Measuring Rates of Sexual Development in Female Mongolian Gerbils

MERTICE M. CLARK
BENNETT G. GALEF, JR.
Department of Psychology, McMaster University, Hamilton, Ontario, Canada

In previous experiments, we have shown that age at vaginal introitus predicts adult patterns of reproductive, maternal, and aggressive behavior of female Mongolian gerbils. In the present series of studies, we explored the relationship in gerbils between age at vaginal patency and three other indices of rate of female sexual development: ovarian weight, occurrence of vaginal estrus, and onset of attractiveness to males. We found that female gerbils, classified on the basis of their ages at vaginal introitus as early-maturing: (a) exhibited greater ovarian weights throughout development than did their late-maturing, female sibs; (b) exhibited estrus and Diestrus I stage vaginal smears and, by inference, ovulation at an earlier age than did late-maturing females; and (c) elicited mounting by males at an earlier mean age than did late-maturing females. The data were consistent with the hypothesis that age at vaginal introitus is a reliable indicator of rate of sexual development in female Mongolian gerbils.

Age at vaginal opening is bimodally distributed in female Mongolian gerbils (*Meriones unguiculatus*) (Clark, Spencer, & Galef, 1986b). Some female gerbils, those we have described in previous papers as early-maturing (E-M), exhibit vaginal opening before reaching 23 days of age; ($\bar{X}$ = 15.9 ± .5 days); other female gerbils, those we have called late-maturing (L-M), fail to exhibit vaginal opening until they are more than 27 days old ($\bar{X}$ = 35.2 ± .7 days). Fewer than 1 in 100 gerbils exhibit vaginal opening when they are between 23 and 27 days of age. (Clark et al., 1986b). Hence, female gerbils can be conveniently defined as either early- or late-maturing depending on whether they exhibit vaginal introitus before or after reaching 25 days of age (Clark et al., 1986b).

Reprint requests should be sent to Dr. Mertice M. Clark, Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

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Meriones unguiculatus is not the only rodent species in which females have been observed to exhibit vaginal introitus prior to reaching weaning age. For example, in the laboratory, steppe lemmings (*Lagurus lagurus*) have become pregnant when only 19 days old and individuals pregnant at 21 days of age are "quite common" (Gebchinska, 1967, p. 521). Some female *Microtus californicus* become sexually active at a weight of 15 g, i.e., possibly as young as 14 days of age (Greenwald, 1956) and female prairie deer mice (*Peromyscus maniculatus*) have been observed with their vaginas patent before reaching weaning age (Whitsett & Miller, 1982).

There has been a tendency to treat such precocious females as aberrant and disregard them. Whitsett and Miller (1982), for example, discarded data from any female exhibiting vaginal opening prior to weaning and Greenwald (1956) treated precocious females as unimportant because they weighed significantly less at vaginal introitus than did the lightest visibly pregnant females in his sample. Gebchinska (1967), on the other hand, considered the very precocious female lemmings he observed to be important. He treated early and late vaginal opening as possibly indicative of alternative reproductive life histories available to females.

The present series of studies were undertaken to gather further evidence as to whether, in Mongolian gerbils, early vaginal introitus is indicative of a general acceleration of sexual maturation. Although we have shown previously that age at vaginal introitus predicts adult patterns of maternal, reproductive, and aggressive behavior in female gerbils (Clark & Galef, 1986; Clark et al., 1986a,b), we do not know whether, in our subjects, age at vaginal opening correlates well with other more frequently employed indices of sexual development. We have previously reported that a female gerbil's age at vaginal opening is predictive of her age at first parturition ($r = .48, p < .001$). However, the former measure accounts for less than 25 percent of the variance in the latter. Of course, first parturition does not occur for some weeks or months following vaginal introitus, depends on interaction between females and males, and is often delayed by fetal resorption, miscarriage, etc. Thus, one might not expect a female's age at first parturition to be highly correlated with other measures of her sexual development.

In the present article, we determined whether gerbils classified as early- or late-maturing on the basis of their respective ages at vaginal opening also differed in their ovarian weights (Exp. 1), ages at first vaginal estrus (Exp. 2), and ages when first mounted by males (Exp. 3).

Method

Multiparous breeding pairs of Mongolian gerbils (*Meriones unguiculatus*), acquired from Tumblebrook Farm (Brookfield, MA), bred and maintained in the McMaster colony, served as the source of all subjects. Breeding pairs were housed in polypropylene, shoe-box cages (35 × 30 × 15 cm), closed with 1/2-in. hardware cloth (1.27 cm), and carpeted with a thin layer of woodchip bedding (Betta-chip, Northeastern Products Corp., Warrensburg, NY). The colony was maintained on ad lib Purina Laboratory Rodent Chow and water in a temperature-controlled colony room illuminated on a 12/hr light–dark cycle (lights on at 0700 hours). Each breeding pair was examined daily and, when a female was visibly

pregnant (i.e., in her last week of pregnancy), her mate was removed from her cage. Each cage containing a pregnant female was examined twice daily (1000 hr and 1700 hr) to determine the date of parturition of its occupant.

Pups in each litter were toe-clipped on the day of their birth (Day 1) for individual recognition and were weaned to a cage separate from that of their dam during the morning of their 25th day. Each female in each litter was examined daily from Day 15 to weaning at Day 25 to determine her age at vaginal perforation. Examination for vaginal perforation was accomplished by applying gentle pressure just below the vagina. Those females exhibiting vaginal introitus before weaning on Day 25 were classified as early-maturing (E-M). Those females achieving vaginal perforation after weaning on Day 25 were classified as late-maturing (L-M).

Experiment 1

In the present experiment, we examined the relationship between age at vaginal patency and ovarian weight in female gerbils. In Study 1 we compared the ovarian weights of L-M females on the day on which their vaginas became perforate with the ovarian weights of both: (a) their E-M sisters and (b) age-matched, unrelated, L-M females with imperforate vaginas. In Study 2, we compared the ovarian weights of E-M females with those of L-M females with imperforate vaginas at 25, 30, 35, and 40 days of age. In Study 3, we determined ovarian weights adequate for successful reproduction.

Method

Subjects and Rearing Conditions

Subjects were 98 E-M females and 109 L-M females selected from 151 litters born in the McMaster colony and reared as described in General Method. To control for litter effects, we used no more than two females (where possible, one E-M and one L-M) from any one litter.

Procedure

Study 1. Eighteen L-M females selected from 18 litters were sacrificed by anesthetic overdose on the day on which their vaginal introitus occurred. The ovaries of these females were removed and weighed to the nearest milligram. We also measured the weights of the ovaries taken from: (a) 18 E-M sisters of the 18 L-M females on the day that the latter exhibited vaginal patency, and (b) 18 age-matched, unrelated, L-M females with imperforate vaginas.

Study 2. On each of Days 25, 30, 35, and 40, 15 L-M females with closed vaginas and 15 E-M females were sacrificed by anesthetic overdose and their ovaries were removed and weighed.

Study 3. To determine ovarian weights typical of reproductively competent E-M and L-M females, we sacrificed 20 E-M and 13 L-M females one week following weaning of their respective litters at Day 25 postpartum. Each of these 33 females had given birth before reaching 84 days of age.

Results

The main results of Studies 1 and 2 of Experiment 1 are shown in Figure 1. Figure 1 presents data describing both the absolute (upper panels) and relative (lower panels) ovarian weights of subjects in Studies 1 and 2. The left-hand panels of Figure 1 present data from Study 1, describing the absolute and relative ovarian weights: (a) of L-M females on the day of their vaginal opening, (b) of their E-M sisters, and (c) of age-matched L-M females with imperforate vaginas. We found that on the day of vaginal opening L-M females had heavier ovaries ($\bar{X}$ = 10.1 ±

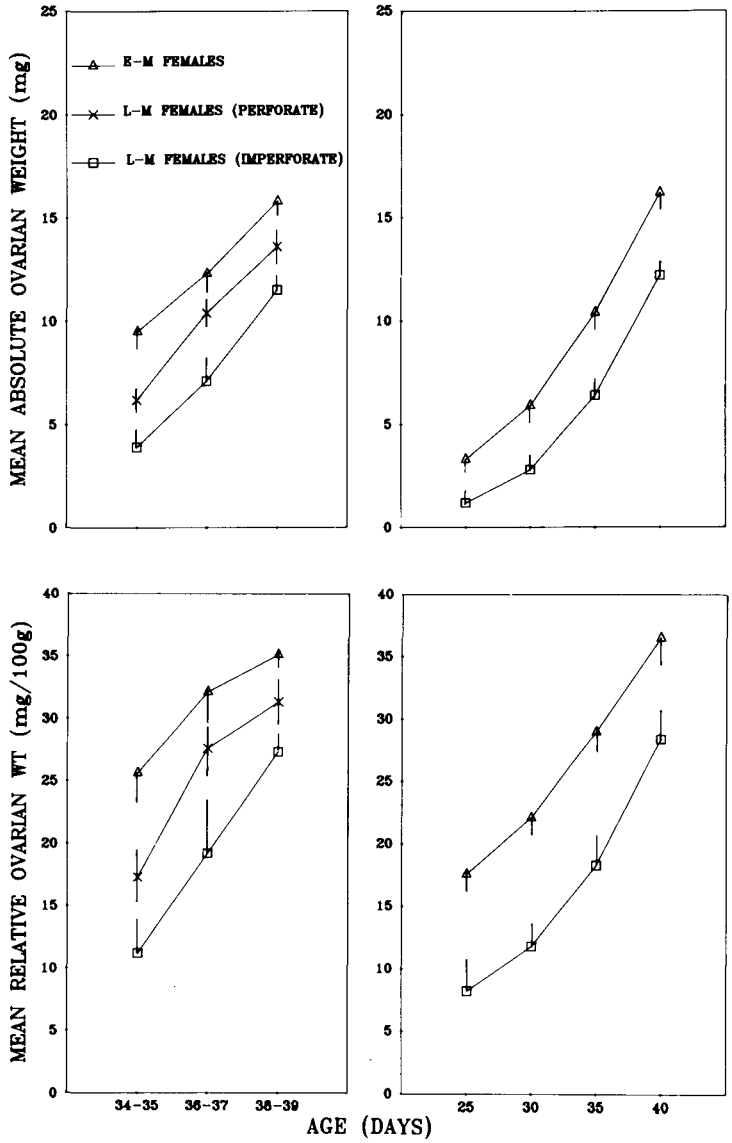

Fig. 1. Mean absolute (upper panels) and mean relative (lower panels) ovarian weights as a function of age in E-M and L-M females in Study 1 (left-hand panels) and Study 2 (right-hand panels) of Experiment 1. Flags = ± SEM; *n*'s = 6/group (left-hand panels), 15/group (right-hand panels).

0.8 mg) than age-matched L-M females whose vaginas were imperforate ($\bar{X}$ = 7.5 ± 1.0 mg; $df = 34$, $t = 2.00$, $p < .05$) and lighter ovaries than their E-M sisters ($\bar{X}$ = 12.6 ± 0.8 mg, correlated t-test, $df = 17$, $t = 3.79$, $p < 0.01$).

As can be seen in the lower, left-hand panel of Figure 1, differences in absolute ovarian weight were not due to differences in the body weights of subjects in the three groups. Relative ovarian weights (ovarian weight/body weight) of L-M females on the day of their vaginal opening ($\bar{X}$ = 25.4 ± 1.8 mg/100 g) were both higher than those of age-matched L-M females with imperforate vaginas ($\bar{X}$ = 19.2 ± 2.4 mg/100 g; $df = 34$, $t = 2.05$, $p < 0.05$) and lower than those of their E-M sisters ($\bar{X}$ = 30.9 ± 1.9 mg/100 g, correlated t-test, $df = 17$, $t = 3.24$, $p < 0.01$).

Ovarian development of E-M and L-M females in Study 2 is described in the right-hand panels of Figure 1. On each of Days 25, 30, 35, and 40, E-M females had, in comparison with L-M females with imperforate vaginas, both heavier ovaries (all df's = 28, all t's > 4.09; all p's < 0.01) and elevated relative ovarian weights (all df's = 28, all t's > 3.71; all p's < 0.01). We do not know whether such differences in the absolute and relative ovarian weights of early- and late-maturing nulliparous gerbil females are maintained into adulthood.

The ovaries of 40-day-old E-M females in Study 2 did not differ significantly in weight ($\bar{X}$ = 16.2 ± 0.7 mg) from the ovaries of primiparous E-M females in Study 3 ($\bar{X}$ = 16.3 ± 1.1 mg; $t = 0.84$, n.s.). In contrast, ovaries of primiparous L-M females in Study 3 ($\bar{X}$ = 24.1 ± 1.6 mg) were twice as large as those of 40-day-old L-M females in Study 2 ($\bar{X}$ = 12.2 ± 0.7 mg).

Discussion

The results of the present experiment indicate that in female gerbils age at vaginal opening is related to degree of ovarian development. At each age examined, ovarian weights were greatest for E-M females, smallest for L-M females with imperforate vaginal openings, and intermediate for L-M females with perforate vaginas. Ovaries of 40-day-old E-M females were equivalent in weight to the ovaries of primiparous E-M females, while the ovaries of L-M females weighed only half as much as the ovaries of reproductively active L-M females. If ovarian weight is indicative of reproductive capability, then our E-M females were reproductively competent long before their L-M sibs. That the ovaries of 40-day-old E-M gerbils are, in fact, fully functional is indicated by the observation that E-M females in our colony occassionally give birth when 60–65 days of age, following a 25-day period of gestation. The data of the present experiment are entirely consistent with the view that from Day 25 through Day 40 the ovarian development of E-M gerbil females is considerably advanced relative to that of L-M females of the same chronological age.

Experiment 2

Perhaps the most commonly employed index of age of sexual maturation in rodents is their age at first vaginal estrus. In the present experiment, we monitored changes in the vaginal cytology of young E-M and L-M female gerbils to determine the relationship between age at vaginal introitus and age at first vaginal estrus in our subjects.

Experiment 3

If it is the case that early vaginal opening is associated with truly precocious sexual development in female gerbils, one might expect males to express a sexual interest in younger E-M females than L-M females (Vick & Banks, 1969). In the present experiment, sexually-proven, adult males were placed with E-M and L-M females throughout their development to establish the age at which E-M and L-M females first elicited attempted mounting by males.

Method

Subjects

We determined retroactively that 10 E-M and 12 L-M 15-day-old females had been selected as subjects. In addition, 20 sexually-proven, adult males, each maintained with a reproductively active female, were used to provide evidence of the sexual attractiveness of subject females.

Procedure

Beginning on Day 15, when all subject females had sealed vaginas, each was placed for 15 min (between 1600 and 1800 hr) in the cage of a randomly-selected, sexually-proven male whose mate had been removed from his cage 5 min prior to introduction of the subject female. An observer, unaware of the maturational rate of subject females, observed each pair and recorded any instances of attempted mounting of the female by the male. Testing of each female was repeated daily either for 40 days or until 15 days after a female's vagina had opened, whichever occurred last. Females were each tested at least twice with each male in the course of the experiment. Order of presentation of females to individual males was counterbalanced across subjects.

Results

The main results of Experiment 3 are presented in Figure 3 which shows, as a function of females' ages, the cummulative percentage of E-M and L-M females that males attempted to mount. As is evident from inspection of Figure 3, E-M females were first mounted at a younger mean age ($\bar{X}$ = 23.9 ± 1.1 days) than were L-M females ($\bar{X}$ = 36.7 ± 1.7 days; $df = 20$, $t = 5.92$, $p < .01$). We also found that age at vaginal opening was significantly correlated with age at first mounting ($r = .67$, $p < .02$) even though 8 of 12 L-M and 1 of 10 E-M females were mounted at least once prior to their vaginal opening. In the 15-day period immediately following vaginal opening, the number of times males attempted to mount E-M ($\bar{X}$ = 2.2 ± .3 mounts) and L-M ($\bar{X}$ = 2.9 ± .5 mounts) females did not differ significantly ($df = 20$, $t = 1.14$, n.s.).

General Discussion

The results of the present series of studies are entirely consistent with the hypothesis that age at vaginal opening is a meaningful index of rate of sexual

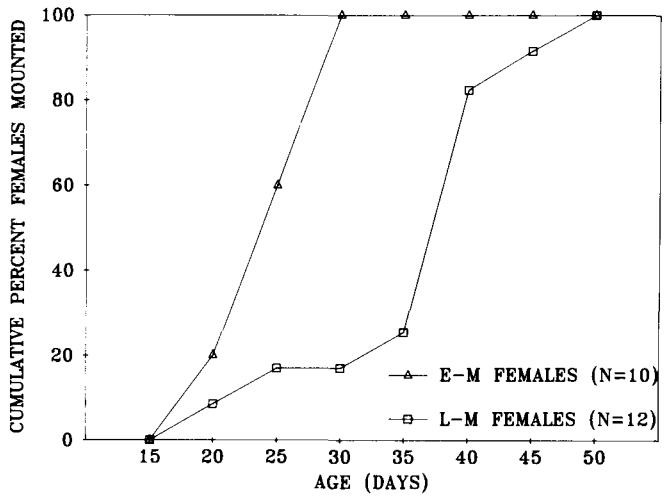

Fig. 3. Cumulative percentage of E-M and L-M females mounted by mature males during testing in Experiment 3.

development in female Mongolian gerbils. Females classified as early-maturing (E-M) on the basis of their ages at vaginal introitus: (a) exhibited greater ovarian weights throughout development than did their late-maturing (L-M) sisters; (b) exhibited both estrus and Diestrus I stage vaginal smears and, by inference, ovulation, at an earlier mean age than did L-M females; (c) elicited mounting by males at an earlier mean age than did L-M females; and (d) gave birth for the first time at an earlier mean age than did L-M females (Clark et al., 1986b). Although no single piece of evidence is conclusive and each is open to alternative interpretation, taken together the observations in the present paper strongly suggest that female gerbils described as early-maturing on the basis of their ages at vaginal introitus are functionally sexually capable at an earlier mean age than those described as late-maturing on the same basis. Age at vaginal opening is not only a predictor of adult reproductive profiles in female Mongolian gerbils, it is also an index of the rate of their sexual development.

The preceding discussion suggests that observations of precocial vaginal opening in rodents should not be ignored or explained away as lacking in functional significance. Females of rodent species other than *Meriones unguiculatus* may share their facultative capacity for precocious puberty. Investigation of the conditions under which this capacity is expressed may be of importance in understanding the life historical strategies of rodents in natural circumstances.

Notes

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