE VOLES *MICROTUS CANICAUDUS*

I. INTRODUCTION

From an evolutionary point of view, the most significant accomplishment any organism can achieve is successful reproduction. In light of this, one would anticipate finding a wide diversity of highly specialized reproductive patterns in nature. A cursory study of the biological literature confirms this. Yet despite the wealth of information we possess on the subject of reproduction, the theoretical basis for our knowledge of reproductive patterns is superficial at best.

Nevertheless, a few generalizations can be made. In r-selected species of small prey-mammals such as the microtine rodents reproductive output can be maximized in a number of ways. First of all, developmental time can be shortened. This not only minimizes the energy output required for parental care, but also shortens generation length, thereby increasing the reproductive potential of an individual and her offspring. Secondly, litter size can be increased. Finally, the reproductive season can be extended. Combined with physiological changes allowing for uninterrupted consecutive pregnancies this can greatly increase a female's yearly reproductive potential. Apparently, various species of microtines have taken advantage of all of these possibilities.

Development

Typically, development is extremely rapid in microtine rodents. In Microtus canicaudus young are born hairless and blind. Within 9 to 12 days the eyes open and by 16 to 18 days the young are independent of the mother (personal observations). Voles of this and related species are considered adults by 9-10 weeks of age. However, individuals may be reproductively active long before this time. Negus and Pinter (1965) successfully bred juvenile M. montanus (4-6 weeks old) in the laboratory.

Factors contributing to sexual development have not been investigated intensively in microtine rodents. However, investigations using laboratory mice have yielded results that may indicate general trends applicable to other rodent species. Exposure to adult males during early development decreases the time of first vaginal estrus in mice (Vandenbergh, 1967; Kennedy and Brown, 1970; Fullerton and Cowley, 1971). The odor of an adult male transmitted via soiled bedding is a sufficient stimulus to elicit this effect (Vandenbergh, 1969).

Litter Sizes

Liter sizes in microtine species tend to be relatively large and variable. Colvin and Colvin (1970) reported mean litter sizes of 6 for M. montanus, 5.5 for M. pennsylvanicus, 4.7 for M. californicus, 4.0 for M. longicaudus, and 3.9 for M. ochrogaster. Negus and Pinter (1965) found that in adult M. montanus the size of

consecutive litters increased to a peak mean litter size of 5.8 for the fifth litter. Individual fifth litters ranged in size from 3 to 10 offspring. Similar findings have been reported for the closely related M. canicaudus (Gilston, 1976). However, Storm and Sanderson (1968) found no evidence of increasing litter size in consecutive litters for M. pennsylvanicus.

Breeding Season

Generally microtine species have breeding seasons extending from early spring to late fall with an extended period of anestrus during the winter months. However, in their southern ranges M. montanus and M. pennsylvanicus may breed year round (Asdell, 1964). In the laboratory M. canicaudus shows consistent breeding success throughout the year (personal observation).

Reproductive Patterns

Estrus

Much effort has been expended in elucidating characteristics of estrous cycles in various mammalian groups. So much attention has been directed toward this study that the true significance of the estrous cycle may be overstated. In a farsighted review, Conaway (1971) terms the nonpregnant cycle a "pathological luxury" that is rarely encountered in nature. Particularly in short-lived prey species (whose reproductive lives may be measured in weeks) time spent in a nonreproductive state must be drastically curtailed. Small

herbivorous mammals have developed two strategies to minimize time spent in the nonfertile state: short, consecutive estrous cycles and long periods of uninterrupted estrus (Conaway, 1971). Certain genera in the families Muridae and Cricetidae have reduced the estrous cycle to 4-7 days. Although ovulation and corpus luteum formation are spontaneous, the corpora lutea regress quickly and a new follicular phase begins without delay. In other cricetid genera, most notably the microtines, an alternative solution to the problem of infertile periods has developed. In these groups there is no well defined estrous cycle. Females remain in the anestrous state until environmental stimuli induce an extended period of sexual receptivity. Estrus is generally maintained as long as appropriate behavioral stimulation occurs or until copulation takes place.

There has been some controversy as to the nature of the reproductive cycle in the genus Microtus. Early investigators considered the short-tailed vole, M. agrestis, to be a spontaneous ovulator exhibiting typical estrous cycles (Brambell and Hall, 1939, cited in Milligan, 1974). Chitty and Austin (1957) claimed that depending on the social context M. agrestis might exhibit either the short well-defined estrous cycle found in rats or mice or prolonged periods of estrus. A 3-4 day estrous cycle during lactation was also reported by Chitty (1957). Subsequently, Breed (1967) found no correlation between social environment and estrus type. He reported indefinite periods of estrus, up to 4 weeks in length, in all females.

Further studies with other microtine species tend to confirm Breed's findings. It now appears that M. montanus (Gray et al., 1974b), M. ochrogaster (Richmond and Conaway, 1969; Gray et al., 1974a), M. pennsylvanicus (Clulow and Mallory, 1970), M. pinetorum (Kirkpatrick and Valentine, 1970), M. californicus (Greenwald, 1956), and Arborimus longicaudus (Hamilton, 1962), all show indefinite states of estrus punctuated by periods of anestrus.

In the laboratory anestrous females can be stimulated into estrus in a number of ways. The most effective method is to place the female in close proximity to males (Richmond and Conaway, 1969; Hasler and Conaway, 1973; Gray et al., 1974b). Female M. ochrogaster placed adjacent to male cages showed persistent vaginal cornification and sexual receptivity for periods extending up to 30 days (Richmond and Conaway, 1969). Physical presence of the male is not required; soiled bedding from a male's cage is a sufficient stimulus for estrus induction (Richmond and Conaway, 1969). Exogenous hormones have also been used to induce estrus. Hasler and Conaway (1973) found that injection of 1 μg estradiol cyclopentylpropionate brought most females into heat within 3 days. Negus and Pinter (1966) showed that addition of sprouted wheat to the females' diets stimulated estrus within 24 hours. Richmond and Conaway (1969) found that in M. ochrogaster even such a mundane treatment as changing the litter in a female's cage could bring about an estrous response. It was concluded that it might be adaptive for a female to respond to any change in the environment by coming into estrus.

Unstimulated anestrous M. ochrogaster females tend to remain in this condition. This phenomenon is usually observed in isolated females or those maintained in large stable groups (Richmond and Conaway, 1969; Hasler and Conaway, 1973). Hasler and Conaway (1973) suggested that it would be advantageous for a female to avoid estrus and the consequent energy cost of uterine build-up in lieu of a sufficiently strong environmental stimulus.

Ovulation

Induced ovulation is generally associated with those species showing long indefinite periods of estrus. Induced ovulation has been observed in M. agrestis (Breed, 1967), M. montanus (Cross, 1972; Gray et al., 1974b), M. ochrogaster (Richmond and Conaway, 1969; Gray et al., 1974a), M. pennsylvanicus (Clulow and Mallory, 1970; Lee et al., 1970), M. pinetorum (Kirkpatrick and Valentine, 1970), M. californicus (Greenwald, 1956), and Arborimus longicaudus (Hamilton, 1962). In all cases copulation was necessary to produce ovulation.

Ovulation has also been induced by injection of hormones. Cross (1972) obtained an ovulatory response in montane voles following administration of 5 I.U. human chorionic gonadotropin. Ovarian response to HCG was significantly less pronounced than that produced by copulation.

Ovulation occurs in M. pennsylvanicus following copulation with vasectomized males (Lee et al., 1970). Pseudopregnancy involves an increased ovarian weight immediately following ovulation. However ovarian regression follows after seven days. Ovulation does not

occur in female M. pennsylvanicus caged with castrated males (Lee et al., 1970).

Mechanical stimulation of the vagina is an insufficient stimulus for ovulation in M. montanus (Cross, 1972) and M. agrestis (Milligan, 1974). Vigorous mounting by the male without vaginal penetration also fails to elicit an ovulatory response in M. ochrogaster (Gray et al., 1974a). However, Milligan (1975a) found that parous females of M. agrestis ovulated following mounting without intromission. Similarly it appears that the frequency of ovulation may be determined in part by the length and intensity of copulatory behavior in M. montanus (Davis et al., 1974), M. ochrogaster (Gray et al., 1974a), and M. agrestis (Milligan, 1975a). Recent studies have indicated that ovulation may occur in some female M. agrestis when separated from males by a wire screen (Milligan, 1974; 1975b).

Interspecific and intraspecific variation in stimulus requirements for ovulation are not too surprising. Conaway (1971) rightly suggests that spontaneous and induced ovulation are arbitrary designations. Undoubtedly a continuum of responses exists in nature on which these are but two extremes.

Post-Partum Estrus and Delayed Implantation

Post-partum estrus is common in animals giving birth to multiple litters during a single breeding season. There are, however, inherent problems with this system since the female must expend energy not only for maintenance of pregnancy but also for lactation.

Some species (most notably certain mustelids) have circumvented this problem through delayed implantation; the fertilized ova remain in a state of arrested development until conditions in the uterus are conducive to implantation.

Post-partum estrus has been observed in at least some individuals of most microtine species studied. Female receptivity and subsequent mating after parturition occur in M. agrestis (Chitty, 1957), M. montanus (Negus and Pinter, 1965; Gray et al., 1974b), M. ochrogaster (Richmond and Conaway, 1969), M. pinetorum (Kirkpatrick and Valentine, 1970), M. pennsylvanicus (Lee et al., 1970), M. californicus (Greenwald, 1956), M. canicaudus (Gilston, 1976), and Arborimus longicaudus (Hamilton, 1962).

Information concerning the occurrence of lactational delay in implantation is a bit more contradictory. Chitty (1957) reported no difference in the length of gestation for first and second litters in M. agrestis. This pattern seems to hold true for M. pinetorum (Kirkpatrick and Valentine, 1970) and M. montanus (Gray et al., 1974b). However, Negus and Pinter (1965) noted some differences in lengths of gestation in M. montanus. They were unable to determine whether these discrepancies were due to lactational delay in implantation or mating failure during the immediate post-partum period. Richmond and Conaway (1969) reported a similar pattern in M. ochrogaster and suggested that mating failure was a more reasonable explanation than delayed implantation. It was postulated that females of permanent breeding pairs experience a form of anestrus during mid-lactation. This may be due to subliminal stimulation of the

female by her mate. Hamilton (1962) reported delayed implantation of up to two weeks in Aborimus longicaudus. He claimed that delayed implantation is a reproductive adaptation to the energy conversion difficulties inherent in a diet composed entirely of fir needles.

Statement of Purpose

The major intent of laboratory investigations of wild animals is to gain some insight into characteristics of the organism in its natural setting. Yet, we can have only limited confidence in the application of our findings beyond the confines of the artificial world we have created. Most studies on microtine reproductive physiology have been made on adult individuals. However, we have little information concerning the relative contribution of adults to population growth for any microtine species. Certainly it can be argued that in a population of relatively defenseless voles subject to intense predation, 10-week-old individuals may be a rarity. It may well be that much younger individuals contribute the bulk of reproductive output in most populations.

The purpose of this study was to investigate reproduction in very young female gray-tailed voles, M. canicaudus Miller. Specifically, two sets of experiments were undertaken. First, the reproductive success of females mated at 18 days of age was compared to that of females mated at 10 weeks of age. Secondly, the effects of various social conditions on the reproductive development of 18-day-old females were studied.

II. MATERIALS AND METHODS

General Laboratory Procedures

Maintenance of Animals

Animals used in this project were members of an outbred colony of *M. canicaudus* maintained in #635 Weniger Hall on the Oregon State University campus. The colony consists of the descendants of animals trapped in Benton County, Oregon in 1973.

All animals were housed in fiberglass flower boxes (60 cm x 15 cm x 15 cm) fitted with 1.5 cm mesh hardware cloth tops. Bedding material included hardwood shavings and upholstery cotton. Animals were provided with Purina Rat Chow, Purina Rabbit Chow, and tap water *ad libitum*. The animal room was maintained at constant conditions of temperature (20°-22°C) and photoperiod (16 hours light).

In addition one group of experimental animals was housed at the Oregon State University Laboratory Animal Resources Center. These animals were caged in an identical manner to those in the main colony. Conditions of temperature and photoperiod were maintained as close to those of the Weniger Hall facility as possible. No microtine rodents had previously been kept in the Laboratory Animal Resources Center.

Experimental Animals

Animals used in this study were obtained from established breeding cages. In order to insure the outbred nature of the colony

none of the breeding pairs consisted of siblings or first cousins. Offspring from these parents were weaned at 18 days of age. Those animals to be used as adults were toe clipped for identification and caged in groups of five according to sex. Females to be used as weanlings (henceforth termed 18-day-old females) were not toe clipped but were immediately transferred to experimental cages.

Specific Laboratory Procedures

Twelve-week Breeding Experiment

Two groups of breeding pairs were established. In the first group, 18-day-old females were mated to adult, sexually inexperienced males. The second group consisted of adult, virgin females paired with adult, sexually inexperienced males. All adult animals were at least 10 weeks of age and had been reared according to the procedures outlined above.

All animals were weighed and examined for signs of ill health at the beginning of the experiment. Eighteen-day-old females weighing less than 12.5 g were not used (range: 12.5-19.5 g). Individual breeding cages were left undisturbed, except for daily feeding and watering, for 20 days. At the end of this period females were checked for signs of pregnancy by palpation. Thereafter cages containing females in advanced stages of pregnancy were checked twice daily for litters.

Each litter was weighed as a group at birth. The offspring were then immediately returned to the breeding cage. After this the

parents and offspring were left undisturbed until weaning. At 18 days of age young were removed and the breeding pair was transferred to a clean cage. Young were weighed individually and sexed. Survival of offspring from birth to weaning was determined and recorded at this time.

Data for subsequent litters were obtained in a similar manner. Breeding runs were terminated at the end of the 12-week period or when death occurred in a breeding pair.

Ovarian Changes During Pregnancy and Pseudopregnancy

To investigate histological changes in the ovary during pregnancy and pseudopregnancy three experimental groups were established:

1) Eighteen-day-old females mated to adult, sexually inexperienced males.
2) Eighteen-day-old females mated to vasectomized, adult, sexually inexperienced males.
3) Adult, virgin females mated to adult, sexually inexperienced males.

Males in Group 2 were vasectomized at approximately 10 weeks of age. Anesthesia was achieved by intraperitoneal injection of Nembutol at an initial dosage of 50 mg/kg. An incision was made through the body and abdominal walls. The vas deferens were cut and the free ends tied with surgical thread. All males were allowed to recuperate for 10 days before mating. At the end of the experiment these males were killed and inspected for signs of testicular

regression. No consistent pattern of regression was noted although in a few individuals one testis was markedly smaller than the other.

Experimental cages were left undisturbed until termination. Females in each group were killed at 1, 2, or 3 weeks post mating. Body weight was recorded immediately following death. The reproductive tract was dissected out *in toto*, fixed in AFA (alcohol-formalin-acetic acid), and stored in 70% ethanol. The number of embryos in each uterine horn was recorded for females killed at 2 and 3 weeks.

Ovaries were prepared for sectioning using a Fisher Tissuematon (Appendix I) and embedded in Fisher paraplast. Sections were taken at 8 μm. Tissue sections were stained using Eosin-Hematoxylin (Appendix II).

All corpora lutea and Graafian follicles were counted. Follicles exceeding 300 μm in diameter with well-developed antra were considered to be Graafian folicles. Mean diameters of both histological structures were calculated using a micrometer scale and the low power objective on the microscope. Mean diameters were calculated as the average of two perpendicular diameters through the largest section of each structure. The number of corpora lutea found in each ovary was compared to the number of embryos found in the corresponding uterine horn for females in Groups 1 and 3.

Sexual Development

To investigate the effects of various social environments on the sexual development of young females three experimental groups were established:

1) Two 18-day-old females caged together.

2) An 18-day-old female caged with an adult, virgin female.

3) An 18-day-old female caged with an adult, sexually inexperienced male but separated from him by a hardware-cloth barricade.

Animals in Group 1 were housed in a room at the O.S.U. Laboratory Animal Resources Center. This precaution was taken to insure against olfactory stimuli from adult voles. Since voles had not been previously maintained at this facility, females in Group 1 received no adult stimuli from the time they were weaned onward.

Experimental cages were maintained on separate racks according to group. All animals were left undisturbed except for daily feeding and watering. Experimental females were killed at 1, 2, and 3 weeks post mating. Prior to death, each animal was examined for perforation of its vagina. Body weights were recorded immediately following death. The reproductive tracts were dissected out *in toto*, fixed in AFA, and stored in 70% ethanol.

Weights of the fixed reproductive tracts (excluding vagina and cervix) were taken on a Roller-Smith tissue balance. Histological preparations and observations were carried out according to the procedures outlined above.

Statistical Methods

Where appropriate, data obtained from the twelve-week breeding experiment were analyzed using Student's t-Test. One-tailed, 95% confidence intervals were calculated using standardized table values

prepared by Fisher and Yates (Neter and Wasserman, 1974).

Results from all other experiments* were subjected to two-way analysis of variance. F values were obtained to determine significant interactions and main effects. In cases where homogeneity of variance could be assumed significant main effects were tested using standard t values. When homogeneity of variance could not be assumed approximate t values were calculated according to the formula:

$$\text{approximate } t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

$$\text{with degrees of freedom} = \frac{\left[S_1^2/n_1 + S_2^2/n_2\right]^2}{\frac{\left(S_1^2/n_1\right)^2}{n_1 - 1} + \frac{\left(S_2^2/n_2\right)^2}{n_2 - 1}}$$

(Brownlee, 1965).

*Distributions of Graafian follicles by size class were analyzed using the two-tailed Student's t-Test.

III. RESULTS

Twelve-Week Breeding Experiment

A greater percentage of females mated as adults produced at least one litter than did females mated at 18 days (Table I). Surprisingly, the first litters born to young mothers were significantly larger ($P < .005$) than those born to adult mothers (Tables I and II, Figure 1). It might be argued though, that only those litters born within 30 days of initial pairing should be used in calculating mean litter sizes. Litters born after this 30-day period might not be the product of first conception; the first pregnancy may have failed during its early stages. However, given this constraint the mean litter size for the young female group ($N = 13$, $\bar{x} = 5.38 \pm 0.28$ g) remains significantly larger ($P < .005$) than that of the adult group ($N = 16$, $\bar{x} = 4.12 \pm 0.22$ g).

When all first litters are considered, the mean weight of offspring at birth is significantly greater ($P < .005$) for litters born to adult mothers (Table I). However, this may be due in part to the larger litter sizes of young mothers. There is no significant difference in weight at birth ($P > .05$) when litters of equal size are compared (e.g., for a litter size of 5, young females' $\bar{x}$ litter wt = 2.46 ± 0.05 g while adult females' $\bar{x}$ litter wt = 2.48 ± 0.01 g).

Offspring survival from birth to weaning at 18 days of age was considerably greater for litters born to adult mothers. Furthermore, surviving young of both sexes were significantly heavier ($P < .0005$)

Table I. Comparison of reproductive success between young and adult females.

	Females mated at 18 days	Females mated as adults (10+ weeks)
Females producing at least one litter in 12 weeks	68% (21/31)	89% (17/19)
Mean litter size (first litter)	5.14 ± 0.23	4.05 ± 0.18
Mean wt. of offspring at birth (first litter)	2.38 ± 0.03 g	2.48 ± 0.03 g
Offspring survival to 18 days (first litter)	38%	93%
Mean wt. of offspring at 18 days (first litter)		
Males	11.85 ± 0.69 g	16.6 ± 0.21 g
Females	12.68 ± 0.60 g	15.9 ± 0.26 g
Mean days between 1st and 2nd litters	34.22 ± 2.1*	22.75 ± 0.96**
Mean litters produced in 12 weeks	1.47 ± 0.49	2.76 ± 0.13

All values $\bar{x}$ ± S.E.

( ) Number of females producing litters.

* Two of ten females produced a second litter within 30 days after the first litter.

** Fourteen of fifteen females produced a second litter within 30 days after the first litter.

Table II. Distribution of litter sizes for first litters of young and adult females.

ALL FIRST LITTERS

Litter size	Young females	Adult females
1	0	0
2	0	0
3	2	4
4	3	8
5	7	4
6	8	1
7	1	0
	(21/31)	(17/19)

FIRST LITTERS BORN WITHIN 30 DAYS OF PAIRING

Litter size	Young females	Adult females
1	0	0
2	0	0
3	1	4
4	1	7
5	4	4
6	6	1
7	1	0
	(13/31)	(16/19)

( ) Number of females producing litters.

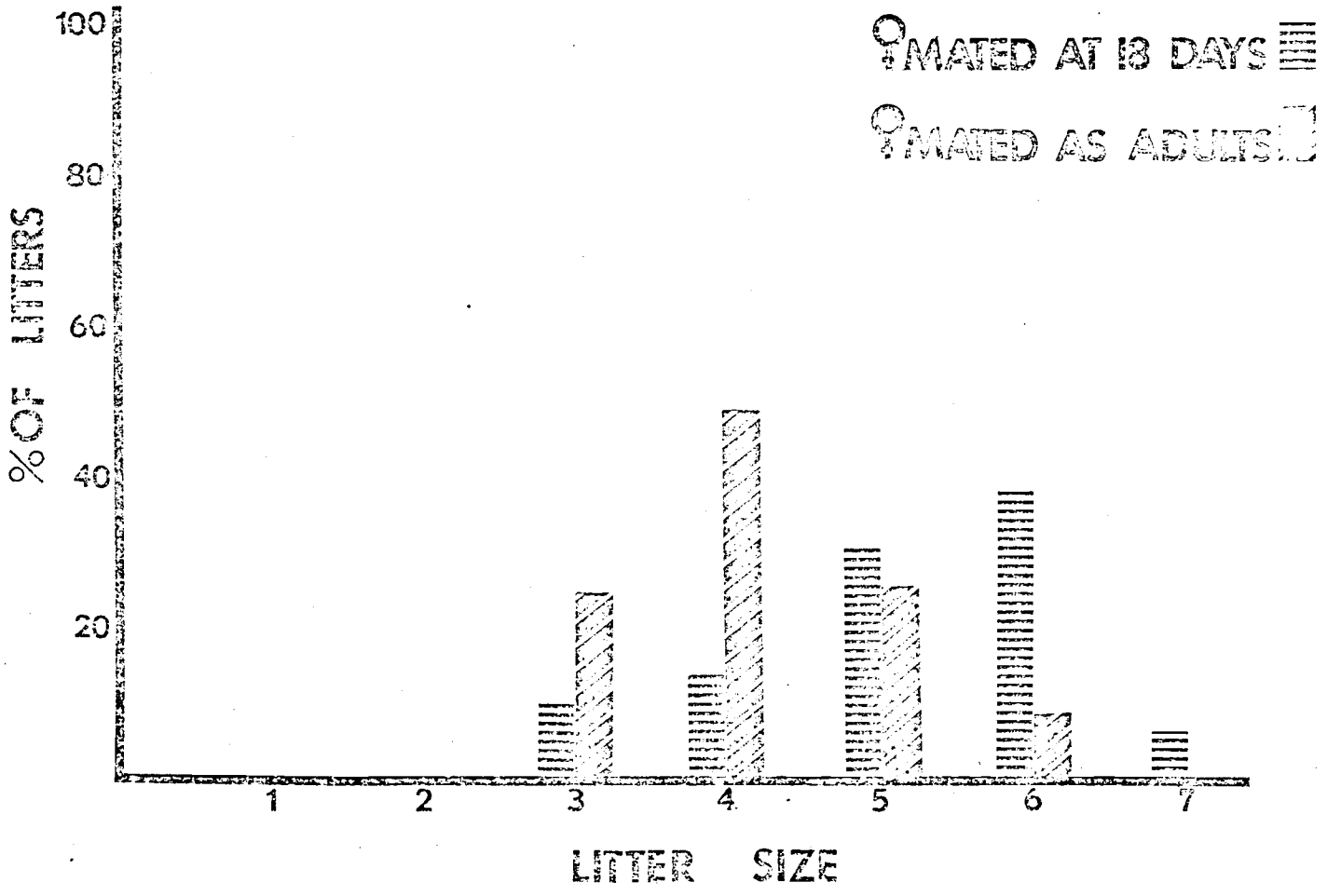

Figure 1. Distribution of litter sizes (first litter) for young and adult females.

in this group (Table I).

The length of time between consecutive litters was significantly longer ($P < .0005$) for young females than adult females. Only 2 of 10 young females produced a second litter within 30 days of the first. In contrast, 14 of 15 adult females gave birth to a second litter during this period. As a consequence of the shorter time period between consecutive litters, adult mothers produced a significantly greater number of litters ($P < .0005$) during the 12-week experiment than did young females (Table I).

Ovarian Histology During Pregnancy and Pseudopregnancy

Experimental results and the accompanying discussion will be given using the following group designations:

Group 1: 18-day-old female mated with adult male.

Group 2: 18-day-old female mated with adult, vasectomized male.

Group 3: Adult female mated with adult male.

Graafian Follicles

The distribution of Graafian follicles into four size classes for the three experimental groups is given in Table III. Group 1 had a significantly greater mean number of follicles ($P < .005$) in the 300-399 μm category during week 1 than did either Group 2 or Group 3. There are no other statistically significant differences between groups in any size category for any week.

Table III. Distribution of follicular diameters during pregnancy and pseudopregnancy.

Group	N	300-399 µm	400-499 µm	500-599 µm	600+ µm
Week #1					
1	4	9.0 ± 3.18 (4/4)	2.0 ± 1.35 (3/4)	0.25 ± 0.25 (1/4)	0
2	4	15.75 ± 3.09 (4/4)	3.5 ± 1.19 (4/4)	0	0
3	5	8.6 ± 2.44 (4/5)	4.2 ± 0.91 (5/5)	0.6 ± 0.6 (1/5)	0
Week #2					
1	5	8.4 ± 1.36 (5/5)	5.8 ± 0.58 (5/5)	0.8 ± 0.58 (2/5)	0
2	5	3.4 ± 1.07 (4/5)	4.6 ± 1.32 (4/5)	0.6 ± 0.24 (3/5)	0
3	4	6.5 ± 2.32 (4/4)	5.5 ± 1.55 (4/4)	0.5 ± 0.5 (1/4)	0
Week #3					
1	5	12.6 ± 1.77 (5/5)	4.6 ± 1.74 (4.5)	0	0
2	5	4.2 ± 1.11 (4/5)	3.6 ± 1.02 (5/5)	1.4 ± 0.92 (3/5)	0
3	5	4.2 ± 1.15 (5/5)	5.4 ± 0.5 (5/5)	0.2 ± 0.2 (1/5)	0

All values $\bar{x}$ ± S.E. number of follicles per individual.
( ) Number of individuals per group with follicles.
All females had at least one ovary containing corpora lutea.

There was no significant interaction between week and group on Graafian follicle size ($F_{(2,41)} = .709$, $P > .05$) (Table IV and Figure 2). There were also no significant main effects of week ($F_{(2.41)} = 2.963$, $P > .05$) or group ($F_{(2,41)} = 3.040$, $P > .05$) on follicular development.

Corpora Lutea

There was a significant interaction between week and group on corpus luteum diameter ($F_{(4,37)} = 3.042$, $P < .03$) (Table IV and Figure 3). This interaction was undoubtedly due to the small mean diameters of Group 2 corpora lutea during weeks 2 and 3. When a two-factor analysis was performed on Groups 1 and 3 there was no significant interaction between week and group ($F_{(2,27)} = .083$, $P > .05$). There was also no main effect of group on corpus luteum growth (two-factor analysis:$F_{(1,27)} = 1.585$, $P > .05$). However, there was a significant effect of time on corpus luteum development (two-factor analysis:$F_{(2,27)} = 29.775$, $P < .001$). Mean diameters of corpora lutea increased significantly between weeks 1 and 2 (approximate $t_{(13)} = 5.326$, $P < .001$) and weeks 2 and 3 (approximate $t_{(13)} = 6.972$, $P < .001$).

Correlation between Embryos and Corpora Lutea

During weeks 2 and 3 of pregnancy 8 of 9 adult females had a greater number of corpora lutea than embryos (Table V). During this same period only 2 of 10 young females showed a greater number of

Table IV. Ovarian histology during pregnancy and pseudopregnancy: $\bar{x}$ diameter of follicles and C.L. (μm).

	Group	N	$\bar{x}$ diameter of follicles	$\bar{x}$ diameter of C.L.
Week #1	1	4	355 ± 16	925 ± 102
	2	4	361 ± 12	890 ± 66
	3	5	395 ± 23	878 ± 75
Week #2	1	5	385 ± 5	1302 ± 50
	2	5	411 ± 21	1014 ± 15*
	3	4	404 ± 15	1208 ± 39
Week #3	1	5	371 ± 9	1392 ± 60
	2	5	423 ± 27	966 ± 48*
	3	5	411 ± 8	1339 ± 32

All values $\bar{x}$ ± S.E.

All females had at least one ovary containing corpora lutea.

*N = 3

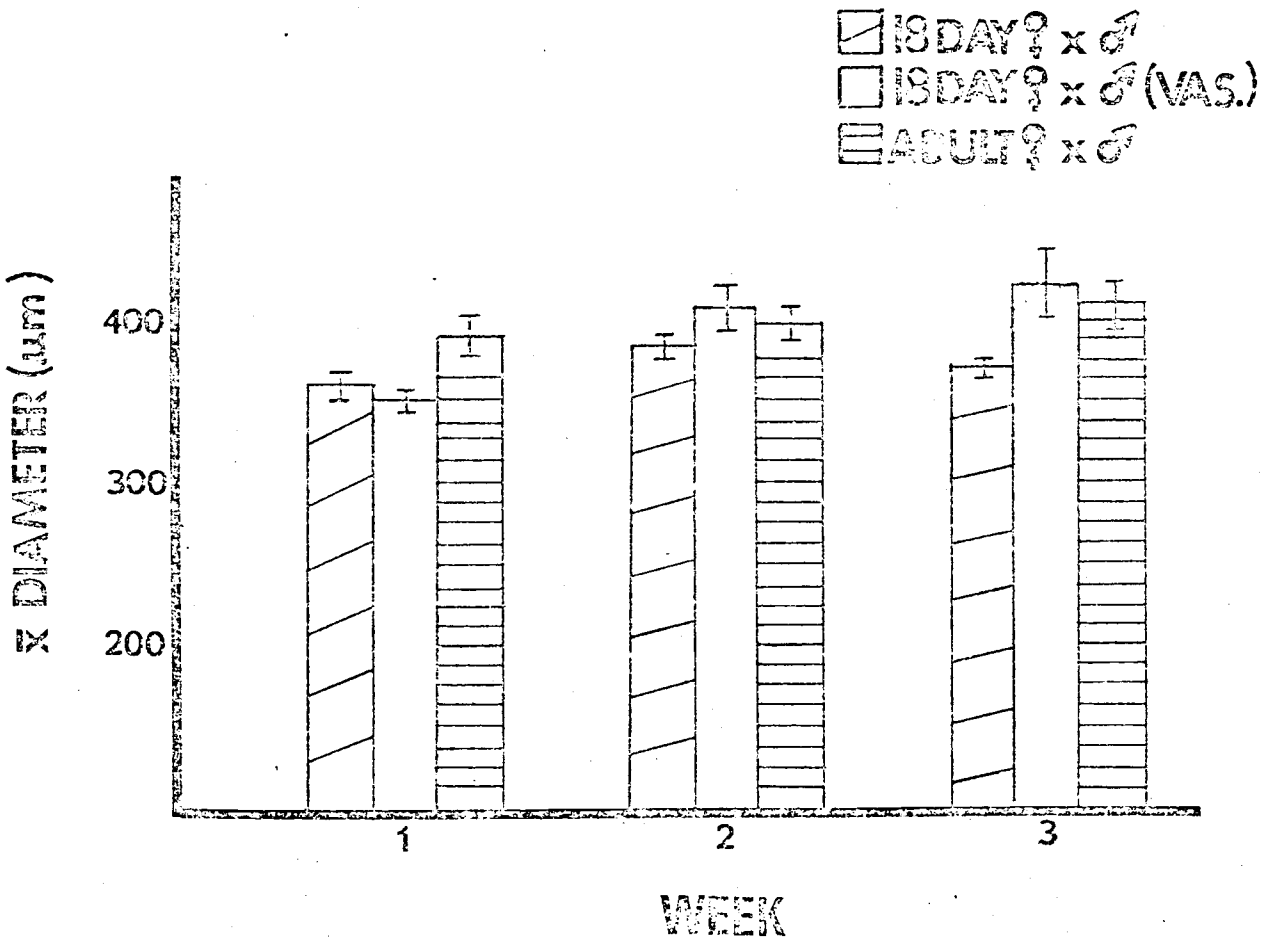

Figure 2. $\bar{x}$ diameter of Graafian follicles during pregnancy and pseudopregnancy.

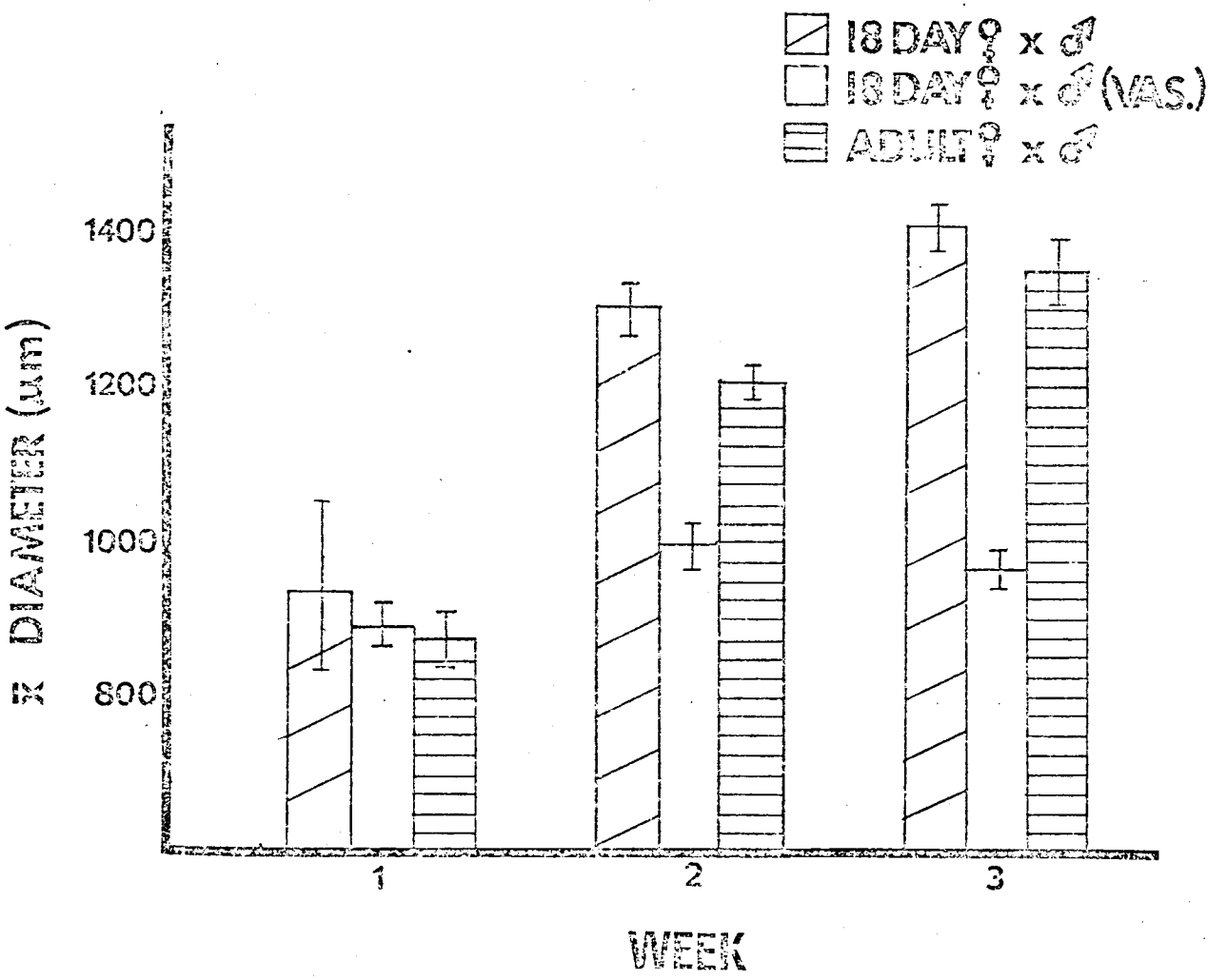

Figure 3. $\bar{x}$ diameters of corpora lutea during pregnancy and pseudopregnancy.

Table V. Comparison of numbers of embryos and corpora lutea in pregnant females.

Individual	# C.L.	# embryos	Embryo/C.L.
Week #2			
Adult female			
1	6	5	
2	7	6	
3	6	5	
4	4	3	
	$\bar{x}$ = 5.75 ± 0.62	$\bar{x}$ = 4.75 ± 0.62	0.82
Young female			
1	4	4	
2	6	6	
3	6	6	
4	5	4	
5	6	6	
	$\bar{x}$ = 5.4 ± 0.4	$\bar{x}$ = 5.2 ± 0.48	0.96
Week #3			
Adult female			
1	5	4	
2	7	6	
3	6	6	
4	4	2	
5	5	4	
	$\bar{x}$ = 5.4 ± 0.5	$\bar{x}$ = 4.4 ± 0.62	0.81
Young female			
1	5	5	
2	6	6	
3	3	4	
4	5	4	
5	7	7	
	$\bar{x}$ = 5.2 ± 0.66	$\bar{x}$ = 5.2 ± 0.41	1.00

corpora lutea than embryos. In one case (young female), a number of embryos greater than the number of corresponding corpora lutea was found. However, a corpus albicans was found in one of the ovaries of this individual.

Effects of Social Conditions on Sexual Development

Experimental results and the accompanying discussion will be given in terms of the following group designations:

Group 1: Two 18-day-old females caged together.

Group 2: 18-day-old female caged with adult female.

Group 3: 18-day-old female caged with adult male.

Body and Reproductive Tract Weights

There was no significant interaction between week and group on body weight ($F_{(4,100)} = .581$, $P > .05$) (Table VI and Figure 4). There was also no main group effect ($F_{(2,100)} = .493$, $P > .05$) on body weight. There was, however, a main effect of time on overall growth ($F_{(2,100)} = 45.692$, $P < .001$). Significant increases in body weight occurred between weeks 1 and 2 (approximate $t_{(57)} = 7.968$, $P < .001$) and weeks 1 and 3 (approximate $t_{(58)} = 9.587$, $P < .001$).

There was no significant interaction between group and week on reproductive tract weight ($F_{(4,100)} = 1.393$, $P > .05$) (Table VI and Figure 5). However, both time ($F_{(2,100)} = 6.415$, $P < .002$) and group ($F_{(2,100)} = 10.543$, $P < .001$) had main effects on uterine

Table VI. Body and reproductive tract weights for young females.

Group	N	$\bar{x}$ Body wt. (g)	$\bar{x}$ Reproductive tract wt. (mg)
Week #1			
1	12	18.91 ± 1.62	17.16 ± 1.32
2	10	20.54 ± 0.47	18.93 ± 1.55
3	11	20.59 ± 0.65	29.65 ± 5.92
Week #2			
1	12	23.9 ± 0.78	16.6 ± 1.25
2	10	25.13 ± 0.75	24.98 ± 2.10
3	11	25.04 ± 0.65	36.52 ± 5.28
Week #3			
1	13	25.86 ± 0.79	29.63 ± 1.85
2	10	26.3 ± 1.0	31.22 ± 4.35
3	12	25.14 ± 0.62	34.24 ± 3.2

All values $\bar{x}$ ± S.E.

Reproductive tracts (excluding vagina and cervix) were weighed in fixed condition.

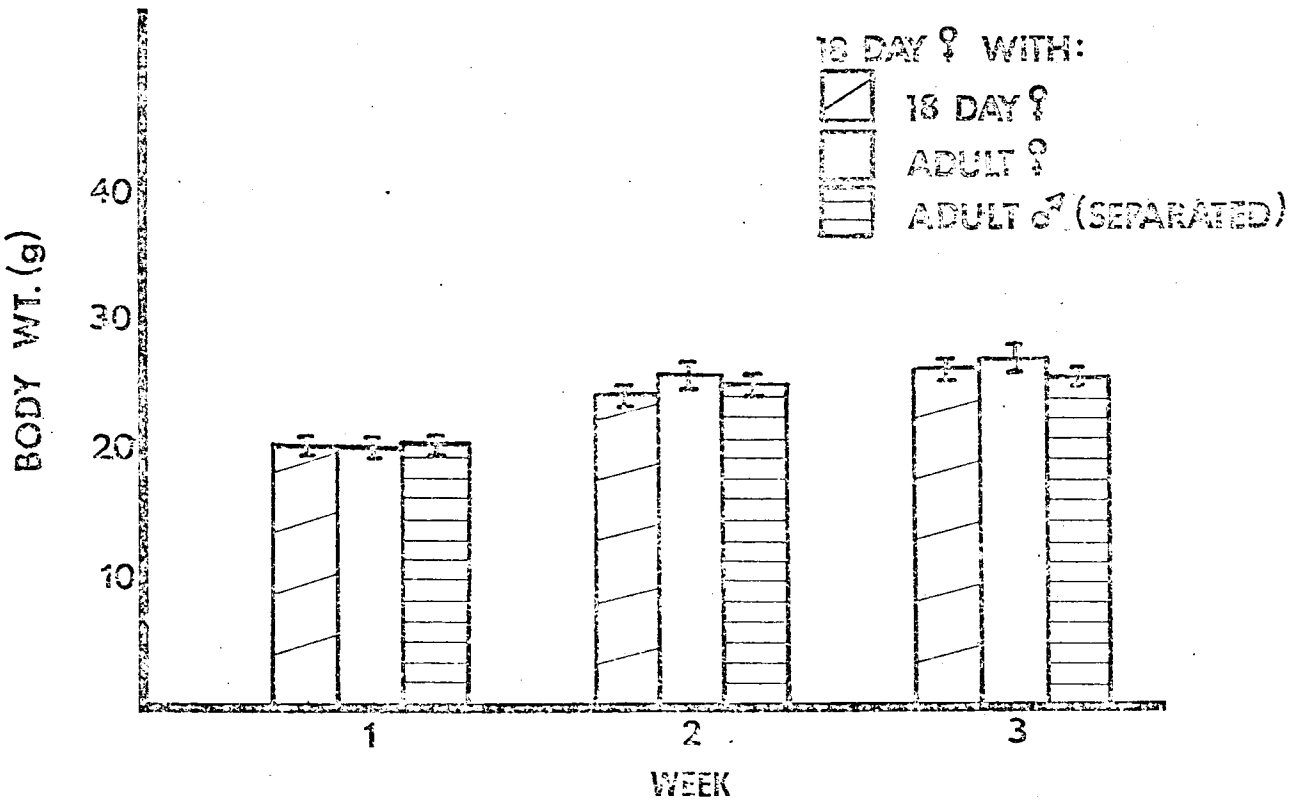

Figure 4. Effects of different social conditions on body weight in young females.

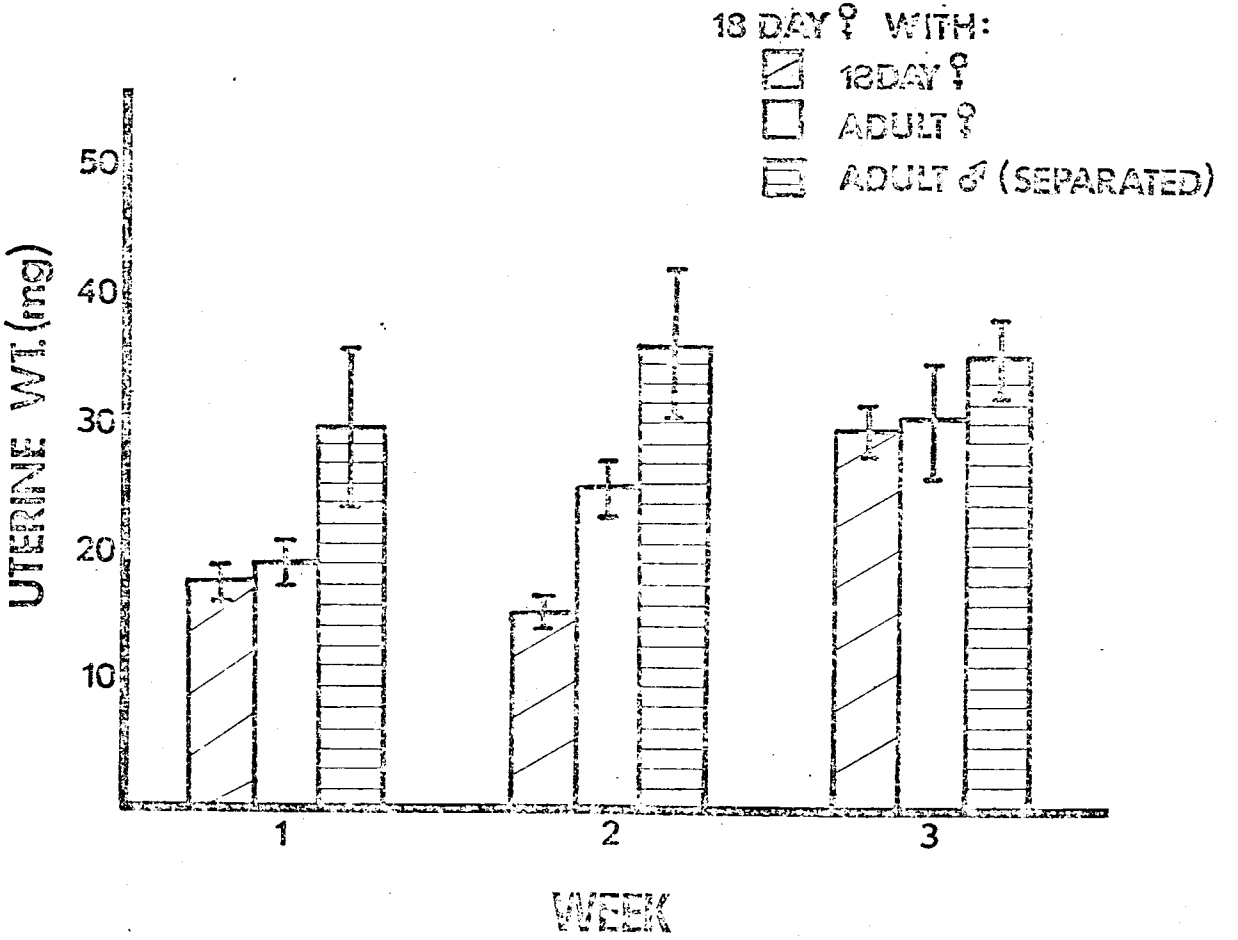

Figure 5. Effects of different social conditions on uterine weight in young females.

weight. Groups 1 and 3 (approximate $t_{(48)} = 3.879$, $P < .001$) and Groups 2 and 3 (approximate $t_{(57)} = 2.466$, $P < .017$) had significantly different uterine weights. There was also a significant difference in uterine weight between weeks 2 and 3 ($t_{(98)} = 2.017$, $P < .017$) and weeks 1 and 3 ($t_{(98)} = 3.260$, $P < .001$).

Ovarian Histology

The distribution of Graafian follicles into four size categories for the three experimental groups is given in Table VII. No significant differences ($P > .05$) occurred among groups in any size class for any week. There was no significant interaction between week and group on mean follicular diameter ($F_{(4,53)} = 2.274$, $P > .05$ (Table VIII and Figure 6). Follicular size was also not significantly affected by group ($F_{(2,53)} = .947$, $P > .05$) or by time ($F_{(2,53)} = 1.508$, $P > .05$).

Vaginal Perforation

A greater proportion of females in Group 3 had open vaginae than did females in either Group 1 and Group 2 during all three weeks of the experiment (Table IX). The number of females with perforate vaginae increased in all groups during consecutive weeks.

Table VII. Ovarian histology of young females: distribution of follicular diameters (μm)

Group	N	300-399	400-499	500-599	600+
Week #1					
1	6	12.16 ± 2.46 (6/6)	0.66 ± 0.49 (2/6)		
2	6	14.83 ± 1.42 (6/6)	0.66 ± 0.33 (3/6)	0	0
3	6	12.00 ± 2.2 (6/6)	2.83 ± 1.19 (4/6)	1.66 ± 1.05 (2/6)	0.16 ± 0.16 (1/6)
Week #2					
1	6	8.33 ± 1.64 (6/6)	1.5 ± 0.42 (5/6)	0.16 ± 0.16 (1/6)	0
2	6	9.16 ± 1.37 (6/6)	2.66 ± 0.76 (5/6)	0.33 ± 0.33 (1/6)	0.33 ± 0.33 (1/6)
3	6	8.38 ± 1.95 (5/6)	2.16 ± 0.79 (5/6)	0.5 ± 0.5 (1/6)	0
Week #3					
1	6	9.83 ± 1.99 (6/6)	4.16 ± 0.94 (6/6)	1.0 ± 0.68 (2.6)	0
2	6	10.0 ± 1.21 (6/6)	2.5 ± 0.42 (6/6)	1.33 ± 0.49 (3/6)	0
3	6	11.5 ± 1.6 (6/6)	3.83 ± 1.01 (6/6)	0	0

All values $\bar{x}$ ± S.E. number of follicles per animal

( ) Number of individuals per group with follicles

Table VIII. Ovarian histology of young females: $\bar{x}$ diameter of follicles.

	Group	N	$\bar{x}$ diameter of follicles	# of individuals with C.L.
Week #1				
	1	6	341 ± 5	0
	2	6	334 ± 3	0
	3	6	391 ± 29	1
Week #2				
	1	6	356 ± 9	0
	2	6	372 ± 11	0
	3	6	382 ± 27	0
Week #3				
	1	6	390 ± 14	0
	2	6	381 ± 9	0
	3	6	370 ± 6	0

All values $\bar{x}$ ± S.E.

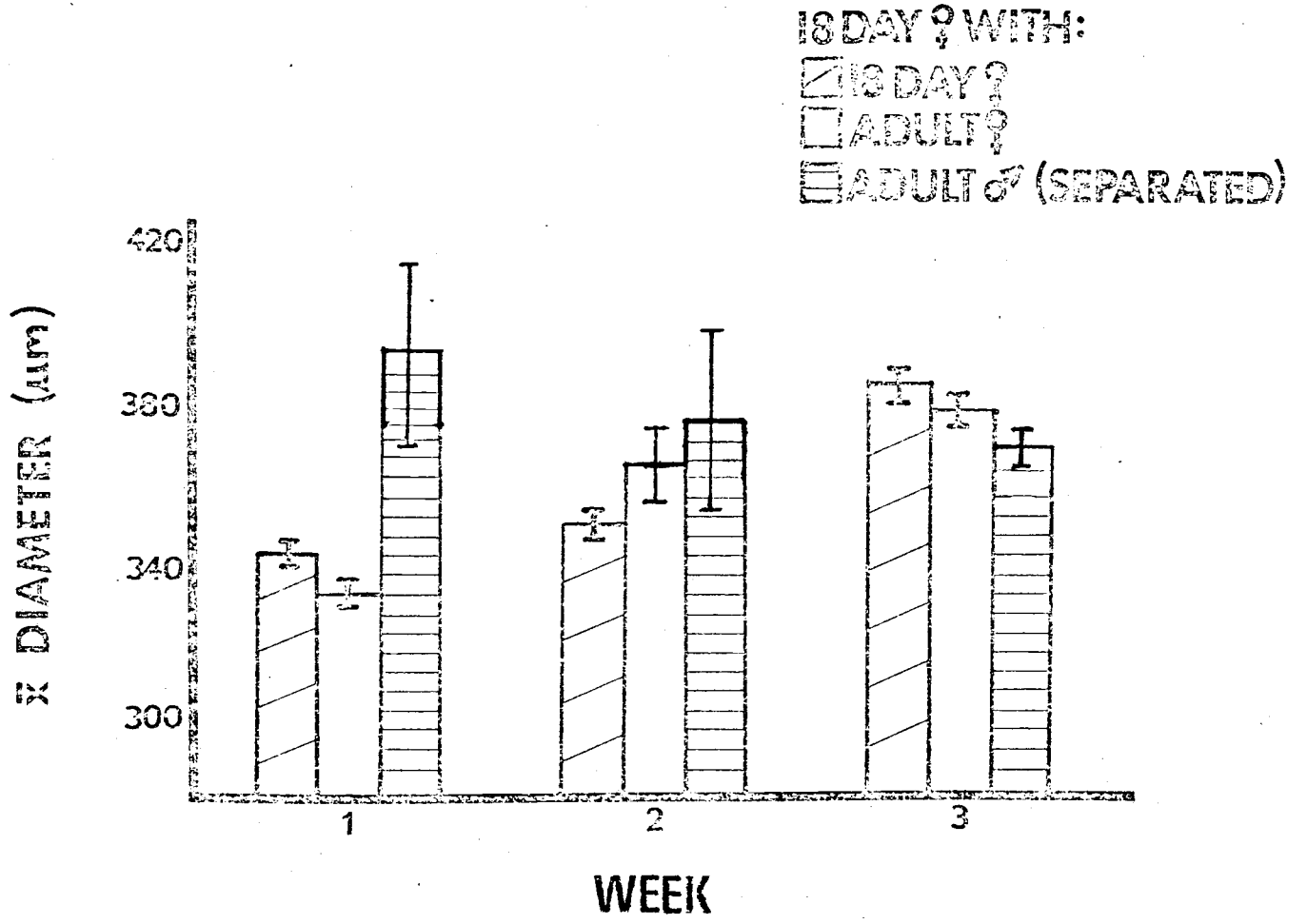

Figure 6. $\bar{x}$ diameters of Graafian follicles in young females.

Table IX. Vaginal perforation in young females.

	Group	N	Open	Closed	Imperforate
Week #1					
	1	12	0	2	10
	2	8	0	2	6
	3	11	1	7	3
Week #2					
	1	12	0	5	7
	2	10	1	6	3
	3	8	3	5	0
Week #3					
	1	12	3	4	5
	2	10	2	6	2
	3	11	5	5	1

Females with imperforate vaginae were assumed not to have reached first estrus.
Females with closed vaginae were assumed to be entering or leaving estrus.
Females with open vaginae were assumed to be in estrus.

IV. DISCUSSION

Twelve-Week Breeding Experiment

As expected, results from this experiment indicate a greater reproductive success in females mated as adults than in those mated at 18 days. However, successful reproduction in young female voles has not been previously reported in the literature. Since some females in this group gave birth to litters 22-23 days after mating it appears that females in this species may, in some cases, reach puberty even before the age at which they are weaned in the laboratory.

The percentage of adult females producing at least one litter falls within the normal range for the breeding colony as a whole. Likewise, the size of the first litter for adult females in this experiment agrees with data presented by Gilston (1976) for this species. An explanation for the larger first litter sizes in the young female group is not immediately clear. It may be that a mechanism has developed in this species to limit litter size by selective embryonic mortality. It would be advantageous for a female to give birth to no more offspring than she can successfully rear. It does appear that some embryonic death occurs in the uteri of adult females since in the majority of cases a greater number of corpora lutea exist than embryos. Similar evidence of embryonic death does not appear in the young female group. Whether this hypothesis can adequately explain these results is clearly open to question; the

matter deserves further investigation.

The similarity between groups in mean weight of offspring at birth would indicate that pregnancy proceeds normally in young females. No apparent differences in general health were detected between the offspring of the two groups at birth. The lower survival rate for offspring born to young mothers may be accounted for by failure in the later stages of lactation. Examinations of the bodies of dead offspring indicated that in nearly all cases death occurred after 9-12 days of age. The stunted appearange and dull pelage of many of the surviving young also evidence a failure of lactation.

Adult females, almost without exception, became pregnant soon after parturition. However, in only one case was successful post-partum mating conclusively exhibited in the young female group. In all other females in this group either mating was unsuccessful or was delayed during lactation. It seems unlikely that delayed implantation could explain the prolonged period between first and second litters, since this phenomenon is not common in the genus Microtus and has not been noted in M. canicaudus. It may also be possible that post-partum mating occurred, but resulted in pseudopregnancy. Pseudopregnancy appears to last for between one and two weeks in M. canicaudus (see below). A pseudopregnancy lasting approximately ten days could explain the discrepancy in time between consecutive litters in young females as opposed to adult females.

Ovarian Histology During Pregnancy and Pseudopregnancy

There appears to be no significant difference in size of Graafian follicles between adult and young pregnant females. Also, during the final week of pregnancy nearly all individuals in both groups had some follicles exceeding 400 μm in diameter. Since all follicles in this size category had large, well developed antra it appears likely that ovulation could occur in young females following parturition. The inability of young females to become pregnant in the immediate post-partum period may therefore, be due to factors other than failure to ovulate.

Neither young nor adult females exhibited a pattern of consistent follicular growth throughout pregnancy. This compares favorably with results from other studies on microtine rodents. Breed and Clarke (1970b) reported a period of rapid follicular growth during the first 3-5 days of pregnancy in M. agrestis. Except for a marked decrease on day 10, the mean size of Graafian follicles remained constant during the rest of pregnancy. The absence of large follicles immediately after mating followed by rapid increase in follicular diameters has also been reported for M. orchrogaster (Martin, Stehn, and Richmond, 1976). A similar pattern of follicular development may occur in M. canicaudus as well. However, since no histological examinations were made until 7 days post-pairing, the growth period would not have been detected in this study.

The mean diameters of Graafian follicles for both adult and young females were smaller than those reported by Breed and Clarke (1970b) for adult M. agrestis. However, in their study, Breed and Clarke considered only those follicles which exceeded 400 μm in diameter. In this study some follicles with well developed antra were found which measured less than 400 μm in diameter; these were considered Graafian follicles and were used in calculations of mean follicular diameters.

The continued growth of corpora lutea throughout pregnancy in both adult and young females is in agreement with results obtained for adult M. agrestis (Breed and Clarke, 1970b). However, the maximum diameter of corpora lutea attained by M. canicaudus appears to be greater than that of M. agrestis. An explanation for the larger size of corpora lutea in young pregnant females than in adult females is not apparent. However, this is yet another indication that pregnancy proceeds normally in young females.

The number of corpora lutea did not appear to increase during pregnancy in either experimental group. However, in the adult female group the number of corpora lutea tended to exceed the number of embryos. Similar findings have been reported for M. agrestis (Breed and Clarke, 1970b).

Pseudopregnancy appears to last for between 1 and 2 weeks in young females mated to vasectomized males. At the end of the first week corpora lutea were present which were indistinguishable from those of pregnancy. No corpora albicans were observed at this time. By the end of the second week corpora albicans were found in all

ovaries. Some second and third week ovaries also contained new sets of corpora lutea. In these cases the corpora albicans were diffuse and an accurate count was not possible. Presumably the constancy in corpora lutea diameter in this group during weeks 2 and 3 is due to the occurrence of second and perhaps third pseudopregnancies in these females.

Breek and Clarke (1970b) reported a 9 to 10 day pseudopregnancy in M. agrestis. Recently, Milligan (1975a) found short-lived corpora lutea lasting 2 to 4 days in female M. agrestis prevented from completing copulation. The absence of degenerating corpora lutea at the end of the first week in this study indicates that at least the first pseudopregnancy in M. canicaudus lasts longer than 7 days.

Effects of Social Conditions on Sexual Development

Exposure to an adult male did not appear to have a stimulatory effect on overall growth in young females. Though the experimental designs were somewhat different, similar results have been obtained in studies on laboratory mice (Vandenbergh, 1967; Kennedy and Brown, 1970).

At the end of one week the reproductive tracts of young females exposed to males were not significantly heavier than those of young females exposed to adult females. However, the greater variability in the male-exposed group indicates that there were some estrous females in this group at the time of sacrifice. Although the mean reproductive tract weight of the male-exposed group did not increase

significantly during the final two weeks of the experiment, the amount of variation decreased. This appeared to be due to a decrease in the number of very light reproductive tracts. This may be an indication that a greater proportion of females in this group either were in estrus or had been in estrus earlier.

Groups of females exposed either to adult females or to other young females showed an increase in mean reproductive tract weights throughout the three-week period. The lack of significant difference in mean reproductive tract weights between groups at the end of the experiment indicates that comparable numbers of females had reached estrus regardless of the social situation under which they had been maintained.

Perforation of the vagina has been used as an indicator of the onset of estrus (Richmond and Conaway, 1969). Females that have never achieved estrus generally have imperforate vaginae. Those in estrus have open vaginae, while those that have left the estrous state have closed vaginae. In this study females whose vaginae were closed were assumed either to have been in estrus earlier or else to have been entering estrus at time of death.

The greater proportion of females with open vaginae in the male-exposed group during all three weeks of the experiment is an indication of a greater incidence of estrus in this group. This is in agreement with findings by Richmond and Conaway (1969) for adult M. ochrogaster. The increase in numbers of females achieving first vaginal estrus during successive weeks in all experimental groups confirms the evidence from mean reproductive tract weights. It

appears that regardless of social stimulus, a large percentage of females have reached estrus by approximately 6 weeks of age.

Females exposed to other females tended to show an increase in size of Graafian follicles throughout the experiment. This is in agreement with data for these groups concerning reproductive tract weights and vaginal condition. As expected, females exposed to males showed a large mean diameter for Graafian follicles initially. Although the decrease in follicular diameters for this group is not significant between successive weeks, it is difficult, in light of data for other reproductive parameters, to explain the smaller size of follicles at the end of the experiment. However, at the end of three weeks all females in this group had some follicles that exceeded 400 μm in diameter. Since follicles of this size appear to be mature Graafian follicles, the smaller mean diameter may not be of great importance.

Recently Milligan (1974; 1975b) has reported spontaneous ovulation in large numbers of single female M. agrestis separated from males by hardware-cloth barriers. In contrast, only one female in this study was found to have ovulated after being exposed to a male behind a barrier. The contradiction in results may be explained by species differences. However, it may also be due to differences in experimental procedures used in the two studies. In Milligan's studies the males were housed in small wire mesh tunnels placed directly in the females' cages. This may have allowed for more direct stimulation than the caging method used in the present study. Also, females in these studies were removed daily for vaginal smears.

It may be that performing vaginal lavages was enough added stimulus to bring about ovulation.

V. SUMMARY

Twelve-Week Breeding Experiment

Female M. canicaudus were shown to be capable of becoming pregnant as early as 18-20 days of age. As expected, reproductive success was much greater for females mated as adults than females mated at 18 days. Pregnancy appeared to proceed normally for young females and healthy appearing litters were produced of comparable weight to those of adult females. Offspring survival to 18 days and mean weight of surviving young were much greater for litters born to adult mothers. Adult females showed a much greater incidence of successful post-partum mating than did young females. Consequently, adult females produced a greater mean number of litters during the 12-week experimental period than did young females.

Ovarian Histology during Pregnancy and Pseudopregnancy

Corpora lutea in both young and adult females increased in size throughout pregnancy. Young females had significantly larger corpora lutea at mid-pregnancy than did adult females. However, by the end of pregnancy there was no significant difference in corpus luteum size.

There was no significant increase in follicular size during pregnancy in adult females. In young females follicles increased in size during the second week of pregnancy but decreased during the

final week of pregnancy. Females in both age groups had Graafian follicles exceeding 400 μm in the later stages of pregnancy. Therefore, it appears that follicular development is not a factor in the failure of young females to become pregnant soon after parturition.

Pseudopregnancy lasts for between 1 and 2 weeks in young females mated to vasectomized males. Ovaries of females killed at 2 and 3 weeks after mating contained corpora albicans and in some cases new corpora lutea. The presence or absence of new corpora lutea did not appear to have an effect on follicular size.

Effects of Social Conditions on Sexual Development

Exposure to a strange male increased the sexual development of 18-day-old females to a greater extent than did exposure to either young or adult females. Reproductive tract weights, mean diameters of Graafian follicles, and incidence of vaginal estrus were all greater in the male-exposed group during either the first or second weeks of treatment. No consistent differences in these parameters were apparent between the two female-exposed groups. By the end of 3 weeks large numbers of females in all experimental groups appeared to have reached first estrus.

Exposure to males seemed to have no stimulatory effect on overall growth of 18-day-old females. There was no significant difference in mean body weight between experimental groups at any week.

Exposure to a male without physical contact does not appear to be an adequate stimulus for ovulation. In only one case were corpora lutea found in females exposed to males behind a hardware-cloth barrier.

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APPENDIX

APPENDIX I

TISSUE PREPARATION

60 min. 85% Ethanol

30 min. 95% Ethanol

30 min. 95% Ethanol

30 min. 100% Ethanol

30 min. 100% Ethanol

30 min. 100% Ethanol

30 min. ½ 100% Ethanol ½ Xylene

15 min. Xylene

15 min. Xylene

15 min. Paraplast

60 min. Paraplast

15 min. vacuum infiltration

Mount in tissue ring

APPENDIX II

STAINING TECHNIQUE

5 min. Xylene

3 min. Xylene

3 min. ½ Xylene ½ Ethanol

3 min. 100% Ethanol

3 min. 95% Ethanol

3 min. 80% Ethanol

3 min. Distilled H_2O

6 min. Hemotoxylin

Rinse in running water to remove excess stain.

3 dips in 1% Acid Alcohol

Rinse in running water to remove excess Acid Alcohol.

Dip in 1% Ammonia water until tissue turns blue.

10 min. running water

3 min. Eosin

2 min. 95% Ethanol

2 min. 100% Ethanol

2 min. 100% Ethanol

3 min. Xylene

3 min. Xylene

Acid Alcohol: 1 ml HCl in enough 70% EtOH to make 100 ml