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I am submitting herewith a thesis written by Kitty S. Parker entitled "Male Influence on Estrous Cycle Phenomena in the Rat." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

R. L. Murphree

R. L. Murphree, Major Professor

We have read this thesis
and recommend its acceptance:

Robert R. Shrode

W. V. Shirley

Accepted for the Council:

L. Evans Pond

Vice Chancellor
Graduate Studies and Research

MALE INFLUENCE ON ESTROUS CYCLE PHENOMENA IN THE RAT

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Kitty S. Parker

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DEDICATION

This thesis is dedicated to Mrs. Grace Hitch Speer, recently retired after 19 years as secretary in the Department of Animal Science, The University of Tennessee, Knoxville. To Grace, befriending graduate students was simply "one of the benefits to the job." We have shared her home, her enjoyment of life and we have grown as individuals by having known her. As one of many who has shared this privileged friendship, the author wishes to dedicate this thesis--as a thank you from all.

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ABSTRACT

Sixty-four colony-bred Sprague-Dawley female rats were used in examining the effect of the male presence on female estrous cycle interval. Females were randomly assigned 4 per cage to 16 cages. Vaginal smears were taken daily during a 28-day period. Detection of cornified cells was assumed to be associated with estrus. There was considerable variability in individual estrous cycle length. The mean number of cycles exhibited was 3.84 during the 28-day premale period. The expected mean number of cycles would have been 4.7 to 5.4 if all females had exhibited regular estrous cycles of 5 to 6 days duration. The average length of estrous interval prior to exposure to males was 5.18 days.

On day 28 a mature intact male was introduced within each pen. Prior to introduction, males were housed separately from females; mixing of colony odors was minimized by room ventilation. Males were removed from females 17 days following introduction. Following introduction of males, an average estrous interval length of 5.28 days was observed. This did not differ significantly from the 5.18-day average interval observed prior to exposure to males. Thus, no significant male influence upon estrous interval length was demonstrated.

One-hundred percent of the females, including those showing abnormal estrous patterns prior to the introduction of the males, showed cornified cells within the first 5 days following introduction of the male and 76 percent of this number conceived within this first 5-day period. The difference between percent showing cornified cells

and the percent conceiving suggests that observation of cornified cells may not be a reliable indicator of sexual receptivity in the rat.

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CHAPTER I

INTRODUCTION

The influence of the male on female reproductive phenomena has been the focus of increasing research interest particularly within the framework of animal breeding and reproduction. Specifically, pheromones produced by the male have been investigated for their possible role in reducing the interval between estrus periods, thus hastening sexual receptivity and ovulation by the female to coincide with the presence of the male. While research has shown that similar components exist to a degree in most species studied, additional investigation has indicated the influence of the male in modifying the estrous cycle may not be attributable to any one specific stimulus but to a combination of many factors.

Fertility is of primary importance within the animal production industries. Accurate detection and synchronization of estrus in large cattle herds would greatly increase production, simplify management and improve marketing by increasing efficiency of artificial insemination techniques with higher conception rates and production of a more uniform calf crop. These advantages are similarly applied in other production species where low fertility rates cause billions of dollars in economic losses annually. The utilization of an efficient estrus inducing component could play a vital role in the future of animal reproduction.

The study here reported was conducted to further examine the influence of the male on estrual cycle phenomena in the female rat.

CHAPTER II

REVIEW OF LITERATURE

Several studies have indicated that the presence of the male may be a major factor affecting the female reproductive cycle (Everett, 1940; Whitten, 1956; Hughes, 1964; McDonald, 1975). Investigators have given the term "pheromone" to the chemical substance secreted externally by one individual which affect the behavior of other animals of the same species. Such stimuli acting via the olfactory senses may convey information of territory, aggression, fear, sex and sexual state and thus produce modifications in development, behavior and reproductive activity (Swyer, 1967; Turner and Bagnara, 1971). It is through such peromonal effects that the male influence is believed to function and that females, within a species, may modify estrual behavior among themselves (Parks and Bruce, 1961).

Two general types of pheromonal actions have been recognized in mammals. They are called signaling and priming pheromones. Bronson and Whitten (1968) and Bronson (1971) distinguished between the two, depending on how directly they acted upon the central nervous system to modify the behavior of the recipient. Signaling pheromones were thus given credit for the immediate alterations in motor activity by the recipient animal, while the adjective, priming, was applied to those substances involved in the induction of endocrine and neuroendocrine activity within species (Bronson, 1971). He postulated the following pheromones and their related effects in the mouse:

Signaling Pheromones	Priming Pheromones
1. Fear substance	1. Estrus-inducer
2. Male sex attractant	2. Estrus-inhibitor
3. Female sex attractant	3. Adrenocortical activator
4. Aggression-inducer	
5. Aggression-inhibitor	

As the male effect upon estrous cycle phenomena is the focus of this paper, the review of literature will largely be restricted to effects of a priming nature.

Although the specific pheromonal component has not yet been chemically identified in the male rodent, studies have indicated the presence of an estrus modifying constituent in the urine of the male (Marsden and Bronson, 1964, 1965; Bronson and Whitten, 1968; Vandenbergh, Whitsett and Lombardi, 1975). Vandenbergh et al. (1975) found this substance to be androgen-dependent and bound to a protein in the male urine. The androgen-dependency was demonstrated by the finding of this component in urine of intact males and adrogenized females but not in the urine of castrated males (Bronson and Whitten, 1968).

Gonadal control of pheromonal activity and sensitivity was investigated by Carr and Caul (1962). Their work indicated that both intact male and female rats could discriminate between odors from sexually active and gonadectomized members of the opposite sex. Bruce (1965) reported that pheromonal activity was altered by castration. In her work, groups of male mice were castrated prior to puberty (4 weeks of age), shortly after puberty (8 weeks of age), while older

males were castrated at 8 months of age. Prior to castration all animals were tested for pheromonal activity. The results indicated that castration of males before puberty prevented the development of pheromonal activity while castration of young mature and older males abolished pheromonal functions. In a similar study, Carr, Loeb and Wylie (1966) found that both prepuberal and castrated male rats were unable to distinguish between receptive and nonreceptive females.

Although pheromone effects may be exerted via ingestion and absorption, olfactory reception of odors is apparently the major pathway of pheromonal transmission in mammals (Marsden and Bronson, 1965; Bronson, 1971). Marsden and Bronson (1964) found that introduction of male urine into the nares of female mice gave the same response as the presence of the male; this suggests the existance of substances in urine that influence pituitary gonadotropin secretions through the olfactory sense. Removal or impairment of the olfactory bulbs has been shown not only to deter sexual behavior but also to abolish the estrous cycle in mice (Parks and Bruce, 1961; Carr et al., 1966; Whitten, Bronson and Greenstein, 1968).

Once the male priming cue is perceived by the recipient female, a series of poorly understood physiological and neuroendocrine reactions apparently occur. The existence of an interrelationship among pheromones, adrenal activation and hormonal function has been the focus of several investigations. Bronson (1971) noted that priming pheromones not only play a role in the transfer of information that must precede insemination but also have relatively direct effects on

anterior pituitary function by altering the release of FSH, LH, and ACTH or prolactin in the recipient female. Evertt (1940) demonstrated that persistent estrous patterns found in female rats isolated from males were restored to normal cycles by the daily administration of progesterone; while Nequin and Schwartz (1971) found that adrenal activation was necessary for LH release via the intermediary action of progesterone. Priming pheromones serve, in part, in an activator capacity by influencing pituitary gonadotrophin secretions (Bronson, 1971).

The male influence in modifying female estrual behavior may be divided into three principal effects:

1. Acceleration of onset of puberty
2. Normalization of the female estrous cycle; reduction of interval between estrus periods with subsequent estrual sychronization.
3. Pregnancy block following exposure to a strange male.

I. ACCELERATION OF PUBERTY IN THE FEMALE BY MALE INFLUENCE

The acceleration of puberty and induction of cycling in immature female mice has been the subject of numerous investigations. Marsden and Bronson (1964) found that exposure to male urine prior to pairing accelerated sexual development in virgin female mice. This effect was not observed in control mice not exposed to male urine or in mice exposed to the urine of females. The same authors later evaluated species specificity in the acceleration of puberty; their results

indicated that only male mice of the same species as the females under study were effective in inducing puberty (Marsden and Bronson, 1965). Bronson and Whitten (1968) attributed the induction of cycling by the immature females to a component in the male urine.

Zarrow, Ester, Denenberg and Clark (1970) examined the effect of male presence on the stimulation of ovulation. In this study, a control group of female mice living in a male-free environment were injected with pregnant mare serum hormone (PMS). Of these, 55 percent ovulated, and an average of 6.8 eggs were recovered upon sacrifice. In the group exposed to a male in addition to injection with PMS, 81 percent ovulated with an average of 12.5 eggs being recovered. In a related study, Zorrow et al. (1970) found that a mature testis was necessary for the male to affect a significant increase in ovulation rate among immature females treated with chorionic gonadotrophin (HCG).

The influence of the female presence upon females has confounded the study of cyclic phenomena in mice. Michael and Keremel (1968) reported that the presence of males increased the rate of sexual maturation and prevented pseudopregnancy which is noted in group-housed females in the absence of males. Conversely, among females not exposed to males, sexual maturation was retarded.

Vandenbergh (1973) found that a male placed with immature female mice accelerated sexual maturation both in isolated and grouped females. The effect was especially pronounced in isolated females, which observation he suggested supported the concept that among grouped females on the onset of sexual maturity is inhibited by the presence of juvenile

female cage-mates or their bedding. Since the presence of the adult female was found to accelerate slightly the attainment of sexual maturity, it was suggested that the inhibitory stimulus was produced by the juvenile rather than by the adult female. The question remains as to whether this inhibitory effect is produced by the individual immature female or solely by immature females living in groups.

II. THE INFLUENCE OF THE MALE IN NORMALIZING THE FEMALE ESTROUS CYCLE, REDUCING INTERVAL BETWEEN ESTRUS PERIODS AND AFFECTING ESTRUAL SYNCHRONIZATION

The role of the male influence upon the female reproductive cycle is to enhance acceptance and fertility by the recipient female. In concurrence with this physiological objective, the male effect has been demonstrated to normalize the aberrant estrous behavior that predominates among females isolated from males.

Everett (1940) noted that separation from males resulted in persistent estrous patterns in the female rats. In mice, "the Lee-Boot effect" is a term used to describe the estrual cycle abnormalities commonly observed in females caged in isolation from males (Lee and Boot, 1955, 1956). Whitten (1957) noted that female mice maintained in groups apart from males showed a higher incidence of anestrus and pseudopregnancy than when caged individually. He later found that although, among grouped females isolated from males, there was a suppression of estrous cycles, in individually caged females, estrous cycles of 5 and 6 days rather than the normal 4-day cycles predominated.

When, however, a male was placed in a basket within the females' cages, the majority of cycles were of 4-day duration (Whitten, 1958).

Lamond (1959) found that a prolonged diestrus was exhibited by female mice previously housed singly and then grouped. This effect was not produced when a male was present. Bronson (1968) suggested that cycles ranging up to 11 to 12 days in the laboratory mouse should not be considered abnormal, depending upon the olfactory environment in which the females are housed and upon the strain under consideration.

A similar response has been described in rats. Hughes (1964) noted that among females crowded in stock colony cages (20 to 22 per cage), the majority exhibited cycles of more than 4 days duration. When the same animals were changed to experimental cages at a lower density (6 to 8 per cage), there was a progressive reduction, from 9.7 to 4.5 percent, in the proportion of animals exhibiting cycles longer than 4 days. Following introduction of the male, the incidence of estrus was not random; the number exhibiting estrus was more than expected for the first 3 days, especially on the first day. Although a shortening of the estrous cycle was evident and was attributed to the presence of the male, Hughes concluded that the estrous cycle of the rat was less susceptible to male influence than that of the mouse.

The shortening of the estrous cycle and pursuant synchronization of heat has been attributed to various factors, the male influence being a primary effect. Bruce (1965) found that shortening of the cycle occurred only in females exposed to intact males. Whitten (1956, 1957) found a concentration of matings (over 50 percent) on the third night following pairing of formerly isolated female mice. The effect did not

depend on age or previous parity of the females, and, also, the frequency of mating was altered by placing the females in a cage recently contaminated by males prior to pairing.

Lamond (1959) also noted that mating frequency of mice was not consistent with a hypothesis of random occurrence over a 5-day period as would be expected if the average cycle length were 5 days. The majority of matings in his study occurred on the third and fourth days after introduction of the male; unlike the previous study by Whitten, age was a significant effect on the distribution of matings.

The synchronization of estrous periods among female mice also had been attributed to the male influence. Marsden and Bronson (1964) found that exposure of virgin females to urine from intact male mice for 2 days prior to pairing significantly altered the expected pattern of estrus. A higher proportion of mice exposed to male urine exhibited estrus during the 2 days after pairing than did females exposed to female urine or control mice not exposed to urine. This same synchronizing effect of the male was confirmed by Chipman and Fox (1966) in their study of the wild house mouse. Similar data from rats were not recorded.

Estrual synchrony due to environmental crowding was examined by Marsden and Bronson (1965). A high degree of estrus synchronization was found in the laboratory mouse on the first night after pairing with males. These authors suggested that apparent synchrony of estrous patterns in grouped females may be attributed to a release from the estrus suppressing effects of crowding. It is apparent from this that

the male effect is only one of many factors which may influence synchronization of heat in animals.

III. PREGNANCY BLOCK FOLLOWING EXPOSURE TO A STRANGE MALE

Pregnancy block in mice was first described by Bruce (1960). Although not reported in other species, it is an important male effect which may have implications in fertility in other species.

Bruce (1960, 1960a; Parkes and Bruce, 1961, 1962) found that both pregnancy and pseudopregnancy failed to occur when recently mated females were housed near or with a strange male, particularly a male of a different strain. If males from a different strain of mice were placed with the female for 2 to 3 days on the following mating, 70 to 80 percent of the females returned to estrus within a week; genetic marking of any offspring that resulted showed that they were sired by the second male, the original pregnancy having been blocked. The smell of the strange male was credited as being the primary stimulus in the block since a similar effect could not be demonstrated in females in which the olfactory bulbs had been removed (Bruce, 1960); Parkes and Bruce (1961) demonstrated that the olfactory stimuli of the strange male must be of 2 to 3 days duration for maximum blocking effect. Bruce (1960) found also that castrate males did not produce this effect; the capacity of the young male mouse to inhibit implantation developed at the same time as sexual maturity (Bruce, 1965).

IV. THE EFFECT OF THE MALE IN ECONOMIC SPECIES

Although investigation of the male influence in altering female reproductive response in species of larger animals has been limited, evidence of a male effect has been reported in swine and sheep.

The role of the boar in stimulating the onset of estrus in prepuberal gilts was examined by Byrum (1957). He found that 68 percent of gilts (average age 174 days) housed in lots with boars exhibited first estrus during a 38-day observation period as compared to 32 percent estrus in gilts not pastured with boars. The gilts with boars had an average interval to estrus of 11.7 days as compared to 29.5 days for gilts not with boars. Kinsey and Zimmerman (1977) made observation also of the hastening of the onset of puberty in prepuberal gilts by exposure to a boar.

Signoret and du Buisson (1961) reported that the odor of the boar elicited the immobilization reflex in the estrous sow so that she would stand rigidly to pressure over the back. Surgical removal of the olfactory bulbs impaired mating ability since the majority of the sows under study demonstrated periods of anestrus or complete anestrus (Signoret and Mauleon, 1962). The immobilization reflex in the female was similarily demonstrated in the absence of the boar in 46 percent of estrous sows by the use of androgen aerosols and in 62 percent of those exposed to a mixture of preputial fluid and urine from the boar (Melrose, Reed and Patterson, 1971).

In sheep, Schinckel (1954) suggested that a synchronization of estrous cycles occurred in teased ewes. Forty-two percent of ewes

placed with vasectomized teaser rams 14 to 16 days prior to the introduction of breeding rams lambed in the first part of the lambing season as compared to only 12 percent early lambing in non-teased ewes.

Ioset (1956), in analyzing the breeding records for a 6-year period in the ewe flocks maintained by the University of Tennessee Experiment Stations, concluded that the presence of the ram during the transition period from non-breeding to breeding season increased fertility during the early part of the breeding season.

Although similar suggestions are commonly heard as to a male influence in cattle, no published studies were found. Much additional research information is needed to provide a complete and clear picture of the male influence on female estrual behavior, especially in economic animal species.

CHAPTER III

MATERIALS AND METHODS

Sixty-four colony-bred, mature female Sprague-Dawley rats were used in this study. Animals were maintained under 10:14 dark-light schedule in a temperature-controlled room (average 25.5° C). A commercial pelleted rat chow and water were provided ad libitum. Rats were individually identified by clipping toes.

Females were randomly assigned 4 females per cage to 16 wire bottom cages. The cages were mounted in a 5-tiered, 2-sided rack. The top and bottom tiers of each side were not used. Each tier contained 3 cages measuring 43 cm wide x 24 cm deep x 19 cm high. The rack was turned daily in an effort to minimize variations in ventilation and lighting.

Vaginal smears were taken daily between 9 and 11 a.m. Smears were obtained with cotton swabs moistened in an isotonic saline solution, inserted into the vagina and rotated to collect cells. The swab was immediately rolled onto a clean 7.62 cm x 2.54 cm glass slide and the slide examined under a light microscope (100x magnification). Prior to examination, one drop of a methylene blue solution was mixed with the cells to facilitate classification of cell types. Detection of cornified cells was assumed to be associated with estrus. Vaginal smears were taken for 28 consecutive days to establish individual estrous cycle patterns. These data were used as the control period prior to the introduction of the male and in determining the effect of the male upon estrous cycle length.

Prior to placing males with females, males were maintained in separate cages positioned in the opposite ends of the colony room. The colony room measured 7.3 x 11 meters. Mixing of air in the room was minimized by an exhaust fan mounted in the center of the ceiling of the room. Although no measurements were made, air movement prevented accumulation of odors within the room.

Following completion of vaginal smearing on day 28, a mature Sprague-Dawley male was placed in each cage containing females. Daily examination of vaginal smears following the introduction of the male were continued for 17 days. Presence of sperm was accepted as evidence of mating. Since, in some females, sperm were detected on more than one day, the data of conception was calculated by subtracting 23 days, the accepted period of gestation within the Sprague-Dawley colony, from the date of birth of the litter.

Females were palpated for pregnancy on the ninth and fifteenth days, following the introduction of the male, and the males were removed from all cages on the seventeenth day. Pregnant females were moved to individual littering cages for the duration of gestation. Date of birth of litters was recorded.

Data were analyzed with the aid of an IBM 360/65 computer using the Statistical Analysis System (SAS) programs. Procedures and options within the SAS package that were incorporated into the analysis included calculations of means and analysis of variance. A t-test was made to determine male effect upon estrous period interval; frequency of estrus and mating following the introduction of the male were tested by Chi-Square analysis. In order to remove position influences, tier-in-the-

rack and cage effects were tested and found to be non-significant; therefore, these effects were not included in further analysis of the data.

Two of the initial 64 females were eliminated from all analysis due to failure to cycle. There were missing values for some variables for a few additional females. Animals with missing data were omitted from analysis of those specific variables only.

CHAPTER IV

RESULTS AND DISCUSSION

The major findings in this study are summarized in Table I. During the 28-day period prior to the introduction of males, the average number of estrous cycles per female was 3.84 $\pm$ 0.5 with an average length of 5.18 $\pm$ 0.6 days. The range in cycle length varied from 2 to 13 days. If all females had been exhibiting regular cycles of 5 to 6 days duration, then the mean number of cycles would have been 4.7 to 5.4 rather than the observed 3.84 cycles. This indicates considerable variability in estrous cycle length. An indication of within-female variability of cycle length is the mean coefficient of variation of 40.7 percent calculated on a within-female basis and averaged.

The mean interval between last detection of cornified cells prior to male introduction and first finding of cornified cells following introduction of the males was 5.28 $\pm$ 0.8 days (Table I). This interval of 5.28 days is not significantly different from the 5.18 days for the pre-male interval, indicating that introduction of the male did not influence estrual cycle length.

The finding of cornified cells in the vaginal smear is generally considered to be synonymous with sexual receptivity to the male. This is not necessarily true, however, as indicated by the percentage of females showing cornified cells as compared with those conceiving within the first 5 days after introduction of the male (Table II). The overall difference is statistically highly significant ($P<.01$).

TABLE I

OVERALL MEANS AND STANDARD ERRORS OF CRITERIA RECORDED FOR FEMALE RATS

Variable	No. of observations	Means ± Standard Error	Range (days) Low	Range (days) High
Pre-male cycles[a]	62	3.84 ± .5	1.0	6.0
Pre-male interval[b]	62	5.18 ± .7	2.0	13.0
Post-male interval[c]	40	5.28 ± .8	2.0	19.0
Post-male first estrus[d]	61	2.07 ± .3	1.0	10.0
Post-male second estrus[e]	25	6.16 ± 1.2	2.0	16.0
Post-male third estrus[f]	5	6.40 ± 2.9	3.0	10.0
Day conceived[g]	57	4.37 ± .6	1.0	17.0

[a]Average number of estrous cycles observed prior to the introduction of the male.

[b]Average number of days between estrous periods prior to introduction of the male.

[c]Average number of days between the last observation of cornified cells prior to the introduction of the male and the first observation of cornified cells or sperm following the introduction of the male.

[d]Average number of days between the introduction of the male and the first observation of cornified cells or sperm.

[e]Average number of days between the first estrus and the second observation of cornified cells or sperm following introduction of the male.

TABLE I (CONTINUED)

[f]Average number of days between the second estrus and the third observation of cornified cells or sperm following introduction of the male.

[g]Average number of days following introduction of the male at which females conceived as calculated from actual littering dates.

TABLE II

RELATIONSHIP OF PRESENCE OF CORNIFIED CELLS AND CONCEPTION FREQUENCY IN RATS

Finding of cornified cells during						Number and Percent Conceiving within first 5 days after introduction of male		
5-day period prior to introduction of male			First 5-days after introduction of male					
Day	No.	Percent	Day	No.	Percent	Day	No.	Percent
-5	12	20	-1	33	55	+1	16	26
-4	9	15	+2	8	13	+2	5	8
-3	9	15	+3	15	25	+3	9	15
-2	2	3	+4	4	7	+4	7	12
-1	17	28	+5	0	-	+5	9	15
	49	81		60	100		46	76

Contrary to the generally accepted statement that female rats show cornified cells only one day during the estrual cycle, 60 of 62 females in this study exhibited cornified cells for 2 or more successive days during one or more cycles prior to introduction of the males. For example, one female exhibited cornified cells for 20 consecutive days prior to introduction of the male and then conceived on the first night with the male.

Data on 22 females were not included in the analysis of the male effect; 17 had cornified cells the night before introduction of the males, and 5 others were eliminated either because they had not exhibited a prior estrous period or had an abnormally long cycle immediately prior to introduction of males.

Sixty-one females exhibited cornified cells at a mean interval of 2.07 $\pm$ 0.3 days following introduction of the male (Table I). Twenty-five of the 61 males subsequently showed cornified cells and/or sperm a second time at an average of 6.16 days; 5 of these females exhibited cornified cells or sperm a third time 6.40 days later. Three of the 25 rats which exhibited cornified cells or sperm a second time may have experienced pseudopregnancy since the interval between detection of sperm was of 10 to 12 days duration. This agrees with Nalbandov (1976) who reported that pseudopregnancy in the rat follows stimulation of the cervix during a sterile copulation. The corpora lutea of pseudopregnancy regresses about 12 days following ovulation, and the estrous cycle is resumed.

The average interval from introduction of the males to conception for 57 females which produced litters was 4.37 days with a range of 1 to 17 days (Table I).

The number of females with cornified cells during the 5-day interval prior to introduction of the males, the number of females with cornified cells and/or sperm during the 5-day interval after introduction of the males and the number of females conceiving during the same period are shown in Table II. Only 81 percent of the females showed evidence of a cyclic pattern of cornification during the 5-day period prior to introduction of the males as compared to 100 percent showing cornified cells during the first 5 days after introduction of the males. One reason for the apparent discrepancy is the inclusion of all females showing cornified cells after introduction of the males. Thus, females which were exhibiting continuous cornifications were excluded from the analysis of the 5-day premale period but were included for analysis of the period after introduction of the male.

Of the 57 females which conceived during the 17-day breeding period, 76 percent (46) did so during the first 5 nights they were with males (Table II). Of these, 35 percent (16 of 46) conceived during the first night of exposure to the males. This agrees with Hughes (1964) who concluded that the frequency of estrus was higher than expected during the first 3 nights and especially on the first night after introduction of the males to previously crowded female rats. However, the animals in his study were crowded (20 to 22 females per cage) and the numbers per cage were reduced (6 to 8 females) prior to introduction of the males. Throughout the current study, the numbers of females per cage were not changed.

This higher conception rate on the first night of exposure to males in the present study is not different from expectation based on

the number of females showing cornified cells 5 days before introduction of the male (assuming a 5-day cycle). Thus, there is no evidence from this study which suggests that males exerted a synchronizing effect on the estrual cycle of the females. This differs from the results reported by Whitten (1956, 1957, 1959) in his study with mice. He found the highest frequency of matings on the third night after introduction of the males. Lamond (1959) reported that frequency of mating in mice was not consistent with a hypothesis of random occurrence over a 5-day period (assuming a 5-day cycle) since the highest frequency of matings occurred on the third and fourth nights after introduction of males.

One possible explanation for the failure to detect a male effect in the current study was the fact that females and males were housed in the same room throughout the study. Thus, the females could have been exposed to male odors (pheromones?) continuously even though there was no direct contact. However, as indicated in the materials section, males and females were housed on opposite sides of the colony room, and the ventilation was such that transfer of male odors to females was minimized.

A further observation was the finding of sperm in 9 females 3 to 14 days after conception. The frequency of females having postconception sperm was: 3 females on the third day, 1 on the third and tenth days, 1 on the seventh day, 2 on the eighth day and 1 each on the twelfth and fourteenth days.

A future study investigating male effect should use groups of females similar in age, regularity of estrous cycles and parity history. This would permit a study of the male effect upon virgin females as compared to females whose reproductive cycle had been completed by previous conception and gestation. The females in such a study could serve as a self control by introducing the male to females only when they are in diestrus. Males should be kept completely separately from the female colony room until their introduction, which would eliminate possible male odor influnece prior to direct contact. It would be additionally useful to utilize both intact (demonstrated fertility) and vasectomized (demonstrated sterility) males. The intact males could thus breed a determined number of the sample which would permit a comparison of estrus as calculated by littering to conception date with estrus as exhibited by traditional histological evidence of cornified cells in a similar number of females placed with vasectomized males and engaging in sterile matings.

CHAPTER V

SUMMARY AND CONCLUSION

The effect of the presence of the male in influencing estrual cycle phenomena in mature female rats was studied.

During a 28-day period to the introduction of males, the average length of estrous interval in 72 females was 5.18 days. Following introduction of the males, an interval of 5.28 days was observed. This was not significantly different from the premale interval. There was considerable variability in estrous cycle length. The mean number of cycles was 3.84 during the 28-day premale period. The expected mean value would have been 4.7 to 5.4 if all females had been exhibiting regular estrous cycles of 5 to 6 days duration.

Although no shortening of the estrous interval was demonstrted by placing males with previously isolated females, 100 percent of the females, including those showing abnormal estrous patterns prior to the introduction of the male, did show cornified cells within the first 5 days following male introduction, and 76 percent of this number conceived within this first 5-day period. The difference between the percent showing cornified cells and the percent conceiving suggests that observation of cornified cells may not be a reliable indicator of sexual receptivity.

From these results it can be concluded that estrous interval in the female rats in this study was not significantly influenced by the presence of the male.

Further study utilizing homogeneous virgin and previously parous females demonstrating regular estrous patterns exposed to intact and vasectomized males might demonstrate a male influence.

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VITA

Kitty Sue Parker was born in Shelby County, Tennessee on August 20, 1942. She attended Oakville Elementary School and graduated from The Hutchinson School, Memphis, Tennessee in 1960.

In September of 1960, she entered the University of Alabama in Tuscaloosa and graduated in 1965 with a degree in Bachelor of Arts.

She was employed by the Tennessee Department of Public Welfare in Shelby County as a social assistance worker from 1965 to 1968. From 1968 to 1970, she was employed by the American National Red Cross, Service to Military Hospitals as a medical social worker serving at Maxwell Air Force Base, Montgomery, Alabama and Ireland Army Hospital, Fort Knox, Kentucky.

In 1970, she began study in Animal Husbandry at the University of Tennessee, Knoxville. During this time she was selected to be a member of Alpha Zeta honorary agricultural society. In 1973, she entered the Master of Science program in Agriculture at the University of Tennessee.

In September of 1974, she initiated study in Veterinary Medicine at the University of Madrid, Spain. Her degree in Veterinary Surgery was completed in Madrid in July of 1978.