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Reproductive Senescence in the Chinese Hamster (*Cricetulus griseus*)[1]

Terry A. Parkening, PhD[2]

The female Chinese (C.) hamster (*Cricetulus griseus*) exhibits a progressive decline in litter size after the third litter. The mean number of litters produced was 4.9 ± .5 for 25 C. hamsters, and the mean age of the C. hamsters at the time of delivering the last litter was 16.4 ± .5 months. Most aged C. hamsters (17 to 20 months old) retained a relatively normal estrous cycle, although fewer ovulated, either spontaneously or after mating, when compared with younger hamsters (5 to 6 months old). Progesterone injections given to aged (16 to 21 months old), otherwise nonproducing, C. hamsters after mating permitted 61% to become pregnant once again and 34% to deliver viable young. Early infant mortality in these litters was greatest, however, and growth of the young was retarded in comparison with earlier litters. It appears as if age-related changes in the ovary and uterus are primarily responsible for reproductive senescence in the female C. hamster.

Key Words: Aging, Litter Size, Ovulation, Estrous cycle

THE Chinese (C.) hamster (*Cricetulus griseus*) was introduced as a laboratory animal in 1919 (Yerganian, 1958). Through the years, it has been maintained and bred primarily for genetic and toxicological research because of its low chromosome number (22). The species would probably enjoy a greater popularity among researchers if it were not for the hamster's aggressive behavior, particularly that of the female.

Several suggestions (Avery, 1968; Belćić & Weihe, 1967; Moore, 1965; Porter & Lacey, 1969) have been made on how to maintain a successful breeding colony of C. hamsters. These suggestions have largely centered on methods to mate the animals in a way that the male hamster is subjected to the least amount of injury. The methods employed generally require additional manpower or specialized cages. For these reasons, little information is available on the long-range reproductive performance of this species. The main purpose of this paper is to provide such data acquired from a colony of breeding C. hamsters over a 4-yr period.

Laboratory rats, and to a lesser extent, mice and Syrian hamsters have been utilized to study age-related effects on their reproductive systems in hopes of obtaining an animal model to better understand aging of the human reproductive system. A secondary purpose of the following study was, therefore, to compare the reproductive performance of the C. hamster with that of other laboratory rodents and to attempt to gain some insight into the possible causes of reproductive senescence in this species. To achieve the latter, experiments were conducted to determine whether the ovary of the older C. hamster was still capable of producing viable ova and if an ovarian hormone, such as progesterone, was being supplied in sufficient quantities to support and maintain pregnancy.

Method

The colony was begun in the fall of 1976 by acquiring eight female and three male, young mature C. hamsters from Chick Line Company (Vineland, NJ). All of the hamsters used in these studies were derived from that stock. The animals were housed in 29 by 19 by 13-cm plastic cages, with sawdust as litter, under uniform lighting (14 hr light/10 hr darkness) and temperature (21 to 23°C), and were provided with food (Formulab Chow #5008, Ralston Purina Company) and water ad libitum. Oatmeal and fresh carrots were provided once a week to supplement their diet.

No specialized cages or breeding procedures were used for propagating the colony of C. hamsters. In general, one mature male was paired with a prepubertal female until it was apparent the female was pregnant, and the male was removed. In some instances, the male was left with the female for several pregnancies if

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[2] Department of Anatomy, University of Texas Medical Branch, Galveston, TX 77550.

they appeared compatible. If a female C. hamster was especially aggressive (had killed a male), she was caged individually, and the vaginal area was examined in the late afternoon for signs of estrus according to the methods of Yerganian (1958). When such an animal appeared to be in estrus, she was housed with two to three males for the evening and examined the next morning for a copulatory plug.

The reproductive capabilities of C. hamsters were analyzed in the following ways. First, the lifetime breeding records of individual female C. hamsters were studied, including the number of young in each litter and the age at which they stopped reproducing. Second, C. hamsters (17 to 20 months old) that were no longer capable of producing young were placed with two fertile males (6 to 10 months old), and their estrous cycles were determined by examining a vaginal smear daily. Those that exhibited sperm in the vagina, or a strong estrus without having mated, were killed at 10 a.m. to 12 p.m. on the morning of estrus, and the oviduct was examined for ova in the manner described by Parkening et al. (1978). A group of young mature female C. hamsters (5 to 6 months old) were treated in an identical manner as a control group for these experiments. Ova recovered from both age groups were fixed, stained, and examined for fertilization by the method of Parkening and Soderwall (1975). In a final study, C. hamsters (16 to 21 months old) that had not produced a litter for at least 3 months were placed with two new males (6 to 10 months old) and examined daily for the presence of sperm in the vagina. On the morning that sperm were detected (9 a.m. to 10 a.m.), the animal received the first of three subcutaneous injections of progesterone (1 mg dissolved in .1 ml of corn oil). The remaining injections were given on alternate days after mating. The number that mated, became pregnant, and subsequently littered was recorded. Since this study was conducted for 8 wk, some C. hamsters that mated and failed to deliver young mated a second time, and one female mated three times. Progesterone was administered according to the same regimen after each mating.

Results

Litter data were collected from 25 female C. hamsters during their reproductive lifespan (Fig. 1). There was a progressive decline in litter size with the age of the C. hamster. Mean litter sizes were 6.2 ± .3, 6.0 ± .3, 4.7 ± .4, 4.0 ± .4, and 3.9 ± .9 after the second, third, fourth, fifth, and sixth litters, respectively. The total number of litters delivered by the 25 C. hamsters during their lifetime is shown in Table 1, with a mean of 4.9 ± .5 litters per C. hamster. In addition to the fact that the number of young per litter gradually declines with age, it is obvious that five or six is the maximum number of litters that this species is capable of producing. The mean age at which they deliver their last litter is 16.4 ± .5 months (Table 2). Two of the 25 C. hamsters died while giving birth to young, one during delivery of her fourth litter and the other during delivery of her fifth. Practically all of the C. hamsters continued to mate after their last litter and soon became pregnant, although none delivered any additional young. In some of these pregnancies, a bloody vaginal discharge was evident for several days midway through the pregnancy, an apparent indication of fetal death, with the subsequent resorption of the fetus(es) by the uterus.

In the study conducted to determine if aged C. hamsters were still capable of producing sufficient numbers of ova, 17 young mature (5 to 6 months of age) and 19 aged (17 to 20 months of age) animals were killed on the morning of estrus. The mean number of ova recovered from 13 young C. hamsters (6.7 ± .4) was almost identical to that from 13 aged C. hamsters (6.6 ± .6). Ninety-seven percent of the ova from younger females were fertilized compared with 74% of the ova from aged females. Male and female pronuclei were visible in 19% of the ova recovered from the younger C. hamsters and 22% from the aged ones. Four young (25%) and six aged (32%) C. hamsters had failed to ovulate, even though sperm were found in their uteri. Relatively prominent follicles appeared to be present on the ovaries of these animals. When oviducts were examined for ova from five young and six aged C. hamsters that were not in the presence of males, but which exhibited a typical estrous vaginal smear, all of the younger C. hamsters were found to have ovulated, whereas only one aged animal had ovulated.

Aged C. hamsters that received exogenous progesterone after mating showed an increased ability to carry fetuses to term. All of the 44 aged female C. hamsters (16 to 21 months of age) used in this study had been caged with males for 2 to 3 months without littering. Twenty-seven (61%) of them became pregnant

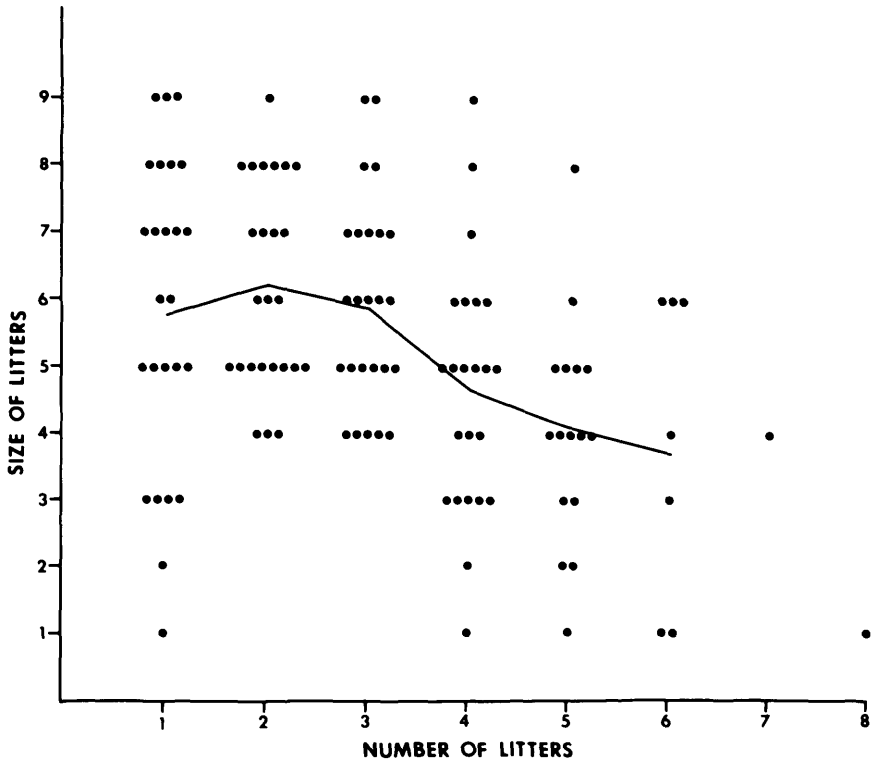

Fig. 1. Lifetime reproductive performance of 25 female C. hamsters. Each dot represents one litter. Only one animal had more than six litters. The line represents the mean litter size for the total number of animals giving birth to successive litters.

Table 1. Number of Total Litters Delivered by Chinese Hamsters during Their Lifetimes

	Number of successive litters					
	3	4	5	6	7	8
Number of C. hamsters	2	8	7	7	0	1

Table 2. Age of the Chinese Hamster when the Last Litter was Delivered

	Age of C. hamsters in months when producing the last progeny										
	11	12	13	14	15	16	17	18	19	20	21
Number of C. hamsters	1	0	3	1	3	2	8	3	1	2	1

after progesterone treatment, and 15 (34%) littered a mean number of 3.6 ± .5 viable young. The entire litter from six of these treated animals died within 1 wk of delivery, either from lack of maternal care or, more probably, from the inability of the mothers to provide sufficient milk. Some early infant deaths also occurred in three of the nine litters, in which young were raised to weaning age. The young from these litters were retarded in their growth when compared with offspring from earlier litters, which is another indication that they were receiving inadequate nourishment from their mothers. Only one of the aged C. hamsters that produced young mated twice and received progesterone injections twice. During the 2-month study, another eight of the 44 females mated twice and subsequently received progesterone treatment twice, but failed to produce young. One-half of them definitely were pregnant, but midway through the pregnancy they exhibited a profuse bloody vaginal discharge indicative of fetal resorption. One aged C. hamster mated three times during the study and received progesterone injections three times without becoming pregnant.

Discussion

Litter size in the female C. hamster declines with advancing age. This pattern of reproductive decline is similar to that reported for the aging

laboratory rat (Ingram et al., 1958; King, 1916), mouse (Biggers et al., 1962; Roman & Strong, 1962), and Syrian hamster (Blaha, 1964; Soderwall et al., 1960). The average number of litters (4.9 ± .5) produced per C. hamster during their reproductive lifetime is less than that reported for the mouse (Biggers et al., 1962) but very similar to the number (5.0) reported for the Syrian hamster (Blaha, 1964). A histological examination of ovaries from 17- to 20-month-old C. hamsters indicated that a sufficient number of ovarian follicles remained in these animals and that the inability of some C. hamsters within this age range to ovulate did not result from a depletion of oocytes. The aged C. hamsters that did ovulate exhibited a mean number of ova similar to that of younger females. The aged C. hamsters, however, had fewer fertilized ova (23%). The male C. hamsters used in these studies had previously been proven to be fertile, so it is doubtful that the lower number of fertilized ova in the older animals occurred because of fertility problems in the male. In all of the younger female C. hamsters and all but one aged C. hamster, some portion of the total ova that were ovulated were fertilized.

The advantageous effects of administering progesterone to permit some otherwise nonproducing C. hamsters to carry fetuses to term agrees with data compiled by Avery (1968). Avery found that various doses of exogenous progesterone (given to C. hamsters after mating at approximately 1 yr of age) improved conception rates 33.3 to 46.7%. In the present study, the percentage of C. hamsters that became pregnant was even greater (61%) in spite of the fact that the C. hamsters were 5 to 8 months older. It should be emphasized, however, that in the present study the animals received a lower concentration of progesterone, given in three injections over a 5-day period rather than a single injection given after mating as in the study by Avery (1968). It would appear that the corpus luteum of the ovary of the aged hamster has lost its capacity to support a pregnancy in its initial stages.

The mean litter size in aged hamsters that were given progesterone was 3.6 ± .5 even though the ovulation rate in those that were ovulating was 6.6 ± .6, an indication that the decline in litter size with age was still inevitable and that other factors, such as an inadequate uterine environment or nonviability of ova or early embryos, may also be involved. In a study on aged Syrian hamsters, a 2 to 5 hr delay that was found in the fertilization of ova was speculated to be a primary cause for the increased incidence of abnormal preimplantation embryos (Parkening & Soderwall, 1975). This delay may also contribute to postimplantation death (resorptions) in this species.

The larger number of aged C. hamsters that failed to ovulate is another indication that concentrations of ovarian steroids or gonadotropins either may be insufficient or may have become asynchronous in their phasic release. The latter possibility is more likely to be true, since in a recent study in this laboratory 67% of 24 senescent C. hamsters (24 to 28 months old) were still showing a regular 4- to 5-day estrous cycle. It would also be of interest to determine prolactin concentrations, since the newborn of several aged C. hamsters died within a few days of their delivery. (None of these dead newborn were eaten or physically abused by the mothers. The maternal instinct of the C. hamster for protecting and caring for her young is exceptionally good in comparison to mice, rats, and Syrian hamsters.) It appeared as if the aged mothers had insufficient milk for nursing the young, since the young that survived did not grow and mature as fast as those raised by the mother when she was younger. The determination of gonadotropin and prolactin concentrations by radioimmunoassay in plasma from 17 to 20-month-old C. hamsters is currently underway in this laboratory.

The female C. hamster appears to be a good rodent to use in further investigations of the effects of aging on the reproductive system, since this species, unlike the aging rat or mouse, has a greater tendency to maintain a relatively normal estrous cycle until late in life. A further investigation of age-related changes in the ovary or uterus may help explain what causes reproductive senescence in this species.

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