1994

An Evaluation of Factors Affecting the Estrous Cycle of the Djungarian Hamster, Phodopus Sungorus

Roberta W. Ellington
Loyola University Chicago

LOYOLA UNIVERSITY OF CHICAGO

AN EVALUATION OF FACTORS AFFECTING THE ESTROUS CYCLE OF THE DJUNGARIAN HAMSTER, *PHODOPUS SUNGORUS*

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

BY

ROBERTA W. ELLINGTON

JANUARY 1994

ACKNOWLEDGMENTS

I am indebted to my academic advisor, Dr. Rosemary Grady, and to the director of the reviewing committee, Dr. Albert Rotermund, for their encouragement, advisement, and worthy assistance throughout the period of thesis research and writing. I am also grateful to the other members of the thesis committee, Dr. John Smarrelli and Dr. Diane Suter, for their time and patience in evaluating my candidacy for the Master of Science degree.

In addition, I wish to thank Dr. John Janssen and Dr. Ian Boussy who provided valuable statistical assistance. And for technical assistance, I wish to thank Ms. Wendy Chiarmonte and the Loyola University Center for Instructional Design.

Further, I am grateful for my family's patience throughout the period of study, research, and the writing of the thesis.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

ABBREVIATIONS

c	cornified epithelial cell
cn nc	mixture of cornified and nucleated cells
cln lnc nlc	mixture of cornified, nucleated, and leucocyte cells
COH	compensatory ovarian hypertrophy
FSH	follicle stimulating hormone
l	leucocytes
lgn	large nucleated cell
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
n	nucleated epithelial cell
nl ln	mixture of nucleated and leucocyte cells
ovax	ovariectomy
ULO	unilateral ovariectomy

CHAPTER I

REVIEW OF LITERATURE

Introduction

A challenge in the design and implementation of an original biology research project is selecting a suitable organism for study. The organism must be: a) easily handled and maintained, b) a source of demonstrable biological phenomena, c) relatively inexpensive, and d) easily reproducible. For many years, small rodents, such as rats, mice, and Golden hamsters, have been used in various aspects of laboratory research. A newcomer, the Djungarian hamster, *Phodopus sungorus*, recently arrived in the United States. Its use in the laboratory may fulfill some or all of the above criteria.

The Djungarian hamster is a dwarf hamster, weighing 30-45 grams when fully grown. They are dark grey in color with a single black stripe down the back and a white underside. Hamsters in the genus *Phodopus* have been found in Siberia, Kazakh, Northern China, Mongolia, and Manchuria, with the Djungarian hamster, *Phodopus sungorus*, occupying the more northern regions (Niethammer 1990). Their name possibly originates from the Dzungarian Region of China, located in the

northernmost part of Sinkiang Province, which is south of Siberia, east of Kazakh, and west of the Mongolian border, and from the Dzungarian Alat Mountains, which run in an east-west direction along a portion of the western Sinkiang Province border with Kazakh.

There is controversy regarding the nomenclature of the hamster. In two articles, Wynne-Edwards, Terranova, and Lisk (1987) and Wynne-Edwards and Lisk (1987) state that the Djungarian hamster is actually *Phodopus campbelli* and that the Siberian hamster is *Phodopus sungorus*. Other authors continue to use the Djungarian hamster, *Phodopus sungorus* nomenclature. Corbet and Hill (1991) list *Phodopus campbelli* as a questionable subspecies of *Phodopus sungorus*. They state that *Phodopus campbelli* comes from Mongolia, whereas *Phodopus sungorus* comes from East Kazakh and Southwest Siberia. Wilson (1992) lists the same range for *Phodopus sungorus*. He further lists *Phodopus campbelli* as a separate species and gives a wider range than Corbet and Hill, namely, from Sinkiang Province in the West, to Mongolia, to Heilongjiang Province in the East. A third species, *Phodopus roborowski*, has the same range as *Phodopus campbelli* with the addition of the Tuva Region of Russia, located north of the Mongolian border (Wilson 1992).

The source of hamsters for these experiments was the Fred Turek laboratory, Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois. The breeding stock originated near Omsk, located in Southwest Siberia, north of Kazakh (see Materials and Methods). The breeding stock for the Wynne-Edwards hamsters at Princeton University originated in Tuva. In a recent conversation with Dr. Turek, this author was told that his laboratory sees no reason to discontinue the Djungarian hamster, *Phodopus sungorus* nomenclature. Further, the Turek laboratory considers the Djungarian and Siberian hamsters to be the same. This author, therefore, will also call *Phodopus sungorus*, the Djungarian hamster.

The Djungarian hamster was chosen for this investigation because little is known about its reproductive patterns. Yakovenko (1974) originally reported a range of 3-5 day estrous cycles in *Phodopus sungorus (campbelli)*, although Wynne-Edwards, Terranova, and Lisk (1987) later reported 4-5 day cycles in *Phodopus campbelli*. An unpublished observation by the Fred Turek research laboratory at Northwestern University, Evanston, Illinois, states that when the males are left with the pregnant females and new pups as a family, the male assists the new mother. Wynne-Edwards and Lisk (1987) report this same phenomena in *Phodopus campbelli*.

While little has been published about the female Djungarian hamster, males are being studied extensively. Some examples of current Djungarian hamster (*Phodopus sungorus*) research include: photoperiod and daily torper (Kirsch, Ouarour, and Pevet 1991; Ouarour, Kirsch, and Pevet 1991); photoperiod and pineal gland (Stieglitz *et al.* 1991); photoperiod and circadian rhythm (Kliman and Lynch 1991; Puchalski and Lynch 1991); and male gonadotropin-releasing hormone (Yellon, Lehman, and Newman 1990; Buchanan and Yellon 1991; Yellon and Newman 1991).

Various factors that may affect the estrous cycle of the Djungarian hamster, *Phodopus sungorus*, are the subject of this thesis. These include pheromones, estrus suppression, photoperiodism, and unilateral ovariectomy. The estrous cycles of small rodents, such as rats, hamsters, and mice show a similar four or five day pattern divided into four phases (Long and Evans 1922; Kent and Smith 1945; Bingel and Schwartz 1969). During each of the four phases, changes occur in the cells of the endometrial lining of the uterus as well as in the lining of the vagina. Histological examination of cells obtained from vaginal lavage, shows that the reproductive epithelium undergoes continual change throughout the estrous cycle. This same method, which has been successful in other small mammals, is employed in studying the Djungarian hamster.

Vaginal Cytology Of The Estrous Cycle

The day of estrus marks the beginning of a new cycle and is designated as Day 1. On this day the Djungarian hamster (Yakovenko 1974), the golden hamster (Kent and Smith 1945), rats (Long and Evans 1922), and mice (Bingel and Schwartz 1969; Nelson *et al.* 1982) show cornified epithelial cells (c) or a mixture of cornified and nucleated epithelial cells (cn) from vaginal lavage (Table 1). In a mixture of cells, the initial of the most prevalent type appears first. Cornified cells, characteristically found in sheet form, are large, non-living, squamous epithelial cells from which the nucleus has been extruded. Cornification can be defined as the transformation of the vaginal epithelial cells from the cuboidal to the squamal type. Nucleated cells (n) are cuboidal epithelial cells from the basal layer of the vaginal lining (Kent and Smith 1945). In the mouse, the cornified-nucleated combination is late proestrus:early estrus (Nelson *et al.* 1982). The day of estrus in the golden hamster is marked by an odorous creamy vaginal discharge that is expelled when the flanks are squeezed. In the rat the cornified cells are always associated with high estrogen levels, indicative of follicular activity (McClintock 1983b). The Djungarian hamster shows a large nucleated cell (lgn) as well as the cornified cells (Yakovenko 1974). These cells are living

Table 1. Summary of Vaginal Changes in the Estrous Cycle of Djungarian Hamsters, Golden Hamsters, Rats, and Mice as reported in the literature.*

Day/Stage	Djungarian Hamster a	Golden Hamster b	Rat c	Mouse d
1/Estrus	c c,lgn	nc c	c	c cn
2/Metestrus	nc lnc nl	l	lc ln lnc	lnc c cl
3/Diestrus	ln-early l -late	ln l	ln	ln l
4/Proestrus	lgn lgn,c nc	lgn,n nc lgn,c,l	n	n nlc nc

* Page ix contains a list of abbreviations.
a. Djungarian hamster--Yakovenko 1974.
b. Golden hamster--Kent and Smith 1945; Ward, 1946.
c. Rat--Long and Evans 1922.
d. Mouse--Bingel and Schwartz 1969; Nelson et al. 1982.

squamous epithelial cells which will become the cornified cells (c). On Day 1 the mature follicle ruptures and expels the ovum. Only at this time of the cycle can fertilization take place and the female Djungarian hamster, golden hamster, rat, or mouse be receptive to the male.

Day 2 is metestrus. Cells observed from vaginal lavage in the Djungarian hamster (Yakovenko 1974) are:

1. nucleated-cornified (nc) and nucleated-leucocyte (nl) mixtures in early metestrus and

2. leucocytes (l), which are lymphocytes and appear as very small nucleated cells, in combination with nucleated and cornified cells in late metestrus (Table 1). In the golden hamster (Kent and Smith 1945; Ward 1946) leucocytes are found. In the rat (Long and Evans 1922) leucocyte-cornified, leucocyte-nucleated-cornified, and leucocyte-nucleated combinations of cells are found. And in the mouse are:

1. cornified cells (c) in early metestrus,

2. leucocytes (l), or

3. a leucocyte-nucleated-cornified (lnc) mixture in later metestrus (Nelson *et al.* 1982).

During metestrous the walls of the evacuated ovarian follicle collapse into the now empty follicle. Vascularization begins and the collapsed follicle is called the *corpus hemorrhagicum*. This body begins producing

progesterone and is called the *corpus luteum*. In the rat, leucocytes are associated with progesterone and indicate luteal activity (McClintock 1983b).

Day 3 (or Days 3 and 4 in a 5-day cycle) is diestrus. Cells observed from vaginal lavage are combinations of leucocyctes and nucleated cells (Table 1). Bingel and Schwartz (1969) stated that no cornified cells are found during diestrus. If there is no pregnancy, the *corpus luteum* decreases in size while estrogen and progesterone levels also decrease. In the mouse the uterus regresses in size to its lowest weight (Bingel and Schwartz 1969).

Day 4 (or Day 5 in a 5-day cycle) is proestrus. More nucleated cells (n) are observed from a vaginal smear in this stage. The Djungarian hamster shows:

1. large nucleated cells (lgn) in early proestrus; and

2. a nucleated-cornified cell (nc) combination in late proestrus (Yakovenko 1974) (Table 1). Golden hamsters show nucleated-cornified and large nucleated cell mixtures (Kent and Smith 1945; Ward 1946). The rat shows nucleated (n) cells (Long and Evans 1922); and the mouse shows nucleated-cornified (nc) and nucleated-leucocyte-cornified combinations (Nelson *et al.* 1982). In the mouse, estrogen levels increase and uterine weight is at its highest (Bingel and Schwartz 1969).

Factors Affecting Cycling

A. Pheromones

It has been known for more than twenty years that chemical cues can elicit endocrine responses. Pheromones are a chemo-olfactory form of communication acting as signaling devices by mammals to regulate reproductive events. They are present in the urine of both sexes and are volatile, air-borne substances which act through the olfactory receptors (Whitten, Bronson, and Greenstein 1968).

There are two types of pheromones. One type is known as a releaser or signaling pheromone because an immediate change in behavioral or sexual activity is elicited in the recipient animal. The other type is known as a primer pheromone which initiates a chain of neuroendocrine or endocrine responses (Aron 1979). Urine of male mice contains estrus-accelerating priming pheromones that activate the LH-estradiol neuroendocrine pathway. On the other hand, urine of female mice contains estrus-decelerating priming pheromones which inhibit cycling and ovulation in other female mice (Bronson and MacMillan 1983). Pheromones may have female-to-female, male-to-female, and female-to-male effects.

In rats, female-to-female pheromonal effects can be either stimulatory or inhibitory. For example, constant exposure to diestrous or follicular odors causes very regular

four day cycles in rats. Continued exposure to constant proestrous or ovulatory odors will lengthen the cycle to 5.4 days (McClintock 1984). Exposure to pheromones from pregnant rats shortens the estrous cycle, whereas exposure to the lactating pheromone lengthens it (McClintock 1983a).

Female-to-female pheromones act in a negative manner on mice living in a group. Either the cycle is non-existent or its length is increased by extending the life of the *corpus luteum* during the diestrous phase. This phenomenon is known as estrus suppression or the Lee-Boot effect (Parkes and Bruce 1961; Bronson and Whitten 1968; Champlin 1971; McClintock 1983b). In the absence of those pheromones, female mice living alone will ovulate spontaneously and regularly. An alternative possibility is that group-housed mice emit an excess of odors which impair olfactory cues that normally stimulate estrus (Aron 1979).

Group-housed female mice experience a delay in puberty that disappears within 10 days of housing the mice singly. The length of delay is related to the population density and the duration of group-housing (Coppola and Vandenbergh 1985).

An example of a stimulatory female-to-female pheromone is found in mice. Urine from singly-housed pregnant and lactating mice, when applied daily directly to the external nares of the test mice, lengthens the fertile estrous phase

of the cycle but does not change the length of the cycle (Hoover and Drickamer 1979).

Male-to-female pheromones may also be both stimulatory and inhibitory. After suppressing the estrous cycle of female mice by group-housing in the absence of a male, cycles are induced within four days by exposing them to a male. This stimulatory phenomenon is called the Whitten effect (Whitten 1959; Lamond 1959). There is agreement that the group-housed estrus suppression (anestrus) is due to the absence of male pheromones and the presence of suppressing female-female pheromones rather than to the stress of overcrowding (Whitten 1959; McClintock 1983b).

In mice, exposure to male urine dripped onto the female bedding, in a room free of male mice, has been shown to initiate and to shorten the female ovulatory cycle (Bronson and Whitten 1968). In prairie voles, male urine applied directly to the upper lip of the female caused increases in serum luteinizing horomone (LH) and in luteinizing hormone-releasing hormone (LHRH) in the posterior olfactory bulb tissue (Dluzen *et al.* 1981).

In addition to the male pheromonal role in influencing the estrous cycle of the female mouse, Bronson and MacMillan (1983) and Bronson and Maruniak (1973) write that an unnamed tactile stimulus also plays an important role. They studied

induction of puberty in mice and concluded that pheromones from male urine work synergistically with tactile stimuli to induce the estrous cycle.

The Bruce effect is an example of the inhibitory male to female pheromonal effect. Exposing the newly mated mouse to a male other than the one with which she was mated, within four days of mating, causes the pregnancy to be blocked by preventing implantation of the fertilized ova. This can occur whether the male is actually caged with the female or only in close proximity (Parkes and Bruce 1961). The same effect occurs when male urine is applied directly to the bedding of the pregnant mouse (Dominic 1966).

Female-to-male pheromones can produce a negative effect, such as the inhibition by females of the ability of the male to induce first ovulation in young female mice. Thus, group housing of young females will inhibit first estrus even though a male is present. However, in adults the presence of a male overrides the inhibitory female effects (Bronson and Macmillan 1983).

B. Estrus Suppression

One of the mechanisms of estrus-suppression in animals is thought to be pseudopregnancy. In pseudopregnancy, the *corpora lutea* are maintained in the absence of pregnancy while

progesterone levels remain elevated instead of decreasing as in a normal infertile cycle. Prolactin levels are also elevated. There is a decidual cell response (DCR) to uterine endometrial trauma in pseudopregnancy (Dewar 1959; Ryan and Schwartz 1977). In addition, a heavy vaginal mucous discharge is seen in pseudopregnant mice (Dewar 1959). The duration of pseudopregnancy in mice is approximately 12-20 days, with a range of 9-30 days (Dewar 1959; Ryan and Schwartz 1977).

Estrus suppression was studied by grouping female mice at different phases of the estrous cycle. Grouping mice in estrus resulted in lengthening of cycles. The diestrous phase was lengthened by increasing the life of the *corpus luteum*, thus delaying the following estrus. There was a high incidence of 10-12 day cycles and normal uterine weights, both typical of pseudopregnancy. *Corpora lutea* were present and a decidual response was noted when the uterus was subjected to trauma (Ryan and Schwartz 1977).

There are two causes of pseudopregnancy: cervical stimulus and extra-coital factors. Cervical stimulus can result from a sterile mating or from the vaginal lavages. Extra-coital causes can result from several possibilities. In group-housed mice in the absence of a male, female-to-female pheromonal effect is a strong possibility. Pseudopregnancy in group-housed female mice has been noted

often (Dewar 1959; Bronson and Macmillan 1983; McClintock 1983b). In one mouse study, excision of the olfactory bulbs decreased pseudopregnancy, indicating an olfactory cause. However, excising the olfactory bulb can also halt all gonadal activity. A second possibility of an extra-coital cause of pseudopregnancy in animals that use olfactory-mediated stimuli more than the other senses is that group housing can produce an excess of odors that impair the ability of the olfactory bulb (Whitten 1958; Dewar 1959; Aron 1979). A third possibility of an extra-coital cause of pseudopregnancy is the chasing and mounting behavior of group-housed female mice (Dewar 1959).

An alternative explanation of estrus suppression is presented by Lamond (1959) and Whitten (1959), who state that the lack of estrus in group housed-female mice caged without a male is a true anestrus because estrus promptly occurred after the introduction of a male. Whitten (1959) stated that the lack of estrus is not due to pseudopregnancy because the presence of a male does not alter the duration of pseudopregnancy. Further, Whitten found no *corpora lutea* in most of the grouped mice; and there was no decidual reaction in response to uterine trauma, which are both indicative of pseudopregnancy.

It is possible that there is not a conflict between these two explanations of estrus suppression. Lamond (1959) and Whitten (1959) studied mice housed in groups of thirty per large cage. The other researchers group housed their mice in smaller numbers per cage. Whitten (1959) and Parkes and Bruce (1961) suggest that when mice are housed in large groups they are truly anestrous, whereas, when housed in a small group, their longer cycles are actually pseudopregnancies. Whitten (1959) further suggests that there could be differences in the strains of mice used.

C. Photoperiodism

The length of daylight has been recognized as a controlling factor for reproductive activities in a number of mammalian species. Animals that are reproductively functional only under certain daylengths are termed photoperiodic. Studies with male golden hamsters (Gaston and Menaker 1967), and male Djungarian hamsters (Hoffman 1978, 1982; Simpson, Follet, and Ellis 1982; Duncan *et al.* 1985) have shown that below a critical daylength, the testes regress, resulting in a loss of spermatogenesis and of reproductive ability. Golden hamsters need around 12.5 hours of daylight and Djungarian hamsters require about 13 hours. The Djungarian hamster testes begin to regress at 14 hours daylight, even though the

hamsters can still reproduce at 13 hours (Duncan *et al.* 1985). This is termed the critical photoperiod. Generally, rats and mice are not photoperiodic.

Sexual maturation in the male Djungarian hamster depends upon the season (number of daylight hours) in which the animal was reared. (No literature is available on the sexual maturation of the female Djungarian hamster). The Djungarian hamster shows delayed gonadal development when reared in short daylengths (Hoffman 1978 and 1982; Yellon and Goldman 1984). However, the Golden (Syrian) hamster and lemming are not affected by the photoperiod in which they were reared (Gaston and Menaker 1967; Darrow *et al.* 1980; Hasler, Buhl and Banks 1976).

D. Unilateral Ovariectomy

In several mammalian species which have been studied, unilateral ovariectomy results in compensatory hypertrophy of the remaining ovary (COH). Much of the increased weight is due to the increased number of maturing follicles and their follicular fluid in the remaining ovary, which ovulates the same number of ova as both ovaries did in the intact animal within the time span of one estrous cycle (Peppler and Greenwald 1970; Varga, Cziszar, and Stark 1976; Bast and

Greenwald 1977; Butcher 1977; Hirshfield 1982; Redmer *et al.* 1984).

In Golden hamsters, unilateral ovariectomy on the morning of any of the first three days of the cycle causes the remaining ovary to release 12 ova, the same number released by the intact pair of ovaries. However, when unilateral ovariectomy was performed on Day 4, only 7 ova were released. This was true only for the immediate cycle. In subsequent cycles, compensatory hypertrophy occured regardless of the day of the cycle in which the unilateral ovariectomy was performed (Bast and Greenwald 1977). They concluded that Day 3 of the hamster cycle was the critical day determining whether follicles would develop or undergo atresia. The same results had been obtained in rats (Peppler and Greenwald 1970). Further, Meredith *et al.* (1992) stated that COH in rats occurs at all ages and does not decrease as the rats become older.

There are two suggestions to explain the basis for compensatory hypertrophy. First, an increased number of developing follicles is found in the rat, guinea pig, and mouse (Bast and Greenwald 1977; Gosden *et al.* 1989). Second, fewer atretic follicles were found in the hamster (Bast and Greenwald 1977), rat (Hirshfield 1982, 1983), and mouse (Gosden *et al.* 1989). These statements are not contradictory. In the intact animal an FSH surge on estrus recruits double

the number of follicles than actually reach maturity to be ovulated in the following cycle. The half that do not reach maturity undergo atresia on metestrus of the recruitment cycle (Hirshfield 1982). In the unilaterally ovariectomized animal Hirshfield (1982) found almost the same number of small antral follicles as mature follicles; thus there is an increase in number of developing follicles because few or none undergo atresia. Gosden *et al.* (1989) also express the same idea.

The phenomenon of COH is thought to be due to an increase of FSH that occurs between 6 and 18 hours following unilateral ovariectomy. Two rat studies in which surgery was performed on metestrus (Ramirez and Sawyer 1974; Butcher 1977) and a hamster study (Bast and Greenwald 1977) support this. In gilts FSH has been shown to peak at 18 hours after surgery (Redmer *et al.* 1984). Each of these investigators reported a drop in FSH to pre-surgery levels between 30-36 hours after surgery. A prolongation of elevated FSH levels in subsequent cycles maintains the compensatory hypertrophy in rats (Butcher 1977) and in hamsters (Bast and Greenwald 1977). Butcher (1977) suggested that the follicle itself was the source of an unknown (but possibly inhibin) COH control factor.

Hirshfield (1982) explains a possible mechanism for COH. When an ovary is removed, there are, of course, fewer early antral follicles than in an intact animal. The follicles are

a source of inhibin which inhibits pituitary FSH secretion. Therefore, when there is less inhibin there is increased FSH. The increased FSH is needed for follicular maturation from the small antral stage.

The protein, inhibin, has been purified in the female from porcine follicular fluid by Ling *et al.* (1985) and from bovine follicular fluid by Robertson *et al.* (1985). It has been shown to inhibit the basal release of FSH from the pituitary. The above mechanism suggested by Hirshfield has been proven with radioimmunoassays of serum inhibin by Acklund *et al.* (1990). They found that when serum inhibin levels were lower, there were concomitant higher serum FSH levels.

When a rat ovary is removed, there is one less ovary to produce inhibin. Therefore, there is a decline in the level of serum inhibin. Because there is a reduction in the negative feedback signals at the pituitary level, there can be an increase in FSH. The increase in FSH aids in the recruitment and growth of follicles (D'Agostino *et al.* 1989).

CHAPTER II

PURPOSE OF STUDY

The purpose of the present study was to examine, by histological examination of vaginal cells obtained from vaginal lavages, some factors that might affect the estrous cycle of the Djungarian hamster. After a pilot study of group-housed Djungarian hamsters showed that very few hamsters exhibited cornified smears every four days as did Golden hamsters and rats, an explanation was sought. The hamsters for the pilot study were housed at 14L:10D, which was the accepted photoperiod for Golden hamsters. The same photoperiod was used for the Djungarian hamster experiments.

The hamsters were initially group-housed with no male in the room.

Hypothesis I: At 14L:10D the vaginal smear cell pattern of group-housed Djungarian hamsters would be the same regardless of density.

Golden hamsters cycle regularly when group-housed, but group-housed mice experience estrus suppression (Parkes and Bruce 1961; Bronson and Whitten 1968). Because of the

possible negative effect of female-to-female pheromones a comparison of group and singly-housed hamsters was indicated.

Hypothesis II: At 14L:10D, the number of Djungarian hamsters exhibiting cornified cell smears regularly every 4-5 days would be the same whether they were group or singly-housed.

The presence or absence of a male has no effect on the cycling of Golden hamsters or rats, but mice are greatly influenced by a male (Whitten 1959). A comparison of Djungarian hamsters with and without a male was indicated.

Hypothesis III: At 14L:10D, Djungarian hamsters housed with or without a male in a subcage would have the same number of cornified cell smears regularly every 4-5 days.

Hoffman (1982) and Duncan *et al.* (1985) indicated that the critical daylength for male Djungarian hamsters was longer than for Golden hamsters. Experiments testing male pheromones and group *versus* single-housing with an increased photoperiod were indicated.

Hypothesis IV: At 16L:8D, the number of Djungarian hamsters exhibiting cornified cell smears regularly every 4-5 days days would be the same as hamsters in the 14L:10D photoperiod in each of the test groups.

Nelson *et al.* (1982) found differences in the cycles of young and old mice. An age comparison of the estrous cycles of Djungarian hamsters was indicated.

Hypothesis V: Young Djungarian hamsters would exhibit the same number of estrous cycles (as determined by cornified cell smears) as older Djungarian hamsters.

The unilateral ovariectomy experiment was done to ascertain if Djungarian hamsters respond to unilateral ovariectomy as do other mammals and to ascertain if unilateral ovariectomy affected cycling.

Hypothesis VI: Unilateral and sham ovariectomized Djungarian hamsters in each experimental group would show the same number of estrous cycles.

Hypothesis VII: Unilateral ovariectomy would produce compensatory ovarian hypertrophy in all groups tested.

CHAPTER III

MATERIALS AND METHODS

Animal Husbandry

Djungarian hamsters of reproductive age were obtained from the Fred Turek Laboratory at Northwestern University in Evanston, Illinois. These hamsters derived from the Klaus Hoffman Laboratory by way of the Bruce Goldman Laboratory. They were group-housed in plastic cages with ground corn cob bedding at room temperature under a 16L:8D photoperiod with illumination from 0530-2130 unless otherwise stated. Housing was in a well ventilated room measuring 20 x 14 feet in the Animal Care Facility of Loyola University Chicago, Lake Shore Campus. The animals received Purina pellet chow for hamsters, rats, and mice as well as water *ad libitum*. In experiments requiring single housing, the hamsters were housed in cages measuring 5 inches deep by 7 inches wide by 11 inches long. In experiments requiring group housing the animals were housed in cages measuring 5 inches deep by 9 inches wide by 16.5 inches long with a density of 3-10 per cage. Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Loyola University Chicago, Lake Shore Campus.

After an acclimation period of two weeks, hamsters were numbered and assigned to a colony. Colony M, born the first week of October, 1984, had 20 hamsters; colony N, born in March, 1985, had 18; colony P, born in June, 1985, had 9; colony S, born the last week of September, 1985, had 16; and colony T, born in February, 1986, had 36. Each new group of hamsters received a new colony designation.

Vaginal Cytology

Vaginal lavages, using 0.9% normal saline, were performed and results recorded daily for three-week periods in each of the following experiments to monitor the estrous cycle. A regular pattern of cornified (c) cell vaginal smears (Table 2) indicated estrous cycles. A three-week interval between experiments was observed in an attempt to eliminate any effect of a previous study on the hamsters (after Bronson and Whitten 1968). Because some phases of the cycle last only a few hours, care was taken to lavage at the same time each day, between 0900 and 1000. Clean, not sterile, technique was followed. The procedure for the lavages was as follows: a medicine dropper bulb with a micropipette tip was used because of the small size of the vagina of the hamsters. Holding a hamster firmly in the left hand, the dropper was inserted approximately 1 mm into the vagina and approximately 0.25 ml

normal saline was expelled into the vagina, aspirated back into the dropper and dispensed into the well of a slide. This follows the procedure of Nelson *et al.* (1982). The slides were read under 100x power without staining.

A few stained slides were made for preservation. A sample of the material in the well was placed on a flat slide and allowed to dry. The slides were fixed in methanol for 1 minute; stained for 20 minutes with a 2% Giemsa stain, (prepared fresh daily with a phosphate buffer); rinsed in phosphate buffer for 1 minute; and rinsed with running tap water for 3 minutes. After drying they were ready for viewing under 100x or 450x magnification. Colored 35 mm slides were made of selected microscope slides at 100x magnification. These were developed into black and white micrographs.

In addition to the Djungarian hamster vaginal lavages, this investigator lavaged six Golden hamsters for twelve days to obtain comparative cytology data. The procedure was the same as for Djungarian hamsters with one exception. Because Golden hamsters are larger a medicine dropper was used instead of a micropipet tip. Ten rats were lavaged for three weeks using the same procedure as for the Golden hamsters. The data for Golden hamsters and rats are reported in Table 2.

It was discovered during Experiment 1 that when female Djungarian hamsters were group-housed, then singly-housed,

they could not be successfully group-housed again because of fighting. Therefore, when the same hamsters were used for several parts of an experiment, all group-housed variables were tested, followed by single housing for the animals.

Experiment 1: What are the Effects of Density and Males at 14L:10D?

Female hamsters were housed under a 14L:10D photoperiod with illumination from 0630 to 2030. Factors studied were: a) estrous cycles of group-housed hamsters, 3-4 months of age, with no male in the room, at four cage densities: 9 hamsters housed 3/cage (Colony P); 10 hamsters housed 5/cage (Colony M); 8 hamsters housed 8/cage (Colony N); and 10 hamsters housed 10/cage (Colony N); b) estrous cycles with females only in the room (10 hamsters, Colony M), group-housed 5/cage (aged 4-5 months) and singly-housed (aged 5-6 months); and estrous cycles with females only in the room (12 hamsters, Colony M), singly housed (aged 7 months); c) estrous cycles with males housed across the room approximately 20 feet away from the 16 singly-housed, 6 months of age, females (Colony N); and d) estrous cycles with a male in a subcage (4 hamsters, Colony N), singly housed, aged 8 months.

With no males present in the room, the hamsters were group-housed and lavaged daily for three weeks, following the

procedure outlined above. Next, the hamsters were singly housed and lavaged for three weeks.

With males housed in the room, singly-housed hamsters were lavaged daily for 21 days. All male hamsters had sired at least one litter, a criterion of proven fertility. When the hamsters were housed with a male in a subcage, a wire cage (approximately 3 inches deep by 7 inches wide by 9 inches long) was constructed for the males and placed in the bedding of the larger size female cage. The cage had its own water bottle and food supply. The male and female hamsters were able to nuzzle and sniff each other, but were not allowed to mate.

Experiment 2: Does the 16L:8D Photoperiod Increase Cycling?

The results of Experiment I (14L:10D) showed that few hamsters were exhibiting regular cornified smears regardless if a male was present or not. Hoffman (1982) wrote that the critical daylength for male Djungarian hamsters is around 13 hours compared with 12.5 hours for the Golden hamster. It was felt that perhaps the female also needed more light. Therefore, the photoperiod was changed to 16L:8D with illumination from 0530 to 2130. Throughout the experiment, there were approximately 10 males housed in the room 20 feet away from the females. Lavages were done daily for 21 days.

Estrous cycles of 16 hamsters (Colony S) with or without a male in a subcage were compared. The group-housed hamsters were divided into 4 cages of 4 hamsters each, totaling 16. Two of these cages had one male placed in a subcage (as described above). The other 2 cages had no males and were placed approximately 20 feet away from any cages with males. The experiment was repeated with all females singly-housed. The same hamsters that had a male in a subcage when group-housed, had a male in a subcage when singly-housed. The purpose was to study the effects of female-to-female pheromones and the close-up effect of males to females with the increased daylength.

Experiment 3: Does Litter Soiled by a Male Affect Cycling?

The effect of male urinary pheromones (found in soiled litter) was tested by comparing the number of estrous cycles of 16 female hamsters (Colony S) when exposed to soiled litter *versus* clean litter. Lighting was 16L:8D with lights on from 0530 to 2130. Daily lavages were performed for 21 days upon 16 hamsters.

Each day the group receiving soiled litter had approximately one-half of an 8 ounce styrofoam cup of litter removed from her cage and replaced with an equivalent amount of soiled litter from a male's cage. This litter was

sprinkled randomly over the top of the litter in the female's cage. The soiled litter was taken from the corners because it was observed that the male hamsters always urinated in the corners. The same amount of clean litter was then placed in the male's cage by sprinkling it randomly over the top of the litter in the cage. The litter was not weighed, but was measured with a line on the styrofoam cup. A different labelled cup was used for the female's litter, the male's litter, and for the clean litter. Care was taken that litter from the same male was used for the same female throughout the experiment; in other words, litter from one male was used for one female as if they were a pair.

The hamsters receiving clean litter also had one-half of an 8 ounce styrofoam cup of dirty litter removed and received the same amount of clean litter. The litter was removed from random places in the cage and replaced by sprinkling randomly over the top of the litter in the cage.

The experiment was done with animals group-housed (four per cage) and repeated with the hamsters singly-housed. Due to the small size of the animal facility, this experiment was performed with males housed across the room.

Experiment 4: Does Age Affect Cycling?

The effect of aging on Djungarian hamster estrous cycles was studied by comparing the number of estrous cycles (determined by c smears) of 9 singly housed hamsters (Colonies N and P) with a male across the room when they were 6-8 months old and when they were 12-13 months of age. Estrous cycles (again determined by c smears) of nine other singly housed hamsters from the same colonies were compared at the younger and older ages with a male in a subcage.

Lighting was 16L:8D with lights on from 0530 to 2130. Hamsters were lavaged daily for 21 days and vaginal cytology was recorded.

Experiment 5: Does Unilateral Ovariectomy Affect Cycling?

The response of the Djungarian hamsters to unilateral ovariectomy was studied. With males housed across the room, the female hamsters were lavaged for 21 days to ascertain their cycles; then they were divided into four experimental groups plus a sham control group for each experimental group. Each group contained 6 singly housed hamsters for a total of 48 hamsters from Colonies T and S. Lighting was 16L:8D with lights on from 0530 to 21:30.

The groups were:

Group

1 Sham ovariectomy, cycling, male in subcage (control)
Ovariectomy, cycling, male in subcage
2 Sham ovariectomy, cycling, male across room (control)
Ovariectomy, cycling, male across room
3 Sham ovariectomy, non-cycling, male in subcage (control)
Ovariectomy, non-cycling, male in subcage
4 Sham ovariectomy, non-cycling, male across room (control)
Ovariectomy, non-cycling, male across room

Hamsters were classed as cycling if they exhibited at least 3 c smears at consecutive four-day intervals during the 21 day period. They were classed as non-cycling if they exhibited zero to two c smears during the 21 day period.

Left unilateral ovariectomy (ovax) and left sham unilateral ovariectomy (in which the ovary was not removed, but surgery was performed to the point of removal) were performed using an inhalation anesthesia, Metophane, which was recommended and provided by the Loyola University veterinarian. A small amount of anesthesia was poured onto cotton balls and these were placed into a bottle with a mouth large enough to accomodate the hamster's head. The anesthesia was allowed to vaporize for approximately ten minutes. The hamster's head was placed into the bottle and in approximately 30 seconds the hamster was anesthetized. The head was moved close to the mouth of the bottle to allow sufficient oxygen intake. Within ten minutes after surgery, the sleeping

hamsters awoke and were walking around their cages. No hamsters died following this procedure. Following surgery, the left ovaries were cleaned and weighed; then they were pressed between two pieces of gauze to remove follicular fluid and were reweighed to obtain follicular fluid weight.

Surgery was performed between 0900-1000 CST. The day of surgery was designated as Day 0. The cycling hamsters had surgery on metestrus. Those classed as non-cycling were chosen on random days for surgery. The hamsters were lavaged for 12 days after surgery at which time they were euthanized by over-anesthetizing with Metophane. Day 12 was metestrus in the cycling hamsters. The uterus and remaining ovary (ovaries in the sham groups) were removed, cleaned, and weighed. The ovaries were pressed between two pieces of gauze to remove follicular fluid and were reweighed to obtain follicular fluid weight.

Statistics

The statistics used in data analysis were the Chi Square, paired-sample *t* test, randomized block ANOVA, Fisher exact test, three-dimensional (2 x 2 x 2) contingency table using the Chi Square, and the three factor ANOVA (Zar 1984).

CHAPTER IV

RESULTS

General Characteristics

The Djungarian hamster did not produce the same odiferous vaginal discharge on the day of estrus that is characteristic of estrus in the golden hamster. Thus, daily vaginal lavages were necessary to follow the estrous cycle. Figure 1 contains micrographs of selected slides of vaginal lavages of the Djungarian hamster. The results of vaginal lavages in the laboratory throughout the cycles of the Djungarian hamster, golden hamster, and rat, are shown in Table 2. Djungarian hamsters and golden hamsters showed a different type of cell from that of rats and mice: a very large nucleated cell (lgn) was observed in addition to the smaller nucleated cell (n) previously mentioned. All of the Day 1 combinations were used as criteria in counting the number of cornified (c) smears. The estrus c smears of the golden hamster and rat were always seen in sheet form when viewed under the microscope. However, sometimes the Djungarian hamster exhibited a partial sheet instead of a full sheet of the cornified cells.

Figure 1. Micrographs of the Djungarian Hamster Estrous Cycle

a). Estrus

Cornified epithelial (c) cells shown in sheet form with two large nucleated (lgn) cells
Scale = 30 micrometers

b). Metestrus

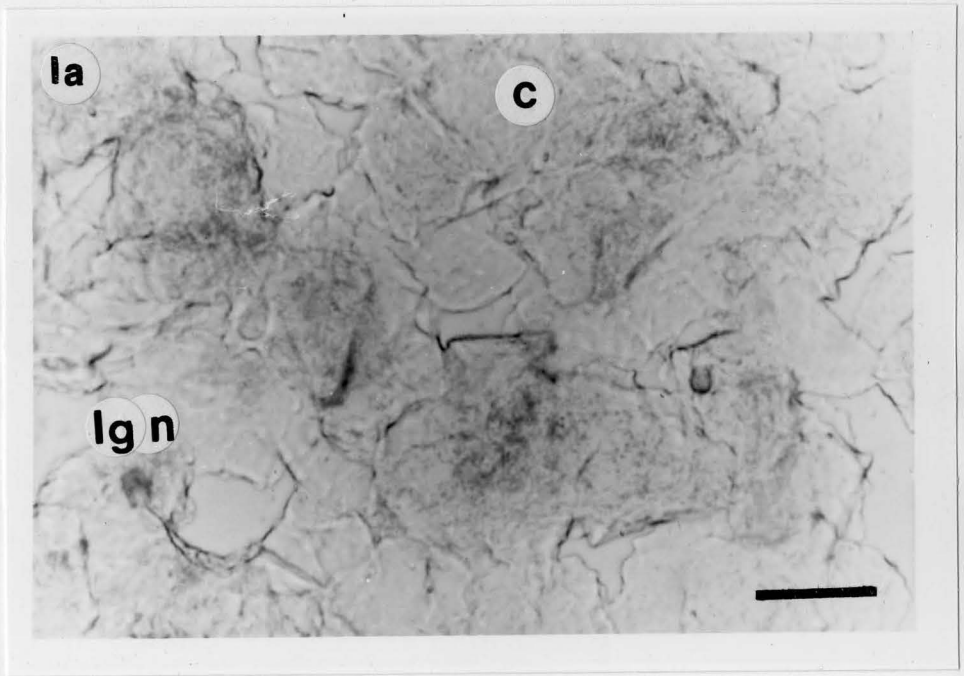
Nucleated epithelial (n) cells with some leucocytes (l) and some large nucleated (lgn) cells
Scale = 15 micrometers

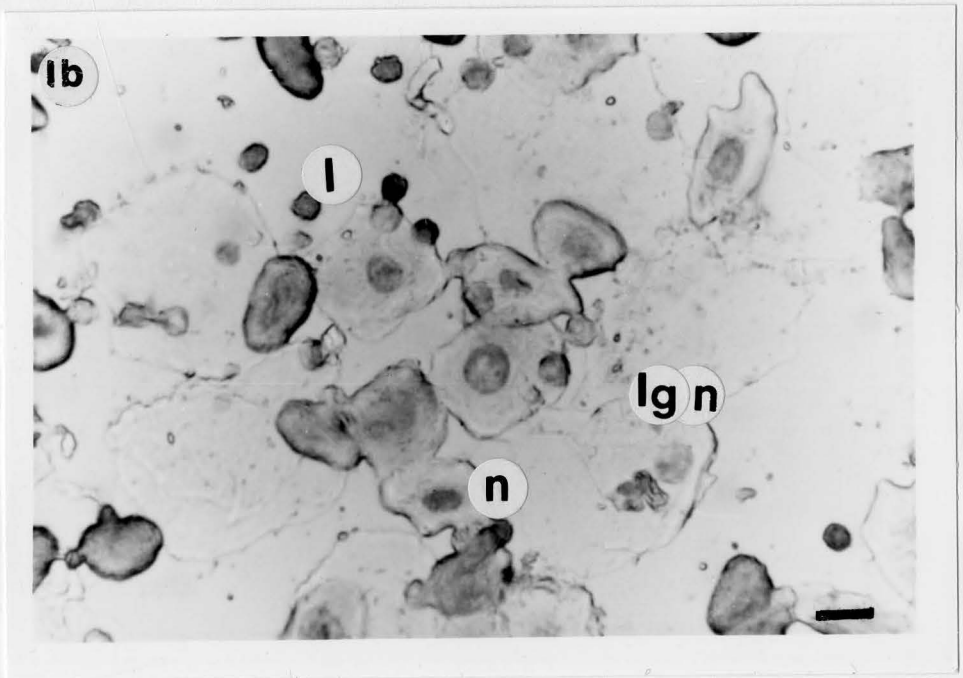
c). Diestrus

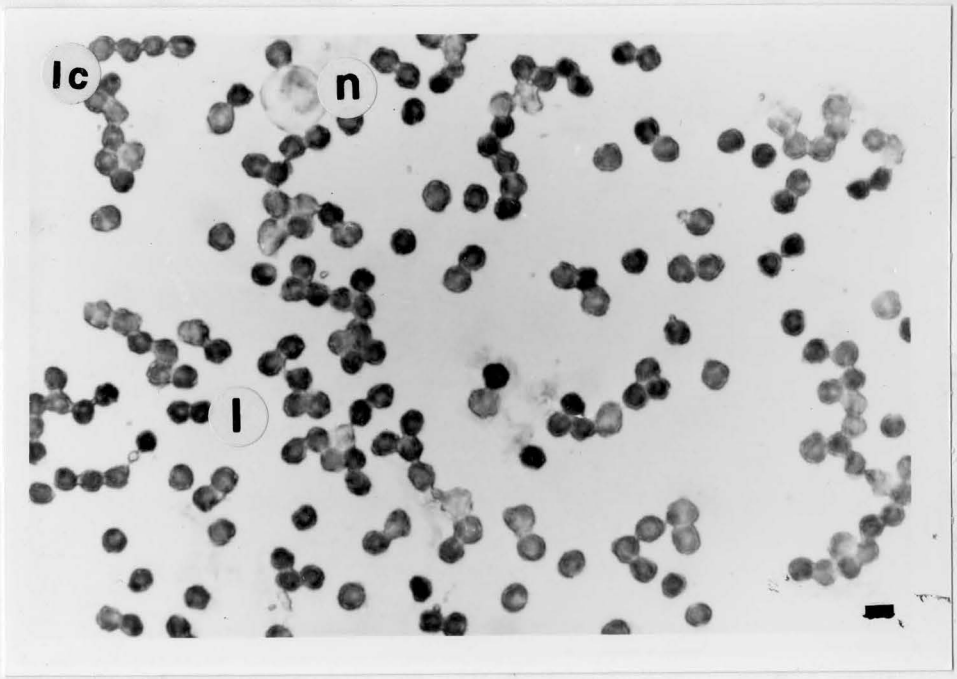
Leucocytes (l) with a few nucleated (n) cells
Scale = 7.5 micrometers

d). Proestrus

Large nucleated (lgn) cells, singular, and few in number
Scale = 30 micrometers


la
c
lg n


lb
l
lg n
n


lc
n
l

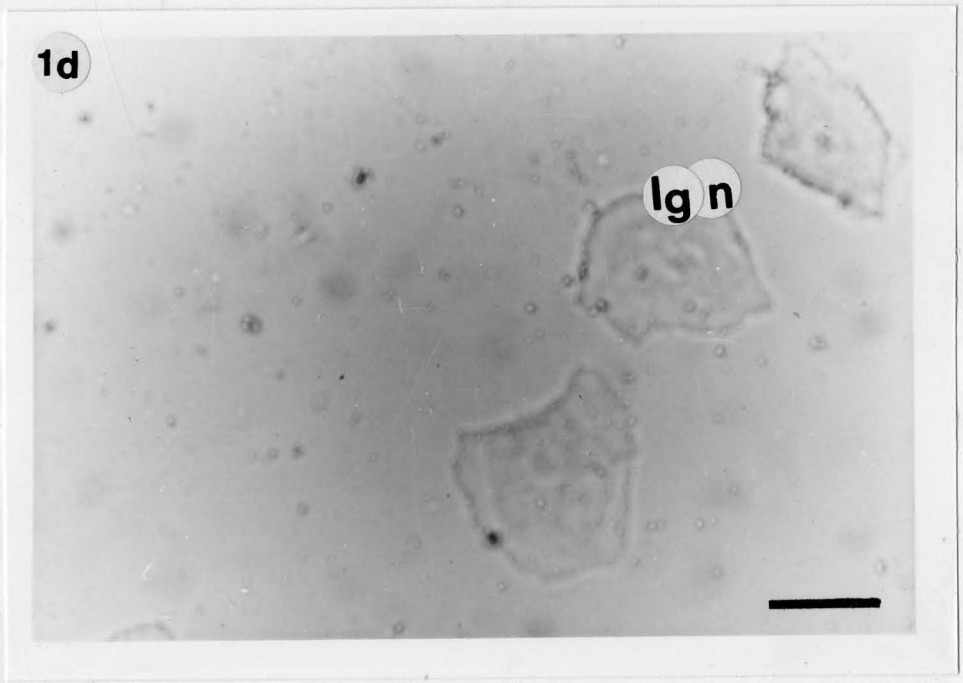

1d
lg
n

Table 2. Comparison of Vaginal Changes in the Estrous Cycle of Djungarian Hamsters, Golden Hamsters, and Rats as seen in the laboratory.

Day/Stage	Djungarian Hamster	Golden Hamster	Rat
1/Estrus	cn partial sheet cnl partial sheet clgn sheet c sheet	+ c sheet cn cnl	c sheet cn
2/Metestrus	nl lgnc lc mucous plug n mucous plug nlgn nclgn mucous plug	n n lgn ncl nlc lgn nc lgn	l lc
3/Diestrus	nl lgn n ln l	nc lnc nlc lgn lnc lgn	l ln lnc
4/Proestrus	n lgn cn n lgn nc lgn	cn lgn cn nlc nc cln	n

Page ix contains a list of abbreviations.
The + on the day of estrus in the Golden Hamster indicates the presence of the heavy odiferous vaginal discharge.

Experiment 1: What are the Effects of Density and Males at 14L:10D?

The number of hamsters showing regular 4-5 day c smears was compared when the hamsters were group-housed at four cage densities, with lighting at 14:10 hr (light:dark) and no males in the room (Figure 2). A Chi Square with 3 degrees of freedom was significant ($P(10.75) < 0.025$), indicating that there is a difference in cycling among the four densities.

Once again, with no males in the room, the number of hamsters showing regular 4-5 day c smears was compared when they were group-housed at a density of five per cage, then subsequently, when they were singly-housed (Figure 3). A paired-sample *t* test showed no significant difference between group housed and singly housed hamsters.

Next, the number of singly housed hamsters showing regular 4-5 day cycles was compared at three conditions: twelve hamsters with no male in the room, sixteen hamsters with males across the room, and four hamsters with a male in a subcage (Figure 4). A Chi Square with 2 degrees of freedom was significant ($P(6.83) < 0.05$) indicating a difference in cycling among the three groups.

In each of the three experiments described in Figures 2-4, a high percentage of hamsters exhibited from 0-2 c smears during the 21 day period.

Figure 2. A comparison of the number of Djungarian hamsters showing regular 4-5 day c smears in 21 days at 14L:10D with no male in the room at four densities of group-housing (Experiment 1). There were 9 hamsters housed 3/cage; 10 hamsters housed 5/cage; 8 hamsters housed 8/cage; and 10 hamsters housed 10/cage. A Chi Square with 3 degrees of freedom was significant ($P(10.75) < 0.025$), indicating a difference in cycling among the four densities of hamsters.

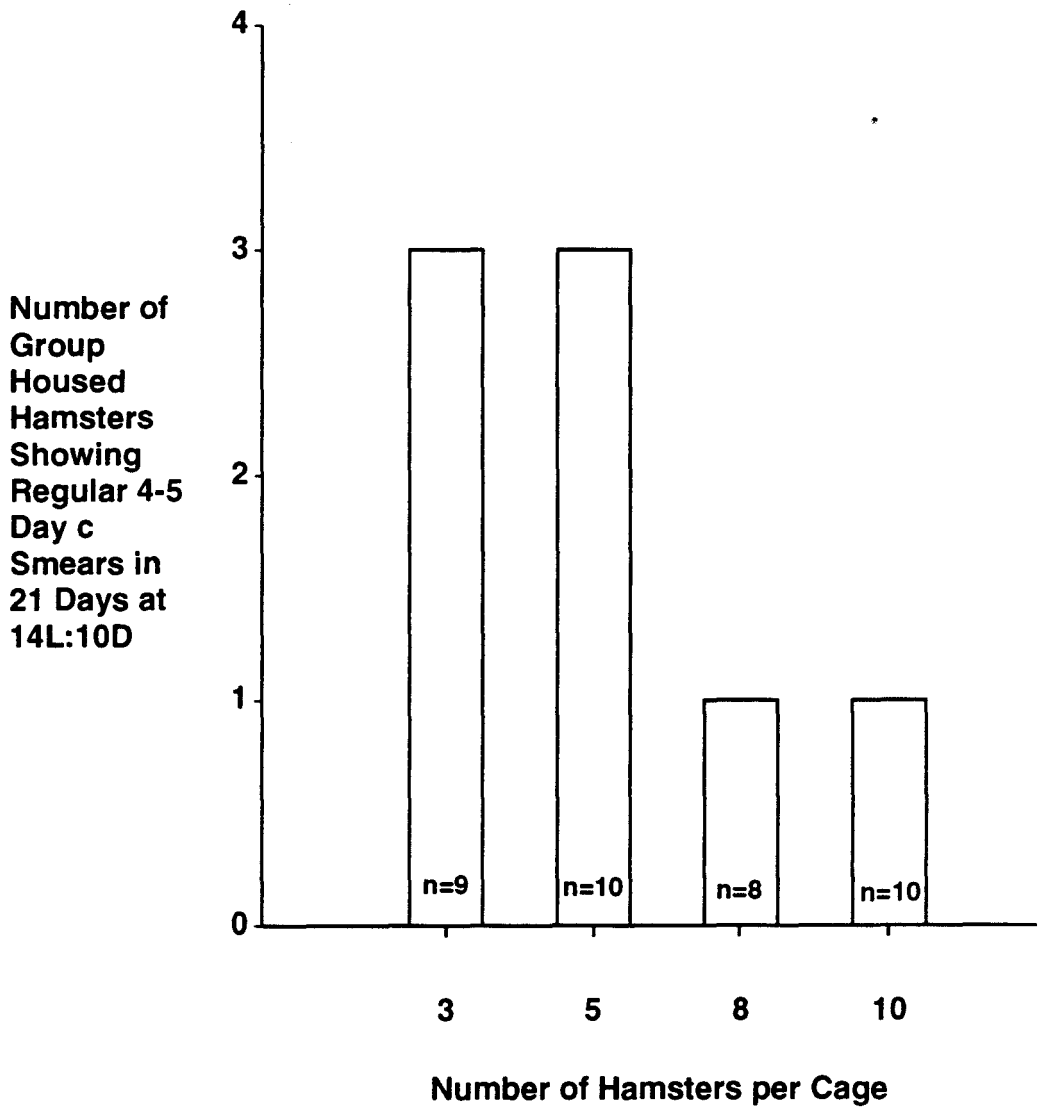

Number of Group Housed Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 14L:10D
4
3
2
1
0
n=9
n=10
n=8
n=10
3
5
8
10
Number of Hamsters per Cage

Figure 3. A comparison of the number of group-housed *versus* singly-housed Djungarian hamsters showing regular 4-5 day c smears in 21 days at 14L:10D with no male in the room (Experiment 1). The density of group-housed hamsters was 5 per cage. Ten hamsters were compared when group-housed, then later singly-housed.

No significant difference in the cycles was found when tested by a paired-sample *t* test.

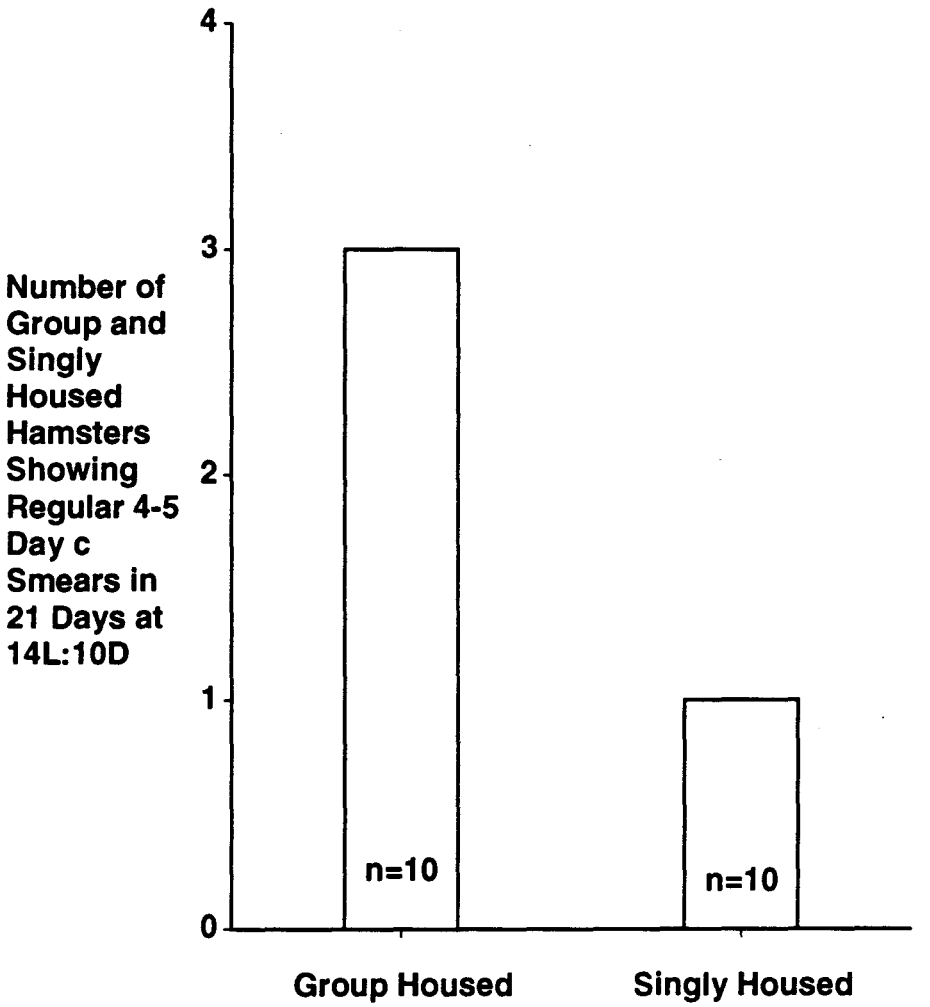

Number of Group and Singly Housed Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 14L:10D
4
3
2
1
0
n=10
n=10
Group Housed
Singly Housed

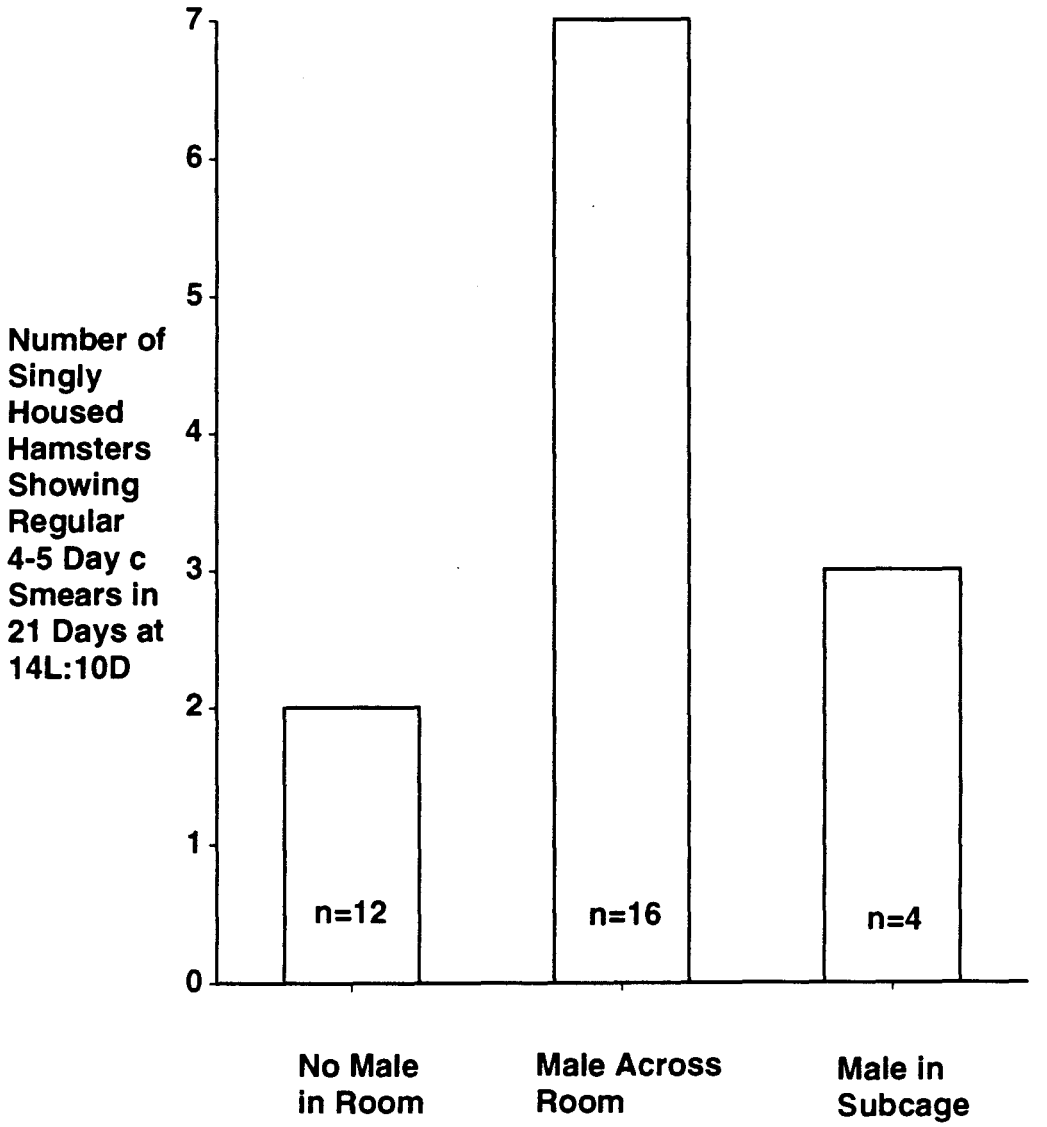
Figure 4. A comparison of the number of singly-housed Djungarian hamsters showing regular 4-5 day c smears in 21 days at 14L:10D with no male in the room, with males across the room, and with a male in a subcage (Experiment 1). The numbers of hamsters contained in each group were:

12--No male in the room;

16--Male across the room; and

4--Male in a subcage.

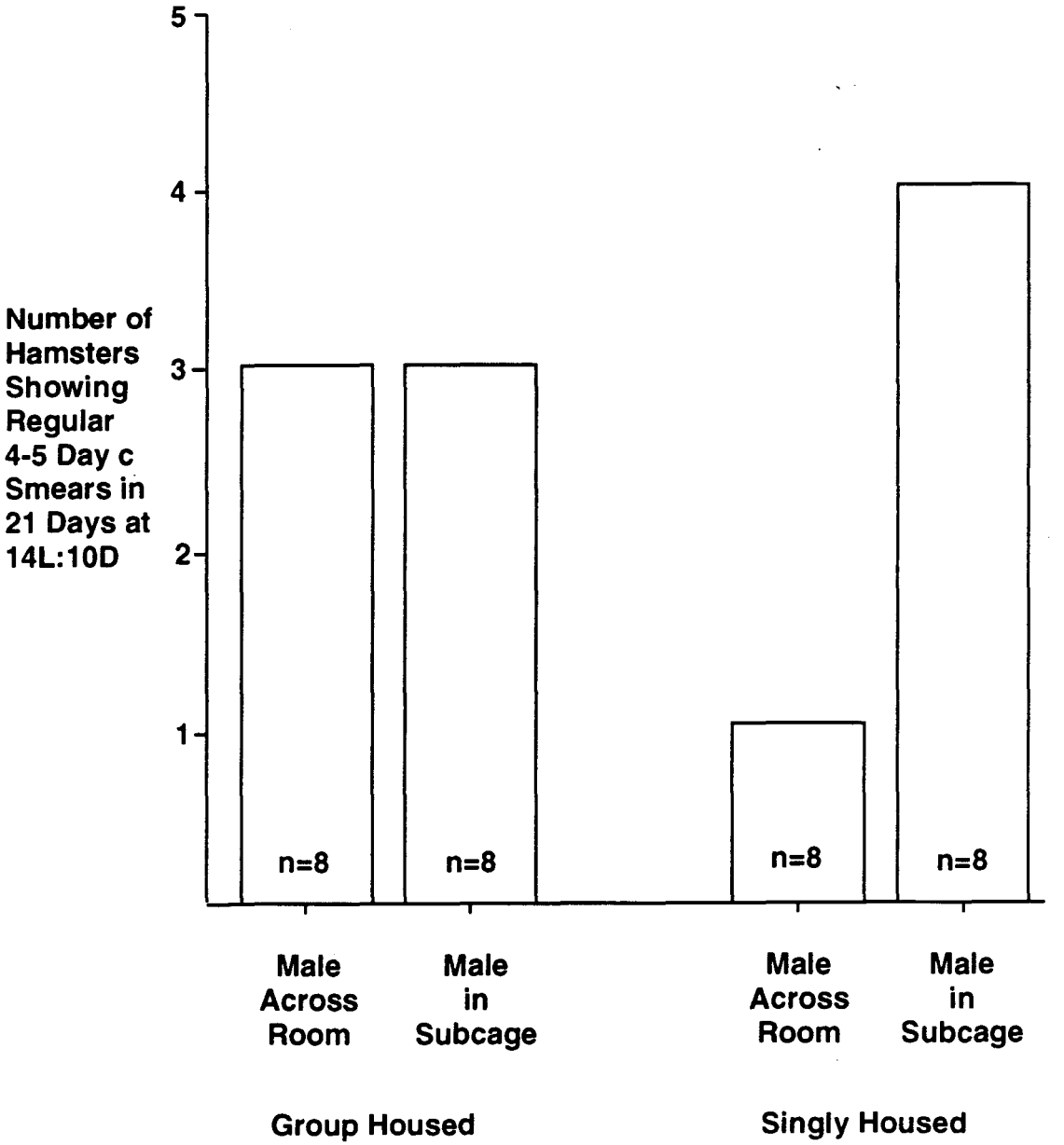
A Chi Square with 2 degrees of freedom was significant ($P(6.83) < 0.05$).


Number of Singly Housed Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 14L:10D
7
6
5
4
3
2
1
0
n=12
n=16
n=4
No Male in Room
Male Across Room
Male in Subcage

Experiment 2: Does the 16L:8D Photoperiod Increase Cycling?

The photoperiod was increased to 16:8 hr (light:dark), and male hamsters were housed in the room approximately twenty feet away. The number of hamsters showing regular 4-5 day cornified (c) smears when group-housed and then singly-housed was compared with males across the room and with a male in a subcage (Figure 5). Once again, as occurred in Experiment 1, a large percentage of hamsters exhibited from 0-2 c smears during the 21 day test period. No statistically significant differences were found among the groups when the results were tested with a Randomized Block ANOVA or with a Fisher Exact Test.

Figure 5. A comparison of the number of group-housed *versus* singly-housed Djungarian hamsters showing regular 4-5 day c smears in 21 days at 16L:8D with a male across the room and with a male in a subcage (Experiment 2). A total of 16 hamsters was used, with 8 in each experimental group. Differences were not significant when analyzed with a Randomized Block ANOVA or with a Fisher Exact Test.

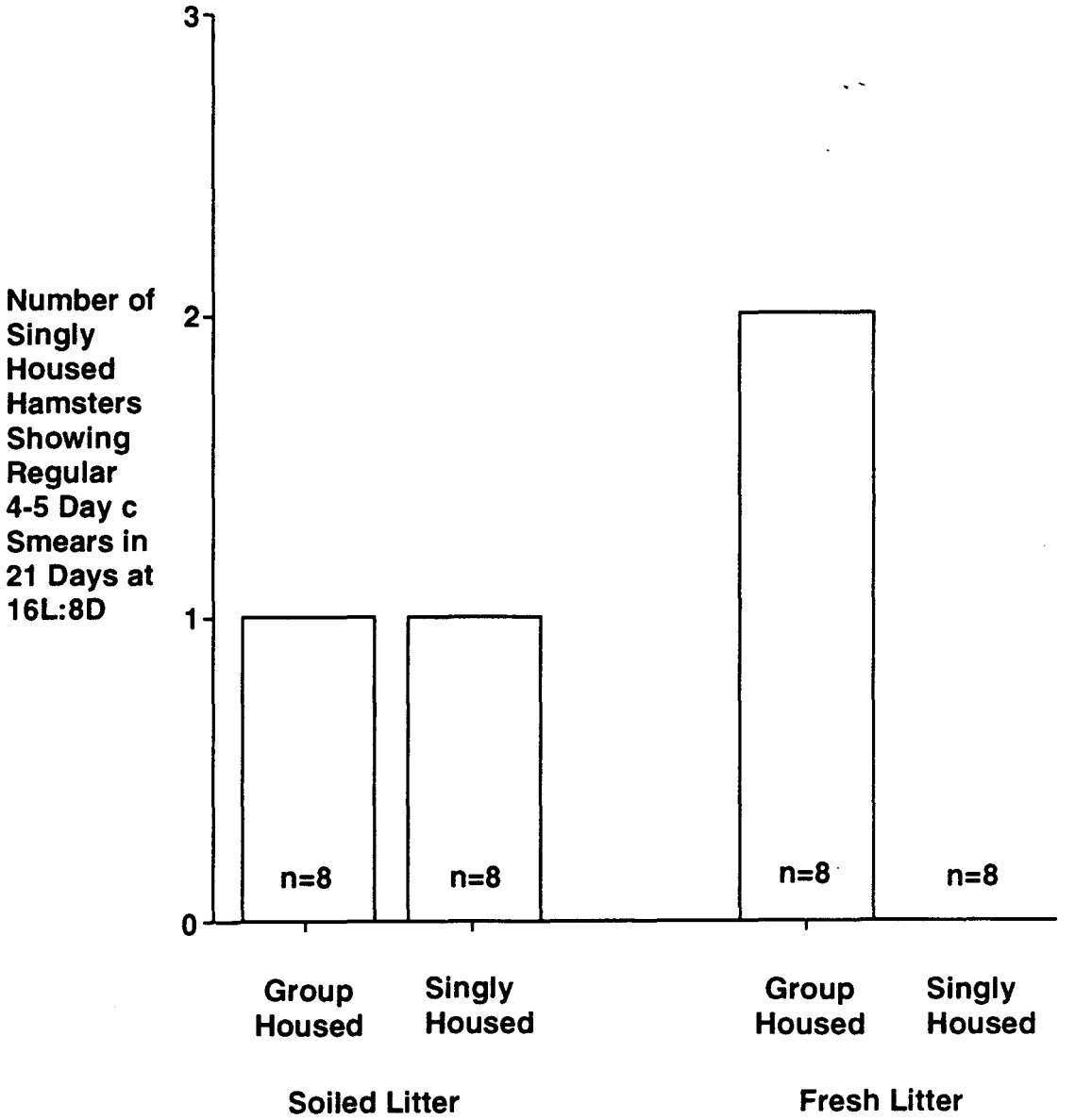

Number of Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 14L:10D
5
4
3
2
1
n=8
n=8
n=8
n=8
Male Across Room
Male in Subcage
Male Across Room
Male in Subcage
Group Housed
Singly Housed

Experiment 3: Does Litter Soiled by a Male Affect Cycling?

This experiment was performed using 16 hamsters (eight in each group) at a photoperiod of 16L:8D. The number of hamsters exhibiting regular 4-5 day cornified (c) smears was compared when hamsters were given fresh litter *versus* hamsters given litter soiled by a male (Figure 6). All females were group-housed initially, then singly-housed later. Males were housed across the room. Each group of hamsters contained a high percentage of females exhibiting from 0-2 c smears during the 21 day period. A Randomized Block ANOVA and a Fisher Exact Test indicated that were no significant differences in the number of cycling hamsters among the groups.

Figure 6. A comparison of the number of Djungarian hamsters showing regular 4-5 day c smears in 21 days at 16L:8D with fresh litter *versus* litter soiled by a male and group-housed *versus* singly-housed (Experiment 3). A total of 16 hamsters was used, with each experimental group containing 8 animals.

According to a Randomized Block ANOVA and a Fisher Exact Test, the differences in cycling were not statistically significant.

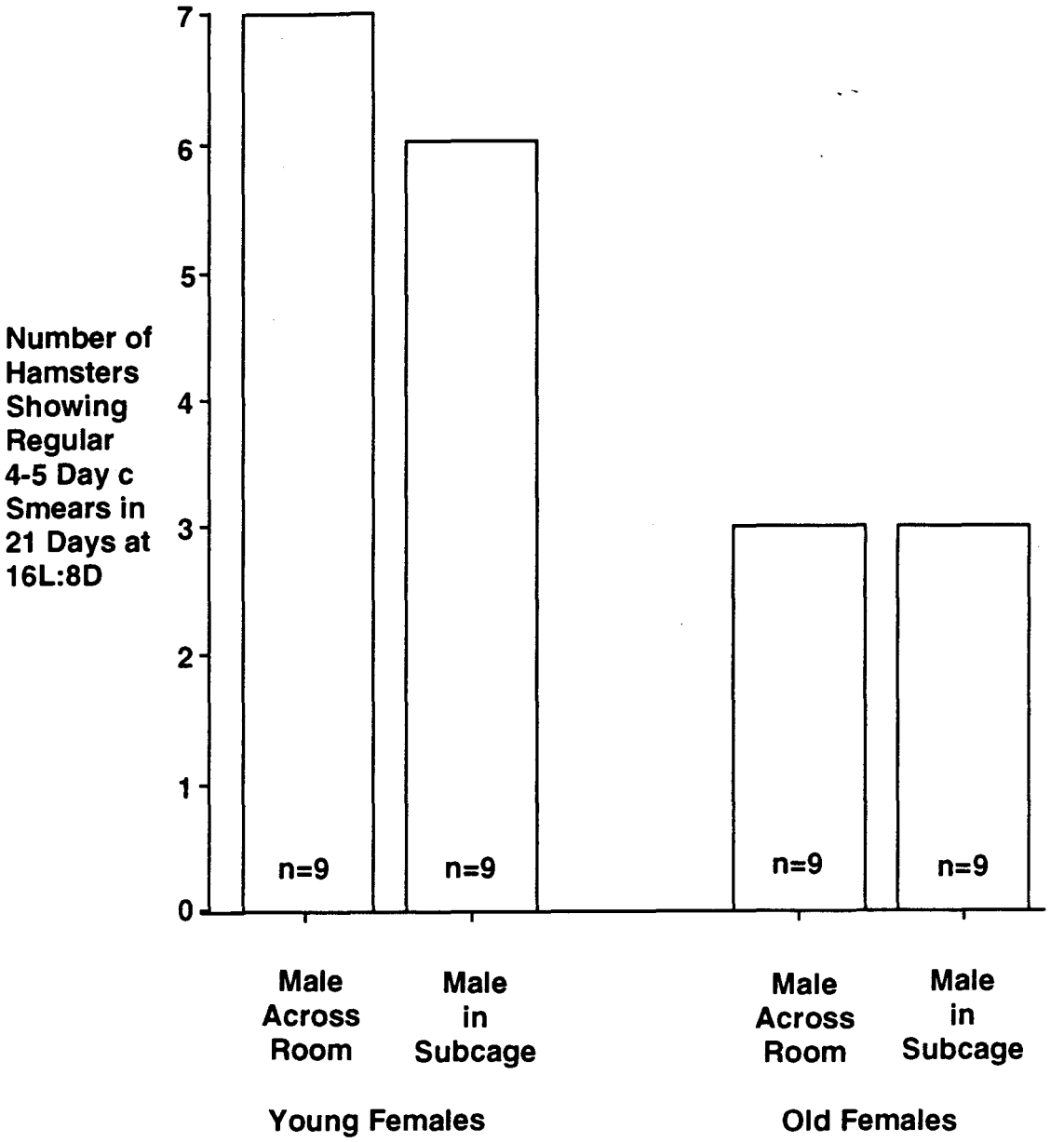

Number of Singly Housed Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 16L:8D
3
2
1
0
n=8
n=8
n=8
n=8
Group Housed
Singly Housed
Group Housed
Singly Housed
Soiled Litter
Fresh Litter

Experiment 4: Does Age Affect Cycling?

The number of singly-housed hamsters exhibiting regular 4-5 day cornified (c) smears was compared with age as a factor. With a male across the room, nine older hamsters aged 12-13 months were compared with data obtained from the same animals at the younger age of 6-8 months (Figure 7). With a male in a subcage, data obtained from nine different older hamsters were compared with the same animals at the younger age (Figure 7). The photoperiod was 16L:8D and the testing period was 21 days. A Randomized Block ANOVA was significant ($P(6.43) < 0.05$) for the age factor with a male across the room, but was not significant with a male in a subcage. A Fisher Exact Test was not significant for either factor. As in the previous experiments, there was a high percentage of hamsters exhibiting 0-2 c smears during the test period.

Figure 7. A comparison of the number of singly-housed Djungarian hamsters showing regular 4-5 day c smears in 21 days at 16L:8D with and without a male in a subcage and at younger *versus* older ages (Experiment 4). Values of young hamsters aged six to eight months were compared with values obtained when they reached 12-13 months. A total of eighteen hamsters was used, with each group having nine animals.

A Randomized Block ANOVA indicated that the age factor was significant ($P (6.43) < 0.05$) with males housed across the room but was not significant with a male in a subcage. A Fisher Exact Test was not significant.


Number of Hamsters Showing Regular 4-5 Day c Smears in 21 Days at 16L:8D
7
6
5
4
3
2
1
0
n=9
n=9
n=9
n=9
Male Across Room
Male in Subcage
Male Across Room
Male in Subcage
Young Females
Old Females

Experiment 5: Does Unilateral Ovariectomy Affect Cycling?

The unilateral ovariectomy (ULO) experiment was performed with a photoperiod of 16:8 hr (light:dark). Forty-eight hamsters were divided into eight groups: four unilateral ovariectomy groups, each containing six hamsters; and four corresponding control sham ovariectomy groups also containing six hamsters. The raw data for this experiment are listed in Appendix I; the mean ovarian and uterine weights in Appendix II; and a summary of the ANOVAS in Appendix III.

Figure 8 evaluated the effect of unilateral ovariectomy by comparing the amount of compensatory ovarian hypertrophy (COH) among groups. COH is defined as the difference between the weight of the right minus left ovaries after a unilateral ovariectomy. An ANOVA was computed for three factors: ovariectomy *vs* sham, cycling *vs* non-cycling, and male in a subcage *vs* male across the room. The ovariectomy factor was statistically significant ($P\ (8.60) < 0.01$).

The amount of follicular fluid (right ovarian weight minus crushed right ovarian weight) is illustrated in Figure 9. A three-factor ANOVA, having the same factors as above, was computed. Two factors were statistically significant: ovariectomy ($P\ (4.78) < 0.05$) and male ($P\ (6.68) < 0.025$).

The number of hamsters exhibiting regular 4-5 day cornified (c) smears in 12 days following unilateral or sham ovariectomy is illustrated in Figure 10. A three-dimensional Contingency Test (2 x 2 x 2) using Chi Square, having the same factors as above, was not significant.

The mean uterine weights are illustrated in Figure 11. A three-factor ANOVA, having the same factors as above, was significant for several factors: cycling ($P(11.11) < 0.0025$); cycling x ovariectomy ($P(11.04) < 0.0025$); cycling x male ($P(7.36) = 0.01$); ovariectomy x male ($P(18.19) < 0.0005$); and cycling x ovariectomy x male ($P(7.08) < 0.025$).

Figure 8. A comparison of compensatory ovarian hypertrophy (COH) of the mean weights (+ SEM) of right minus left ovaries after unilateral ovariectomy (Experiment 5). The experiment was performed at a photoperiod of 16L:8D. The comparison was made among cycling *versus* non-cycling females exposed to a male in a subcage or across the room. Forty- eight hamsters were divided into groups of six each. Four groups were ovariectomized and the remaining four groups contained the corresponding sham hamsters.

A three-factor ANOVA indicated that the sham ovariectomy weights were significantly lower than the ovariectomy weights ($P\ (8.60) < 0.01$).

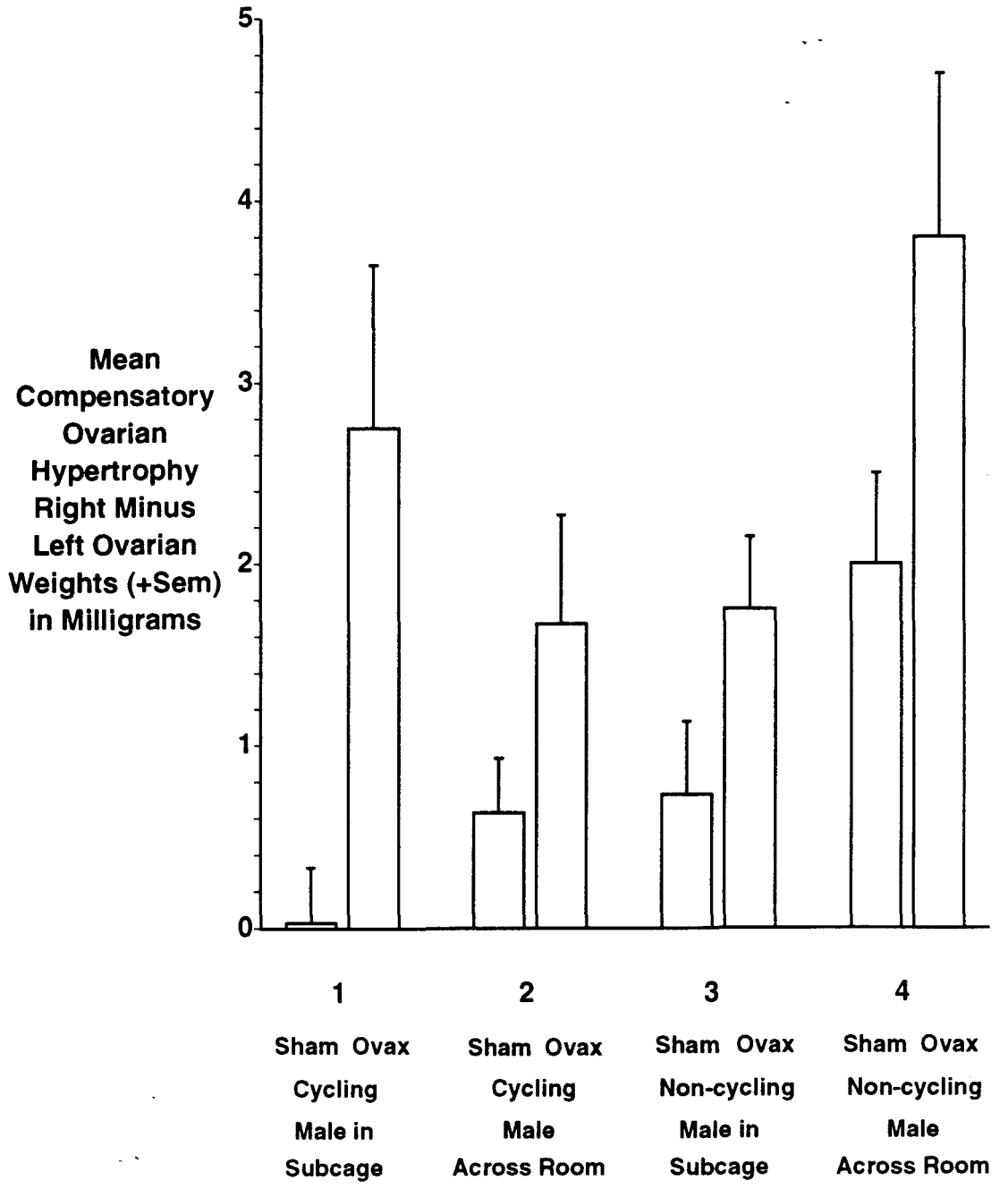

Mean Compensatory Ovarian Hypertrophy Right Minus Left Ovarian Weights (+Sem) in Milligrams
5
4
3
2
1
0
1
Sham Ovax
Cycling
Male in
Subcage
2
Sham Ovax
Cycling
Male
Across Room
3
Sham Ovax
Non-cycling
Male in
Subcage
4
Sham Ovax
Non-cycling
Male
Across Room

Figure 9. A comparison of follicular fluid amounts by comparing the mean weights (+ SEM) of the right ovaries minus the crushed right ovaries after unilateral ovariectomy (Experiment 5). The experiment was performed at a photoperiod of 16L:8D. The comparison was made among cycling *versus* non-cycling females exposed to a male in a subcage or across the room. Forty-eight hamsters were divided into groups of six each. Four groups were ovariectomized and the remaining four groups contained the corresponding sham hamsters.

A three factor ANOVA was significant for the ovariectomy ($P(4.78) < 0.05$) and male ($P(6.68) < 0.025$) factors.

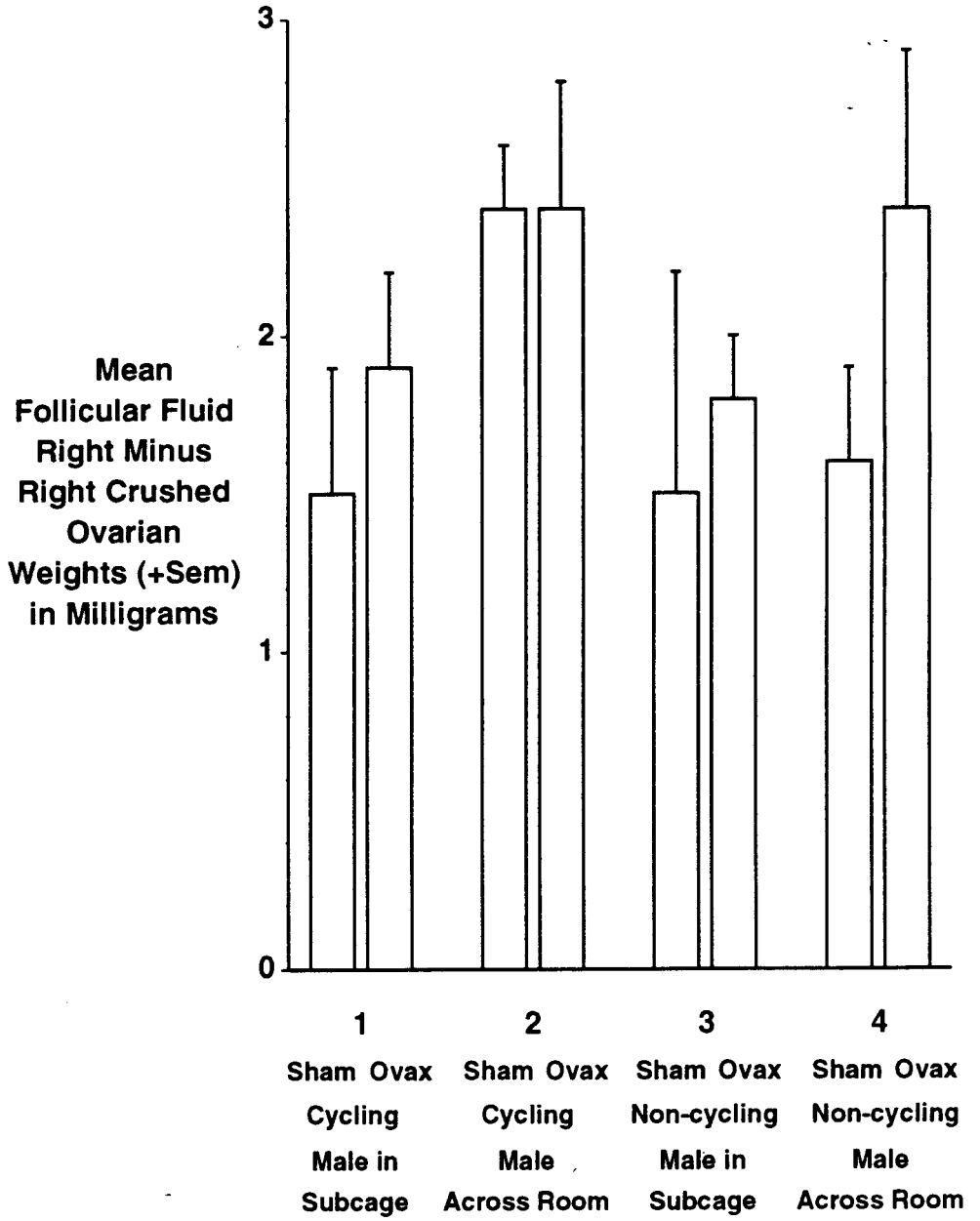

Mean
Follicular Fluid
Right Minus
Right Crushed
Ovarian
Weights (+Sem)
in Milligrams
3
2
1
0
1
Sham Ovax
Cycling
Male in
Subcage
2
Sham Ovax
Cycling
Male
Across Room
3
Sham Ovax
Non-cycling
Male in
Subcage
4
Sham Ovax
Non-cycling
Male
Across Room

Figure 10. A comparison of the number of Djungarian hamsters exhibiting regular 4-5 day c smears in 12 days at 16L:8D following unilateral or sham ovariectomy (Experiment 5). The comparison was made among cycling *versus* non-cycling females exposed to a male in a subcage or a male across the room. Forty-eight hamsters were divided into groups of six each. Four groups were ovariectomized and the remaining four groups contained the corresponding sham hamsters.

A three-dimensional contingency test (2 x 2 x 2) using the Chi Square was not significant.

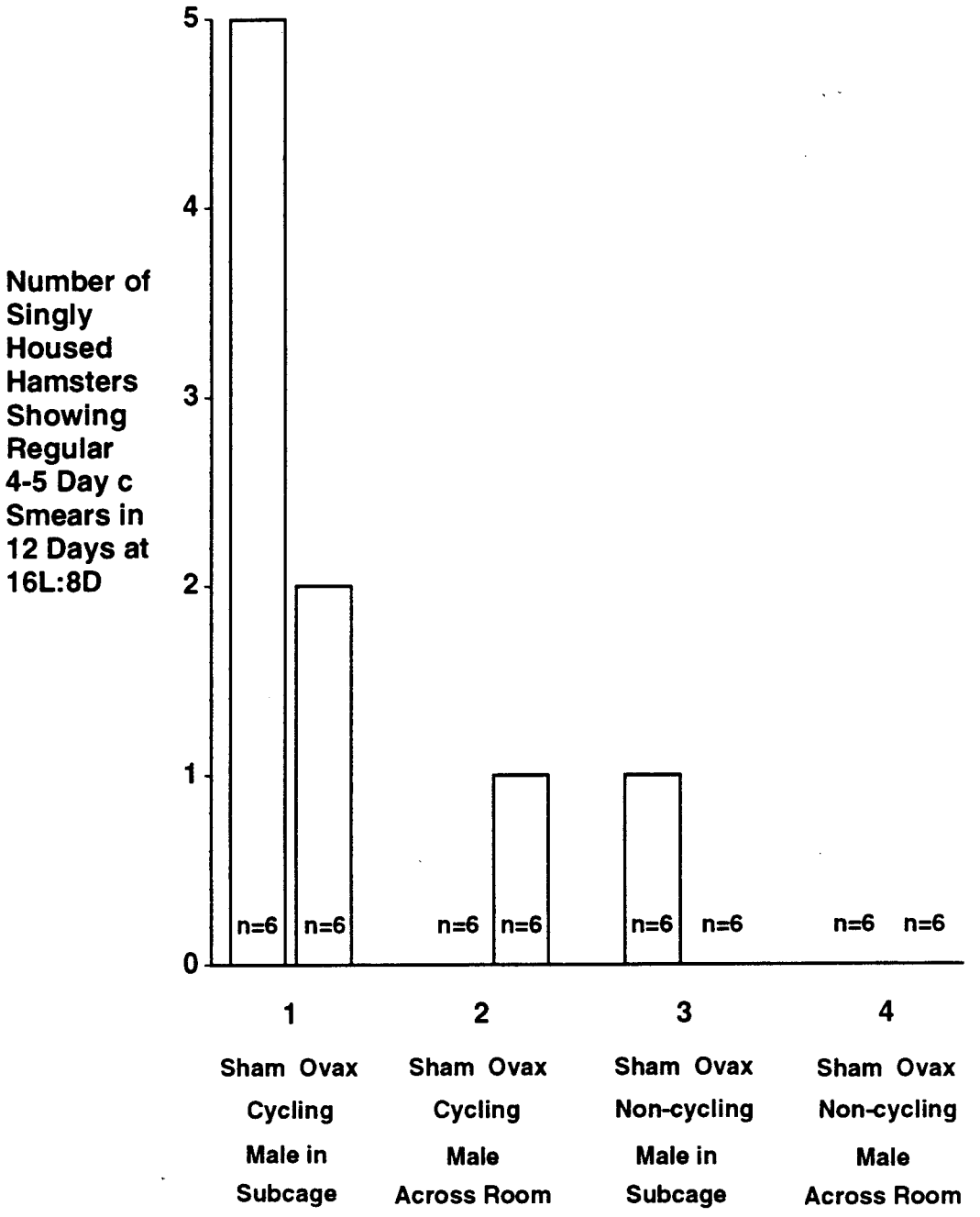

Number of Singly Housed Hamsters Showing Regular 4-5 Day c Smears in 12 Days at 16L:8D
5
4
3
2
1
0
n=6
n=6
n=6
n=6
n=6
n=6
n=6
n=6
1
2
3
4
Sham Ovax Cycling Male in Subcage
Sham Ovax Cycling Male Across Room
Sham Ovax Non-cycling Male in Subcage
Sham Ovax Non-cycling Male Across Room

Figure 11. A comparison of the mean uterine weights (+SEM) after unilateral ovariectomy (Experiment 5). The experiment was performed at a photoperiod of 16L:8D. The comparison was made among cycling *versus* non-cycling females exposed to a male in a subcage or across the room. Forty-eight hamsters were divided into 8 groups of 6 hamsters each. Four groups were unilaterally ovariectomized and the remaining four groups contained the corresponding sham hamsters.

A three-factor ANOVA was significant for:

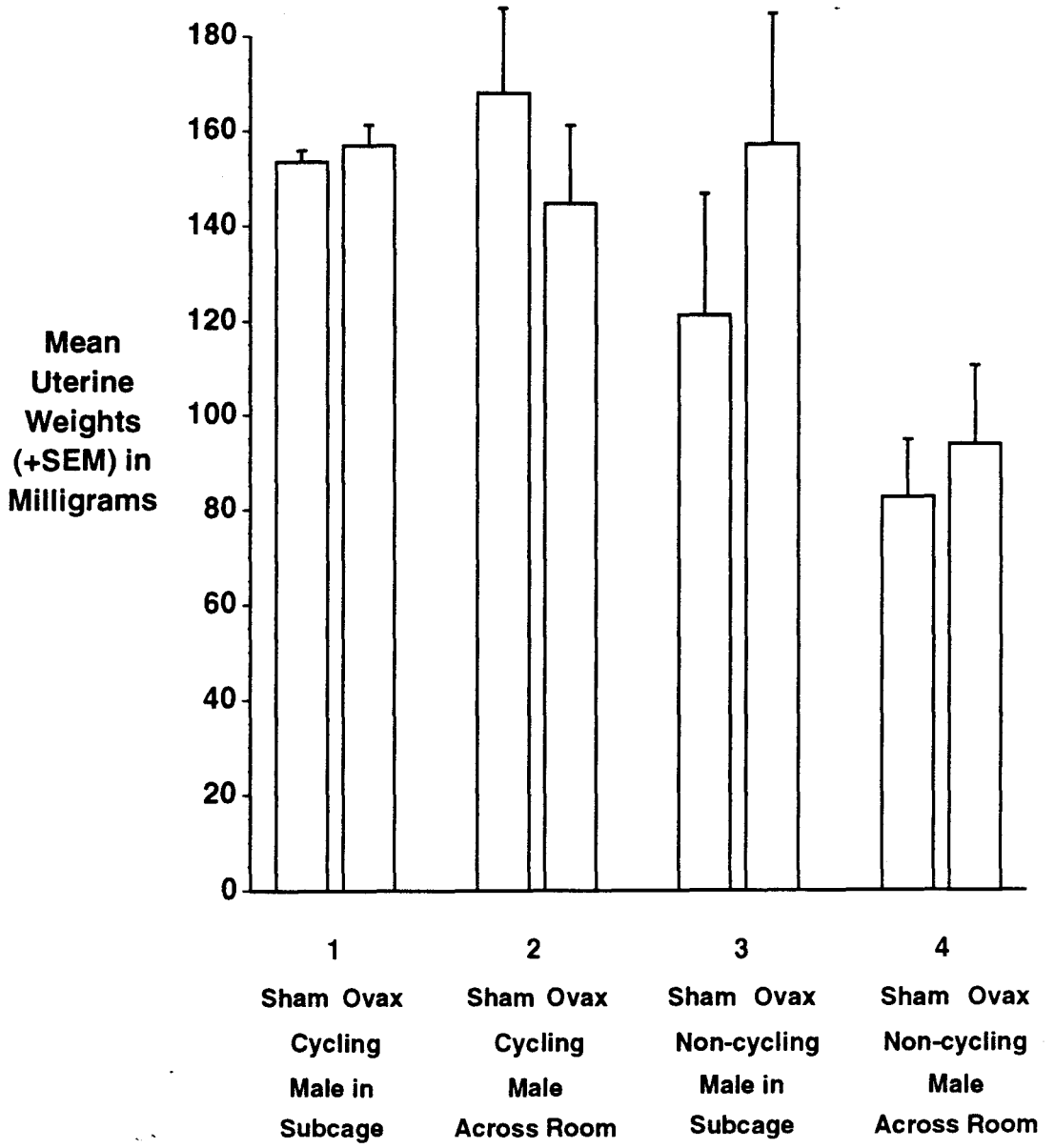
the cycling factor ($P_{(11.11)} < 0.0025$);

the cycling x ovariectomy factor ($P_{(11.04)} < 0.0025$);

the cycling x male factor ($P_{(7.36)} = 0.01$);

the ovariectomy x male factor ($P_{(18.19)} < 0.0005$);

and the cycling x ovariectomy x male factor ($P_{(7.08)} < 0.025$).


Mean Uterine Weights (+SEM) in Milligrams
180
160
140
120
100
80
60
40
20
0
1
Sham Ovax
Cycling
Male in
Subcage
2
Sham Ovax
Cycling
Male
Across Room
3
Sham Ovax
Non-cycling
Male in
Subcage
4
Sham Ovax
Non-cycling
Male
Across Room

CHAPTER V

DISCUSSION AND CONCLUSIONS

Vaginal Cytology

The vaginal cytology of the Djungarian hamsters in Table 2 has a more varied pattern than that reported in the literature (Table 1). Because each stage was not exactly 24 hours long (as designating Day 1, Day 2, etc., might imply), the observed variations may have occurred because the hamsters were in a different phase of a stage from that reported in the literature. By counting tubular ova, the range of the time of onset of ovulation in the Djungarian hamster was determined to be from 2230 h on proestrus to 0430 h on estrus (Wynne-Edwards, Terranova, and Lisk 1987). The hamsters in the present investigation were lavaged at the same time each day. With a range of six hours in a stage that lasts 12 hours (Yakovenko 1974), the variations in the vaginal smears can be explained.

Yakovenko's (1974) cytological description of Day 1 estrus in Djungarian hamsters referred to the presence of cornified cells but not to sheets of cornified cells. Since

the main purpose of his study was to estimate the length of each phase for future embryological studies, he may have considered the sheets to be irrelevant. Because sheets were always manifested in rats and Golden hamsters, but only occasionally in Djungarian hamsters, sheets were included in this investigation to determine if a Djungarian hamster was cycling.

Long and Evans (1922) reported that during metestrus (Day 2) in rats, there was a progression of leucocytes and cornified cells (lc), then leucocytes, nucleated, and cornified cells (lnc), then leucocytes and nucleated cells (ln), and finally, the beginning of the diestrous phase (Day 3). Our investigations did not detect any nucleated (n) cells in the rat during metestrus, although several combinations of n cells were observed in the Golden hamster and in the Djungarian hamster (Table 2). Although leucocytes have been observed by other researchers during metestrus, in both genera of hamsters, the present investigation showed a wider variety of cell combinations than was reported in the literature.

Mucus was observed on Day 2 in some Djungarian hamsters exhibiting c smears or at regular intervals in some hamsters not exhibiting c smears. In contrast, no mucus was observed in the Golden hamster or the rat on Day 2 in our laboratory. Yakovenko (1974) had not reported mucus on Day 2 of the

Djungarian hamster cycle. Since mucus is present in pseudopregnant mice and persists over 12-20 days (Dewar 1959), those hamsters showing only one or two cornified (c) smears, from eight to twelve days apart, in a 21-day period may have been in a state of pseudopregnancy. A possible cycle for such hamsters is: estrus, pseudopregnancy, proestrus, and estrus.

Bingel and Schwartz (1969) stated that no cornified (c) cells were present during diestrus. In our laboratory, the small number of c cells observed during diestrus in the rat and the golden hamster may have resulted from lavaging during late metestrus.

The large nucleated cells (lgn) observed during proestrus (Day 4) are consistent with those previously reported in the Golden hamster (Kent and Smith 1945; Ward 1946) and in the Djungarian hamster (Yakovenko 1974). The absence of lgn in the rat is also consistent with that reported in the literature. Long and Evans (1922) described the origin of these cells. During diestrus the surface epithelial cells were transformed by swelling, since clear fluid accumulated in the uterus during proestrus. Considerable distension occurred, but disappeared quickly during estrus. These lgn cells were then transformed into the c cells seen later.

Yakovenko's statement regarding the length of the Djungarian hamster's cycle is confusing (Yakovenko 1974). By

observing lordosis and lavaging vaginally, he reported three- four- or four-five-day estrous cycles and stated that such cycles were shorter but more regular than those observed in rats and mice. If any hamsters were not exhibiting cornified (c) smears, we have no way of knowing it, since Yakovenko only discussed the positive results. By observing lordosis and counting the number of tubular ova in Djungarian hamsters, Wynne-Edwards, Terranova, and Lisk (1987) reported four-five-day cycles. The present investigation confirmed those findings by demonstrating with lavages that spontaneously cycling Djungarian hamsters showed cornified cell smears in four-five-day cycles. A very small number showed six-day cycles, while no three-day cycles were observed. Thus, the cycles were neither shorter nor more regular than those of rats and mice as Yakovenko reported.

Wynne-Edwards, Huck, and Lisk (1987) reported that it was next to impossible to ascertain the estrous cycles of Djungarian hamsters with vaginal smears. It is possible that Yakovenko, Wynne-Edwards, and this investigator are all correct. First, a comparable statement regarding Golden hamsters (that it was impossible to ascertain the estrous cycle with vaginal smears) has been attributed to the 1930s researcher, Beasley, by Kent and Smith (1945). Within a decade, Beasley was proven wrong by Kent and Smith (1945) and

Ward (1946). Yakovenko (1974) obtained his data by lavaging every 8 hours for two weeks, whereas this investigator lavaged once daily for three weeks. Wynne-Edwards, Huck, and Lisk (1987) do not state the timing of the lavages, but do agree with the frustration of this investigator in not being able to obtain regular cornified (c) smears in all hamsters with daily vaginal lavages. Two questions remain: 1) Why did some hamsters exhibit regular c smears while others did not?; and 2) Did Yakovenko have many unreported negative results?

An answer to the first question is that perhaps two or three populations of hamsters were present, each showing cornified smears at a different time of day. This would explain why Yakovenko could report c smears with lavages every 8 hours, whereas this investigator determined that only some hamsters showed c smears with daily lavages. The cycling pattern of Djungarian hamsters in the wild is presently unknown. Perhaps, because the Djungarian hamster is relatively new to captivity, the hamsters that do not show c smears in the mid-morning hours have not been bred out of the colonies. Further investigation with lavages every 6-8 hours for two to three weeks may resolve this question. An answer to the second question is that perhaps Yakovenko did not have negative results and that lavaging every 8 hours caught all the Djungarian hamsters in their c smear phase.

Factors Affecting Cycling

A. Pheromones

To test whether group-housing affects female-to-female pheromones, hamsters were compared under group-housing conditions at densities of three, five, eight, and ten per cage with no male in the room at a photoperiod of 14L:10D (Figure 2). The difference in the number of hamsters exhibiting regular 4-5 day cornified (c) smears was statistically significant using a Chi Square test with 3 degrees of freedom ($P\ (10.75) < 0.025$). Figure 2 indicates that fewer hamsters cycle at higher densities, suggesting possible inhibitory female-to-female pheromones as are found in mice (Parkes and Bruce 1961). This rejects Hypothesis I. Food and water were plentiful and the cages were large enough to minimize stress from overcrowding.

Because a high percentage of hamsters at each density exhibited no c smears or exhibited only 1 or 2 c smears during the 21 day test period, it is possible that group-housing at any density may have increased odors to overwhelm and inhibit the individual neuro-endocrine pathways resulting in estrus suppression. Aron (1979) has suggested that this occurs in mice. This is only a possibility, however, because Figure 3, which compares group *versus* singly-housed hamsters at 14L:10D, shows no significant difference in the number of cycling

hamsters (Hypothesis II). While an inhibitory effect of female-to-female pheromones cannot be ruled out, it cannot be proven. Other unknown factors may be affecting the hamsters.

An unknown factor that might have an effect is the birth month. As noted in the Materials and Methods, hamsters in different colonies were born in different months. Even though they were housed in controlled lighting, heating, and air circulation conditions in the animal care facilities, the question can be asked whether the hamsters could sense a change in seasons based on an unknown factor in the circulating air. In winter months, the Djungarian hamsters' testes regress (Duncan *et al.* 1985). It is possible that there is a similar phenomenon with females. A study or statistics comparing the number of cycling hamsters with the month of birth was not done.

Another factor that might cause the irregular cycles or anestrus may be attributable to the lack of a male presence (in other words, the lack of male pheromones). Whitten (1959) showed that the absence of male pheromones affected the cycling of mice. Figure 4, which compared cycles of hamsters with no male in the room, with males across the room, and with a male in a subcage at 14L:10D, showed a significant difference in the cycles using a Chi Square test with 2 degrees of freedom ($P\ (6.83) < 0.05$). It would seem that

Hypothesis III could be rejected and that the male pheromones had a positive effect on cycling.

However, at 16L:8D, Figure 5 illustrates that the presence of a male in a subcage had no significant effect upon the cycling of group-housed hamsters. The same number of hamsters cycled with a male across the room as with a male in a subcage. It is possible that the excess odors of group-housed females overwhelmed the male pheromones so that there could be no effect. Further, also at 16L:8D, there was no significant difference in the number of cycling hamsters when fresh litter or male-soiled litter was added to female cages in group-housed or singly-housed hamsters (Figure 6). It is possible that the pheromones in the soiled litter dissipated too rapidly to have an effect. Further, also at 16L:8D, in a comparison of singly-housed younger *versus* older hamsters (Figure 7), a male in a subcage did not significantly affect cycling. With a male across the room, however, the age factor showed a significant difference in the number of cycling hamsters using a Randomized Block Anova ($P\ (6.43) < 0.05$), but not with a male in a subcage (Hypothesis V). Therefore, once again, the male pheromones did not affect cycling.

Even with a fertile Djungarian male in a subcage, a high level of cycling was not achieved in the females. The vaginal smears of a number of hamsters did exhibit a regular cell

pattern but those hamsters were not counted as cycling if they did not conform to the specifications in Table 2. Perhaps those hamsters were cycling, even though there were no cornified smears. If so, this would substantiate the aforementioned possibility of a different biological clock. In contrast, most rats and Golden hamsters exhibited a regular pattern of cells including cornified cells (Table 2). Yakovenko (1974) did not discuss the percentage of cycling although he did lavages. Moreover, Wynne-Edwards, Terranova, and Lisk (1987) did indicate a high percentage of cycling, but observed lordosis and did not lavage.

When comparing Djungarian hamsters with European field mice, indigenous from Eastern Germany to Asia, the Djungarian hamster seems to occupy a field mouse niche in Siberia and in Northern China. Both species are small, stocky, burrowing mammals, whose diet consists primarily of seeds, small tender plants, and insects such as beetles. Both species live in dry areas, especially in planted grain fields, and will move into barns and houses (Niethammer 1990). Possibly the Djungarian hamster reproductive cycle resembles that of mice rather than that of the slightly larger mammals.

B. Estrus Suppression

A high percentage of the hamsters in each experimental group exhibited from zero to two cornified (c) smears during the testing periods. The question arises if the hamsters were anestrous or pseudopregnant.

In mice, anestrus is characterized by no c smears, mucus, and low ovarian and uterine weights. It is reversed within 3-4 days by single housing and the presence of a male (Whitten 1959). In the mouse, grouping may cause tactile and/or sensory stimulation, perhaps interfering with social or territorial requirements (Whitten 1959). In the present investigation, Djungarian hamsters, showing no c smears and without a male in a subcage, had uterine weights ranging from 42.8 to 146.2 mg (Appendix 1 and Figure 11). In contrast, uterine weights, in hamsters showing regular 4-5 day c smears, with males housed across the room, ranged from 116.6 to 246.4 mg. The hamsters classed as non-cycling with the higher uterine weights may have been cycling but not exhibiting cornified cells. There are two possiblities for this phenomenon. First, is the aforementioned suggestion of a different biological clock. Second, it is possible that there was sufficient estrogen to maintain a larger uterine weight but not to elicit cornification. As discussed in the vaginal cytology section, mucus was observed in many of the hamsters

in this investigation. Whitten (1959) stated that mucus is from a dose of estrogen too small to elicit vaginal cornification.

Pseudopregnancy in mice is typified by c smears every 10-12 days, mucus, normal ovarian and uterine weights, and is not reversed by the presence of a male (Ryan and Schwartz 1977). Mucus was observed in many Djungarian hamsters, especially in those with 1 or 2 c smears in the testing period. There are several possible reasons for the apparent pseudopregnancies in Djungarian hamsters which might be drawn by comparing data from mice experiments. First, in group-housed mice, it was thought that sexual excitement from the chasing and mounting behavior of other females but not accompanied by intromission provided enough stimulus to cause pseudopregnancy (Whitten 1958; Dewar 1959). This statement is indirectly supported by Figure 2 which illustrated that housing hamsters at higher densities decreased cycling. However, the statement is not supported by two other examples. At 14L:10D, the highest percentage of 1 or 2 c smears occurred in singly housed hamsters with no male in the room. At 16L:8D, the highest percentage of 1 or 2 c smears occurred in those hamsters singly housed with a male across the room. Second, the actual lavaging technique could stimulate pseudopregnancy (Dewar 1959). This statement cannot be proven nor disproven, but

because of the very small size of the Djungarian hamsters, it cannot be disregarded. And third, too many odors from the group-housed hamsters could have hindered the neuroendocrine pathway as suggested in the previous section.

Another possible cause for anestrus is age. Figure 7 illustrates a significantly higher (P (6.43) < 0.05) number of singly-housed cycling hamsters when housed with a male across the room when tested at a younger *versus* older age.

It would appear that the hamsters used in the present investigation were mixed: some were anestrous, cycling 4 days after a male was placed in a subcage, while others were pseudopregnant, exhibiting 1 or 2 c smears in the 21 day-period. The explanation of the apparent anestrus and pseudopregnancy is possibly a combination of factors which include: the overwhelming odors caused by group-housing; the chasing and mounting behavior of group-housing; too little estrogen; lavaging technique and time of day; a lack of the male influence; and age.

C. Photoperiodism

Although the critical experiments in the present investigation were begun at 14L:10D, the regular c smears obtained by lavaging golden hamsters and rats was not achieved (Figures 2, 3, and 4). Since the testes of male Djungarian

hamsters begin to regress at or below 14L:10D (Hoffman 1978, 1982; Simpson, Follet, and Ellis 1982; Duncan *et al.* 1985), it was hypothesized (Hypothesis IV) that a comparable phenomenon may occur in females, and that more light would increase their cycling. However, there was no significant increase in the number of cycling hamsters when the photoperiod was increased to 16L:8D (Figures 5 and 6).

Wynne-Edwards, Terranova, and Lisk (1987) did observe regular cycling in Djungarian hamsters at 14L:10D by observing lordosis. Unfortunately, Yakovenko (1974) did not state the number of daylight hours in his experiments. Therefore, it was unnecessary to have increased the hours of daylight.

D. Unilateral Ovariectomy

An increase in the right ovarian weight after removal of the left was noted in each of the four experimental groups of Djungarian hamsters (Figure 8). This indicates that the remaining ovary compensates for the loss of the first ovary as in other mammals (Bast and Greenwald 1977; Hirschfield 1982). A three-factor ANOVA indicated that only the ovariectomy factor was significant ($P\ (8.60) < 0.01$). This indicated that cycling and the presence of a male have no influence on the amount of compensatory ovarian hypertrophy (Hypothesis VII).

The ovariectomy factor was also significant ($P(4.78) < 0.05$) in the amount of follicular fluid in the experimental groups (Figure 9). Therefore, at least part of the compensatory ovarian hypertrophy observed in Figure 8 did result from an increase in the amount of follicular fluid after ovariectomy. This is in agreement with observations of other mammals by Bast and Greenwald (1977); Hirshfield (1983); and Gosden *et al.* (1989). The present investigation neither examined follicles nor counted ova. Therefore, it is impossible to conclude whether the compensatory ovarian hypertrophy was based on a proliferation of developing follicles or based on decreased follicular atresia. A third possible explanation for COH is that the same number of follicles grows larger, thereby increasing follicular tissue and fluid, which accounts for the COH. However, this is unlikely because the earlier researchers found that the remaining ovary ovulates the same number of ova as did both ovaries before hemi-ovariectomy (Peppler and Greenwald 1970; Varga, Cziszar, and Stark 1976; Bast and Greenwald 1977; Butcher 1977; Hirshfield 1982; and Redmer *et al.* 1984). Whichever premise is accepted, the result is the same: the remaining ovary has more follicular fluid and increased weight.

Figure 10 illustrates that no significant difference was present in the number of cornified smears in the sham ovariectomy and unilateral ovariectomy hamsters. This indicates that unilateral ovariectomy with resulting compensatory ovarian hypertrophy did not affect vaginal cornification (Hypothesis VI).

CONCLUSIONS

In conclusion, many of the Djungarian hamsters in each experimental group were classed as non-cycling (zero c smears) or as cycling irregularly (one or two c smears during the test periods). Because each stage of the cycle was not exactly 24 hours long, the 0900-1000 time period for lavaging the Djungarian hamsters may not have been optimal, although it posed no problem for golden hamsters and rats. For those not exhibiting any c smears, it is possible that there are two or three populations of Djungarian hamsters, each exhibiting a different biological clock. If so, the cornified smears of hamsters on a different 6-8 hour clock would not be apparent with daily lavages. Further, it is possible that hamsters exhibiting 1 or 2 c smears in a 21 day period are in a state of pseudopregnancy.

Too strict criteria may have been used in reading the c smear slides. More hamsters would have been classed as cycling if those with a regular pattern, but not showing c smears, had been included. The Djungarian hamster seems to exhibit a wider variety of vaginal cytology on the day of ovulation than do Golden hamsters, rats, and mice (Tables 1 and 2). The Djungarian hamsters may have been experiencing a regular 4-5 day cycle although the pattern of vaginal smears

did not conform to the criteria used to evalute rats, mice and Golden hamsters. The regular pattern of cells without any cornified cells does substantiate the possibility of different biological clocks.

Exposure to a male stimulus, whether physical or pheromonal, did not alter the cycling pattern of a hamster exhibiting 4-5 day cornified (c) smears and in most cases did not induce cycling. In those hamsters in which the presence of the male elicited a c smear, the male possibly stimulated estrogen production through pheromonal or tactile nose stimuli (the nose is the only part of the male hamster that could get through the special wire subcage).

Because cornification is indicative of estrogen production, perhaps enough estrogen was produced to initiate ovulation and maintain uterine weights, but not to produce c smears. When a c smear was exhibited, sufficient estrogen was being produced, and the male had no further effect.

It was unnecessary to have increased the photoperiod from 14 hours daylight to 16 because no significant changes were observed.

While the Djungarian hamsters resemble Golden hamsters by being tailless and possessing cheek pouches, they show several differences. First is their lack of the very distinctive mucous discharge on the day of estrus. Second is

the observation that males, females, and new pups live together as a family. Both male Golden hamsters and mice must be removed or they will attack the young. Third is the inability to ascertain cycling with daily mid-morning vaginal lavages. Fourth is reduced cycling when housed at higher cage densities.

Djungarian hamsters do resemble field mice in size and have more resemblances environmentally. The cycling patterns have similarities and differences. First, they are similar in exhibiting reduced cycling at higher cage densities. Second, they are dissimilar in that Djungarian hamsters usually did not increase cycling when singly housed, nor with a male in close proximity when group-housed or singly-housed.

One factor which warrants further study and which could explain all the negative results is the possibility of two or three populations of Djungarian hamsters, each with a different biological clock, thus showing c smears at a different time of day. While showing similarities to Golden hamsters, rats, and mice, it would appear that the Djungarian hamster has unique traits which demand more precise definition in future studies.

APPENDIX 1

RAW DATA FOR EXPERIMENT 5 (a)

	Body wt in gms	Uterine wt in mg	Uterine wt as % of body wt	L ov wt in mg	R ov wt in mg
Cycling hamsters, Sham left ovariectomy, Male in subcage					
1	29	172.8	0.60	5.4	6.4
2	36	144.6	0.40	4.6	4.6
3	30	109.6	0.37	6.4	5.2
4	33	178.0	0.54	4.8	5.2
5	31	162.0	0.52	4.2	4.2
6	32	153.4	0.48	5.1	5.1
Cycling hamsters, Left ovariectomy, Male in subcage					
1	32	168.6	0.53	4.5	3.0
2	35	159.2	0.45	2.6	7.0
3	35	165.6	0.47	3.8	7.4
4	31	145.6	0.47	4.0	7.0
5	33	160.0	0.48	3.2	5.2
6	30	142.0	0.47	3.4	8.4
Cycling hamsters, Sham left ovariectomy, Male across the room					
1	41	246.4	0.60	7.0	6.4
2	52	141.4	0.27	6.4	7.4
3	39	156.2	0.40	6.2	6.4
4	33	130.8	0.40	4.2	5.0
5	37	191.4	0.52	4.8	6.4
6	42	140.8	0.34	6.6	7.4
Cycling hamsters, Left ovariectomy, Male across the room					
1	31	116.6	0.38	4.6	5.8
2	29	151.0	0.52	4.6	8.8
3	38	217.8	0.57	6.4	8.2
4	32	148.2	0.46	6.8	8.2
5	30	131.2	0.44	6.2	6.2
6	31	102.2	0.33	4.8	6.2

RAW DATA FOR EXPERIMENT 5 (b)

	Body wt in gms	Uterine wt in mg	Uterine wt as % of body wt	L ov wt in mg	R ov wt in mg
Non-Cycling hamsters, Sham left ovariectomy, Male in subcage					
1	34	90.6	0.27	3.2	4.6
2	29	101.4	0.35	5.0	3.2
3	27	191.6	0.71	4.4	9.8
4	30	73.0	0.24	3.8	3.2
5	29	64.4	0.22	4.2	3.4
6	33	206.4	0.63	5.6	6.4
Non-cycling hamsters, Left ovariectomy, Male in subcage					
1	28	94.6	0.34	6.4	7.4
2	34	256.4	0.75	3.6	6.6
3	34	219.0	0.64	2.9	4.6
4	34	157.4	0.46	5.8	6.8
5	29	98.2	0.34	3.4	6.4
6	34	116.6	0.34	5.6	6.4
Non-cycling hamsters, Sham left ovariectomy, Male across the room					
1	36	120.0	0.33	5.0	6.2
2	39	53.0	0.14	3.4	6.4
3	32	50.0	0.16	1.6	4.6
4	29	74.6	0.26	5.2	5.2
5	32	114.6	0.36	4.6	7.0
6	34	82.4	0.24	3.4	5.9
Non-cycling hamsters, Left ovariectomy, Male across the room					
1	33	64.4	0.20	4.6	4.6
2	29	42.8	0.15	2.8	6.4
3	35	106.4	0.30	1.2	6.2
4	30	69.2	0.23	3.6	10.2
5	40	146.2	0.37	5.4	8.2
6	31	132.4	0.43	2.4	7.4

RAW DATA FOR EXPERIMENT 5 (c)

	R - L ov wt in mg	Crushed R ov wt in mg	R - Cr R Ov wt in mg	# C smears in 12 days
Cycling hamsters, Sham left ovariectomy, Male in subcage				
1	1.0	2.8	3.6	3
2	0	3.4	1.2	3
3	-1.2	3.8	1.4	3
4	0.4	4.6	0.6	3
5	0	3.4	0.8	2
6	0	3.6	1.5	3
Cycling hamsters, Left ovariectomy, Male in subcage				
1	-1.5	2.2	0.8	0
2	4.4	5.6	1.4	0
3	3.6	5.4	2.0	3
4	3.0	4.2	2.8	3
5	2.0	3.6	1.6	2
6	5.0	5.6	2.8	1
Cycling hamsters, Sham left ovariectomy, Male across the room				
1	-0.6	3.8	2.6	1
2	1.0	5.2	2.2	2
3	0.2	4.0	2.4	2
4	0.8	3.4	1.6	2
5	1.6	4.0	2.4	2
6	0.8	4.4	3.0	1
Cycling hamsters, Left ovariectomy, Male across the room				
1	1.2	3.4	2.4	1
2	4.2	4.6	4.2	2
3	1.8	6.8	1.4	1
4	1.4	5.6	2.6	1
5	0	3.8	2.4	1
6	1.4	4.6	1.6	3

RAW DATA FOR EXPERIMENT 5 (d)

	R - L ov wt in mg	Crushed R ov wt in mg	R - Cr R Ov wt in mg	# C smears in 12 days
Non-Cycling hamsters, Sham left ovariectomy, Male in subcage				
1	1.4	3.6	1.0	0
2	-1.8	2.2	1.0	0
3	5.4	5.6	4.2	1
4	-0.6	3.2	0	2
5	-0.8	3.4	0	0
6	0.8	3.6	2.8	3
Non-Cycling hamsters, Left ovariectomy, Male in subcage				
1	1.0	4.6	2.8	0
2	3.0	5.2	1.4	2
3	1.7	2.6	2.0	2
4	1.0	5.6	1.2	2
5	3.0	4.6	1.8	0
6	0.8	4.6	1.8	1
Non-Cycling hamsters, Sham left ovariectomy, Male across the room				
1	1.2	4.8	1.4	0
2	3.0	4.4	2.0	0
3	3.0	4.0	0.6	0
4	0	3.8	1.4	0
5	2.4	4.6	2.4	0
6	2.5	4.3	1.6	0
Non-Cycling hamsters, Left ovariectomy, Male across the room				
1	0	4.2	0.4	0
2	3.6	3.4	3.0	0
3	5.0	3.8	2.4	0
4	6.6	6.4	3.8	0
5	2.8	5.4	2.8	0
6	5.0	5.2	2.2	0

APPENDIX 2

MEAN OVARIAN AND UTERINE WEIGHTS IN MILLIGRAMS WITH SEM

	Left Ovary + SEM	Right ovary + SEM	Uterine + SEM
Cycling Sham ovax Male in Subcage	5.08 + 0.31	5.12 + 0.30	153.40 + 2.45
Cycling L ovax Male in Subcage	3.58 + 0.27	6.33 + 0.79	156.83 + 4.39
Cycling Sham ovax Male across room	5.87 + 0.45	6.55 + 0.36	167.83 + 17.95
Cycling L ovax Male across room	5.57 + 0.41	7.23 + 0.53	144.50 + 16.51
Non-cycling Sham ovax Male in subcage	4.37 + 0.35	5.12 + 1.07	121.23 + 25.32
Non-cycling L ovax Male in subcage	4.62 + 0.61	6.37 + 0.38	157.03 + 27.51
Non-cycling Sham ovax Male across room	3.87 + 0.55	5.88 + 0.35	82.43 + 12.15
Non-cycling L ovax Male across room	3.33 + 0.62	7.17 + 0.78	93.57 + 16.80

APPENDIX 3

SUMMARY OF ANOVAS FOR EXPERIMENT 5

A. SUMMARY OF ANOVA FOR COH

Source of Variation	Sum of Squares	DF	Mean Square	Calc F	Critical F	P
Total	186.22	47	3.96			
Cells	59.88	7	8.55			
Factor A- Cycling	3.05	1	3.05	0.97	4.08	> 0.25
Factor B- Ovax	27.15	1	27.15	8.60		< 0.01
Factor C- Male	8.25	1	8.25	2.61		< 0.25
A x B	0.09	1	0.09	0.03		> 0.25
A x C	8.76	1	8.76	2.77		< 0.25
B x C	2.95	1	2.95	0.93		> 0.25
A x B x C	9.63	1	9.63	3.05		< 0.10
Error	126.35	40	3.16			

B. SUMMARY OF ANOVA FOR FOLLICULAR FLUID

Source of Variation	Sum of Squares	DF	Mean Square	Calc F	Critical F	P
Total	41.78	47	0.88			
Cells	11.43	7	1.63			
Factor A-Cycling	1.27	1	1.27	1.67	4.08	< 0.25
Factor B-Ovax	3.63	1	3.63	4.78		< 0.05
Factor C-Male	5.07	1	5.07	6.68		< 0.025
A x B	1.27	1	1.27	1.67		< 0.25
A x C	0.02	1	0.02	0.03		> 0.25
B x C	0.03	1	0.03	0.04		> 0.25
A x B x C	0.14	1	0.14	0.19		> 0.25
Error	30.35	40	0.76			

C. SUMMARY OF ANOVA FOR UTERINE WEIGHTS

Source of Variation	Sum of Squares	DF	Mean Square	Calc F	Critical F	P
Total	119096.77	47	2533.97			
Cells	42796.86	7	6113.84			
Factor A-Cycling	21201.61	1	21201.61	11.11	4.08	< 0.0025
Factor B-Ovax	541.36	1	541.36	0.28		> 0.25
Factor C-Male	7550.08	1	7550.08	3.96		< 0.10
A x B	21053.85	1	21053.85	11.04		< 0.0025
A x C	14045.17	1	14045.17	7.36		= 0.01
B x C	34705.42	1	34705.42	18.19		< 0.0005
A x B x C	13503.81	1	13501.81	7.08		< 0.025
Error	76299.91	40	1907.50			

REFERENCES

Ackland, J.F., D'Agostino, J., Ringstrom, S.J., Hostetler, J.P., Mann, B.G., and Schwartz, N.B. (1990). Circulating radioimmunoassayable inhibin during periods of transient follicle-stimulating hormone rise: secondary surge and unilateral ovariectomy. Biol. Reprod. 43: 347-352.

Aron, C. (1979). Mechanisms of control of the reproductive function by olfactory stimuli in female mammals. Physiol. Rev. 59: 229-277.

Bast, J.D. and Greenwald, G.S. (1977). Acute and chronic elevations in serum levels of FSH after unilateral ovariectomy in the cyclic hamster. Endocrinology 100: 955-966.

Bingel, A.S. and Schwartz, N.B. (1969). Pituitary LH content and reproductive tract changes during the mouse oestrous cycle. J. Reprod. Fert. 19: 215-222.

Bronson, F.H. and Macmillan, B., "Hormonal responses to primer pheromones," in Pheromones and Reproduction in Mammals, ed. J.G. Vandenbergh, (New York: Academic Press, 1983), 175-197.

Bronson, F.H. and Maruniak, J.A. (1975). Male-Induced Puberty in Female Mice: Evidence for a Synergistic Action of Social Cues. Biol. of Reprod. 13: 94-98.

Bronson, F.H. and Whitten, W.K. (1968). Oestrus-accelerating pheromone of mice: assay, androgen-dependency and presence in bladder urine. J. Reprod. Fert. 15: 131-134.

Buchanan, K.L. and Yellon, S.M. (1991). Delayed puberty in the male Djungarian hamster: Effect of short photoperiod or melatonin treatment on the GnRH neuronal system. Neuroendocrinology 54 (2): 96-102.

Butcher, R.L. (1977). Changes in gonadotropins and steroids associated with unilateral ovariectomy of the rat. Endocrinology 101: 830-840.

Champlin, A.K. (1971). Suppression of oestrus in grouped mice: the effects of various densities and the possible nature of the stimulus. J. Reprod. Fert. 27: 233-241.

Coppola, D.M. and Vandenbergh, J.G. (1985). Effect of density, duration of grouping and age of urine stimulus on the puberty delay pheromone in female mice. J. Reprod. Fert. 73: 517-522.

Corbet, G.B. and Hill J.E. (1991). A World List of Mammal Species, 3rd ed. p. 164. British Museum (Natural History), London.

D'Agostino, J., Woodruff, T.K., Mayo, K.E., and Schwartz, N.B. (1989). Unilateral ovariectomy increases inhibin messenger ribonucleic acid levels in newly recruited follicles. Endocrinology 124: 310-317.

Darrow, J.M., Davis, F.C., Elliott, J.A., Stetson, M.H., Turek, F.W., and Menaker, M. (1980). Influence of Photoperiod on Reproductive Development in the Golden Hamster. Biol. Reprod. 22: 443-450.

Dewar, A.D. (1959). Observations on pseudopregnancy in the mouse. J. Endocrinol. 18: 186-190.

Dluzen, D.E., Ramirez, V.D., Carter, C.S., and Getz, L.L. (1981). Male vole urine changes luteinizing hormone-releasing hormone and norepinephrine in female olfactory bulb. Science 212: 573-575.

Dominic, C.J. (1966). Observations on the reproductive pheromones of mice. J. Reprod. Fert. 11: 407-414.

Duncan, M.J., Goldman, B.D., Di Pinto, M.N., and Stetson, M.H. (1985). Testicular function and pelage color have different critical daylengths in the Djungarian hamster, *Phodopus sungorus sungorous*. Endocrinology 116: 424-430.

Gaston, S. and Menaker, M.. (1967). Photoperiodic control of hamster testis. Science 158: 925-928.

Gosden, R.G., Telfer, E., Faddy, M.J., and Brook, D.J. (1989). Ovarian cyclicity and follicular recruitment in unilaterally ovariectomized mice. J. Reprod. Fert. 87: 257-264.

Hasler, J.B., Buhl, A.E. and Banks, E.M. (1976). The Influence of Photoperiod on Growth and Sexual Function in Male and Female Collared Lemmings (*Dicrostonyx groenlandicus*). J. Reprod. Fert. 46: 323-329.

Hirshfield, A.N. (1982). Follicular recruitment in long-term hemicastrate rats. Biol. of Reprod. 27: 48-53.

Hirshfield, A.N. (1983). Compensatory ovarian hypertrophy in the long-term hemicastrate rat: size distribution of growing and atretic follicles. Biol. of Reprod. 28: 271-278.

Hoffman, K. (1978). Effects of short photoperiods on puberty, growth, and moult in the Djungarian hamster (*Phodopus sungorus*). J. Reprod. Fert. 54: 29-35.

Hoffman, K. (1982). The critical photoperiod in the Djungarian hamster (*Phodopus sungorus*). In "Vertebrate Circadian Systems: Structure and Physiology." (J. Aschoff, S. Daan and G.A. Gross, eds.), pp. 297-304. Springer-Verlag, Berlin (New York).

Hoover, J.E. and Drickamer, L.C. (1979). Effects of urine from pregnant and lactating female house mice on oestrous cycles of adult females. J. Reprod. Fert. 55: 297-301.

Kent, G.T.C. and Smith, R.A. (1945). A study of the estrous cycle in the golden hamster, *Cricetus Mesocricetus auratus Waterhouse*. Anat. Rec. 92: 263-271.

Kirsch, R., Ouarour, A. and Pevet, P. (1991). Daily torpor in the Djungarian hamster (*Phodopus sungorus*): photoperiodic regulation, characteristics and circadian organization. J. Comp. Phys. A 168: 121-128.

Kliman, R.M. and Lynch, G.R. (1991). Evidence for independence of circadian characters and extent of photoresponsiveness in the Djungarian hamster, *Phodopus sungorus*. J. Biol. Rhythms 6 (2): 159-166.

Lamond, D.R. (1959). Effect of stimulation derived from other animals of the same species on oestrous cycles in mice. Endocrinology 18: 343-349.

Ling, N., Ying, S.Y., Ueno, N., Esch, F., Denoroy, L., and Guillemin, R. (1985). Isolation and partial characterization of a M_r 32,000 protein with inhibin activity from porcine follicular fluid. Proc. Natl. Acad. Sci. 82: 7217-7221.

Long, J.A. and Evans, H.M. (1922). The oestrus cycle in the rat and its associated phenomena. Memoirs of the University of California 6: 1-148.

Meredith, S., Dudenhoeffer, G., Butcher, R.L., Lerner, S.P., and Walls, T. (1992). Unilateral ovariectomy increases loss of primordial follicles and is associated with increased metestrous concentration of follicle-stimulating hormone in old rats. Biol. Reprod. 47: 162-168.

McClintock, M.K. (1983a). Modulation of the estrous cycle by pheromones from pregnant and lactating rats. Biol. Reprod. 28: 823-829.

McClintock, M.K. (1983b). Pheromonal regulation of the ovarian cycle: enhancement, suppression, and synchrony. In "Pheromones and Reproduction in Mammals." (J.G. Vandenbergh, ed.), Academic Press, New York.

McClintock, M.K. (1984). Estrous synchrony: modulation of ovarian cycle length by female pheromones. Physiol. Behav. 32: 701-705.

Nelson, J.F., Felicio, L.S., Randall, P.K., Sims, C., and Finch, C.E. (1982). A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. Cycle frequency, length and vaginal cytology. Biol. Reprod. 27: 327-339.

Niethammer, Jochen, "Family Cricetidae: Burrowing Rodents and Mouse Group Rodents," Grzimek's Encyclopedia of Mammals, vol 3, ed. Sybil P. Parker (New York: McGraw Hill Pub. Co., 1990), 184-191, 206-215.

Ouarour, A., Kirsch, R. and Pevet, P. (1991). Effects of temperature, steroids and castration on daily torpor in the Djungarian hamster (*Phodopus sungorus*). J. Comp. Physiol. A 168: 477-481.

Parkes, A.S. and Bruce, H.M. (1961). Olfactory stimuli in mammalian reproduction. Science 134: 1049-1054.

Peppler, R.D. and Greenwald, G.S. (1970). Effects of unilateral ovariectomy on ovulation and cycle length in 4- and 5-day cycling rats. Am. J. Anat. 127: 1-8.

Puchalski, W. and Lynch, G.R. (1991). Expression of circadian rhythmicity in Djungarian hamsters under constant light: Effects of light intensity and the circadian system's state. J. Comp. Physiol. A Sens. Neural Behav. Physiol. 169 (2): 185-190.

Ramirez, V.D. and Sawyer, C.H. (1974). A sex difference in the rat pituitary FSH response to unilateral gonadectomy as revealed in plasma radioimmunoassays. Endocrinology 94: 475-482.

Redmer, D.A., Christenson, R.K., Ford, J.J. and Day, B.N. (1984). Effect of unilateral ovariectomy on compensatory ovarian hypertrophy, peripheral concentrations of follicle-stimulating hormone and luteinizing hormone, and ovarian venous concentrations of estradiol-17 in prepuberal gilts. Biol. Reprod. 31: 59-66.

Robertson, D.M., Foulds, L.M., Leversha, L., Morgan, F.J., Hearn, M.T.W., Burger, H.G., Wettenhall, R.E.H., and de Kretser, D.M. (1985). Isolation of inhibin from bovine follicular fluid. Biochem. and Biophys. Res. Comm. 126: 220-226.

Ryan, K.D. and Schwartz, N.B. (1977). Grouped female mice: demonstration of pseudopregnancy. Biol. Reprod. 17: 578-583.

Simpson, S.M., Follet, B.K. and Ellis, D.H. (1982). Modulation by photoperiod of gonadotrophin secretion in intact and castrated Djungarian hamsters. J. Reprod. Fert. 66: 243-250.

Stieglitz, A., Steinlechner, S., Ruf, T. and Heldmaier, G. (1991). Cold prevents the light induced inactivation of pineal N-acetyltransferase in the Djungarian hamster, *Phodopus sungorus*. J. Comp. Physiol. A 168: 599-603.

Varga, B., Csiszar, K., and Stark, E. (1976). Ovarian blood flow in compensatory hypertrophy in the rat. J. Reprod. Fert. 46: 409-410.

Ward, M.C. (1946). A study of the estrous cycle and the breeding of the golden hamster, *Cricetus auratus*. Anat. Rec. 94 (2): 139-161.

Whitten, W.K. (1958). Modification of the oestrous cycle of the mouse by external stimuli associated with the male. Changes in the oestrous cycle determined by vaginal smears. Endocrinology 17: 307-313.

Whitten, W.K. (1959). Occurrence of anoestrus in mice caged in groups. J. Endocrinol. 18: 102-107.

Whitten, W.K., Bronson, F.H., and Greenstein, J.A. (1968). Estrus-inducing pheromone of male mice: transport by movement of air. Science 161: 584-585.

Wilson, D.E. and D.M. Reeder, eds., Mammal Species of the World, (Pennsylvania: Smithsonian Institute Press, 1992), 539.

Wynne-Edwards, K.E., Huck, U.W., and Lisk, R.D. (1987). Influence of pre- and post-copulatory pair contact on pregnancy success in Djungarian hamsters, *Phodopus campbelli*. J. Reprod. Fert. 80: 241-249.

Wynne-Edwards, K.E. and R.D. Lisk (1987). Male-female interactions across the female estrous cycle: a comparison of two species of dwarf hamster (*Phodopus campbelli* and *Phodopus sungorus*). J. Comp. Psych. 101 (4): 335-344.

Wynne-Edwards, K.E., Terranova, P.F., and Lisk, R.D. (1987). Cyclic Djungarian Hamsters, *Phodopus campbelli*, lack of the progesterone surge normally associated with ovulation and behavioral receptivity. Endocrinology 120: 1308-1316.

Yakovenko, V.V. (1974). The estral cycle of the Djungarian hamster. Translated by Irwin Weil. Arkh. Anat. Gistol. Embriol. 66: 32-36.

Yellon, S.M. and Goldman, B.D. (1984). Photoperiod control of reproductive development in the male Djungarian hamster (*Phodopus sungorus*). Endocrinology 114: 664-670.

Yellon, S.M., Lehman, M.N. and Newman, S.W. (1990). The gonadotropin-releasing hormone neuronal system of the male Djungarian hamster: distribution from the olfactory tubercle to the medial basal hypothalamus. Neuroendocrinology 51: 219-225.

Yellon, S.M. and Newman, S.W. (1991). A developmental study of the gonadotropin-releasing hormone neuronal system during sexual maturation in the male Djungarian hamster. Biol. Reprod. 45 (3): 440-446.

Zar, J.H. (1984). Biostatistical Analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J. 07632.

VITA

The author, Roberta Whaley Ellington, graduated from the University of Virginia School of Nursing in Charlottesville, Virginia.

For the next 18 years, she did professional and volunteer work in Obstetrics and Gynecology, and in Maternal and Child Health Care in various countries including the United States, Germany, and Indonesia. While in Djakarta, Indonesia, she wrote and administered a program for Food Handlers aimed at adults with a second grade education.

In May, 1983, she received the degree of Bachelor of Science with a major in Biology from Barat College in Lake Forest, Illinois.

In January, 1984, she entered the Loyola University of Chicago Department of Biology as a graduate student where she was granted a graduate teaching assistantship the following year. For two years, she was a teaching assistant in the anatomy and physiology laboratories. By October, 1986, she had completed the academic requirements for the Masters Degree. Since then, she has been a lecturer in Northwestern University's Undergraduate Program in Biological Sciences.

THESIS APPROVAL SHEET

The thesis submitted by Roberta W. Ellington has been read and approved by the following committee:

Dr. Albert Rotermund, Director
Associate Professor, Biology Department
Loyola University Chicago

Dr. John Smarrelli
Associate Professor, Biology Department
Loyola University Chicago

Dr. Diane Suter
Associate Professor, Biology Department
Loyola University Chicago

The final copies have been examined by the director of the Committee and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is, therefore, accepted in partial fulfillment of the requirements for the degree of Master of Science.

8-27-93
Date

Albert J. Rotermund
Director's Signature