Improving the productivity of breeding colonies of Mongolian gerbils (*Meriones unguiculatus*)

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Summary

The productivity of breeding colonies of Mongolian gerbils can be substantially enhanced by using as breeding stock only the 40% of females that exhibit vaginal opening before reaching 25 days of age. Early-maturing females are more likely to breed successfully on first pairing. The lifetime fecundity of early-maturing females is more than twice that of their late-maturing sisters. In those cases in which early-maturing females fail to breed with the first male with which they are paired, they (but not late-maturing females) can be mated with a second male with a high probability of success. Two-thirds of the early-maturing females that failed to reproduce following a first pairing became pregnant following a second. Only 11% of late-maturing females did so.

Keywords: Gerbillinae; Breeding

The laboratory breeding of Mongolian gerbils poses problems rarely encountered in the breeding of rats or mice: (1) only 50%–75% of gerbil females produce litters following first pairing with a proven male (Arrington, Beaty & Kelly, 1973; Norris & Adams, 1982; Clark, Spencer & Galef, 1986); (2) approximately 60% of reproductively active gerbil females consistently both produce small litters and exhibit extended interpartum intervals throughout their reproductive life (Clark, Spencer & Galef, 1986); (3) females failing to reproduce with a first partner will frequently kill an unfamiliar male when a second pairing is attempted (Robinson, 1975). The productivity of gerbil colonies would be significantly enhanced if such problems could be overcome.

The results of recent studies in our laboratory indicate that female gerbils exhibiting vaginal opening before reaching 25 days of age (early-maturing females) are more likely to bear young following a first pairing than those exhibiting vaginal introitus at a greater age (late-maturing females) (82% versus 70%). Further, reproductively active early-maturing females had more than twice the average lifetime fecundity (25·1 versus 11·4 pups) of late-maturing females (Clark, Spencer & Galef, 1986). The exclusion of late-maturing females from breeding stock would clearly be a useful first step in enhancing the productivity of gerbil colonies.

Received 13 August 1985; in revised form 25 March 1986. Accepted 12 May 1986.

Even among early-maturing females, 18% failed to deliver any young in the 6 months following first pairing with a proven male (Clark, Spencer & Galef, 1986). Colony productivity could be further enhanced if those females failing to breed with the first male with which they were paired could be successfully bred with a second male. The present experiment was undertaken to determine whether those early-maturing female gerbils failing to breed at first pairing could be successfully re-mated and bred.

Materials and methods

The subjects were 38 (20 early-maturing and 18 late-maturing) primiparous female gerbils born in the McMaster colony to breeding stock acquired from Tumblebrook Farms (West Brookfield, MA, USA) and 18 (nine early-maturing and nine late-maturing) nulliparous females that had failed to breed within 180 days of pairing with a sexually proven male. The 56 subjects were randomly selected from 50 litters of four to eight pups born in the spring of 1984. Late-maturing females were taken from litters averaging 43·1% ± 5·5% males and early-maturing females from litters that averaged 44·4% ± 5·7% males at birth.

The rearing, maintenance and breeding conditions have been described at length elsewhere (Clark & Galef, 1985; Clark, Spencer & Galef, 1986). In brief, at weaning on day 25 postpartum, each female was examined for vaginal introitus by applying gentle pressure just below the vagina and was then placed in the cage of a proven adult male. Breeding pairs were housed individually in polypropylene cages (35 cm × 30 cm × 15 cm) lidded with a 1·27 cm screen and carpeted with a thin layer of wood-chip bedding. All breeding pairs were maintained on Purina Laboratory Rodent chow (Ralston-Purina Canada Ltd, Longueuil, Quebec, Canada) and water *ad libitum* in a single temperature-controlled colony room illuminated on a 12 h light–dark cycle. Each female was examined daily for evidence of pregnancy and males were removed from the cages of gravid females during the

third trimester, when females were obviously swollen.

Each of the 38 females successfully bearing litters were tested for response to a strange adult male 7 days after weaning of her litter at 25 days post-partum, approximately 39 days after we had removed each female's original mate. Each of the 18 females failing to breed for 180 days following first pairing was tested for response to a strange male 7 days after her original partner had been removed from her cage.

On the test day, each female was introduced into the cage of a strange male that had previously sired a litter. We observed the newly constituted pair for 15 min and recorded whether a fight occurred. Fights were easily discriminated, as they involved vigorous tumbling about the cage, vocalizations and attempts at biting. Following observation, pairs were left undisturbed for 3 weeks. Each female was then sacrificed by anaesthetic overdose and her uterus was examined for the presence of fetuses.

Results

The main results of the present study are presented in Table 1 which shows the percentage of early-maturing and late-maturing nullipara and primipara (1) that attacked their new mate during the 15 min period of observation on the test day, (2) that were pregnant at autopsy 3 weeks following pairing and (3) whose consorts died during the 3 weeks after pairing.

Table 1. Response of nulliparous and primiparous early- and late-maturing female gerbils towards unfamiliar adult males

	Nullipara		*Primipara*	
Maturing	Early	Late	Early	Late
n	9	9	20	18
Number fighting (15 min test) (%)	11·1	66·7	15·0	66·7
Number pregnant (%)	66·7	11·1	75·0	33·3
Number of males dying (%)	11·1	55·6	5·0	44·4

As can be seen in the table, early-maturing nulliparous and primiparous females were (1) more likely to become pregnant (nullipara, Fisher exact probability test, $P < 0{\cdot}05$; primipara, $\chi^2 = 6{\cdot}6$, $P < 0{\cdot}01$), (2) less likely to fight with a strange male (nullipara, Fisher exact probability test, $P = 0{\cdot}05$; primipara, $\chi^2 = 10{\cdot}6$, $P < 0{\cdot}01$) and (3) less likely to kill a strange male (nullipara, Fisher exact probability test, not significant; primipara, $\chi^2 = 8{\cdot}1$, $P < 0{\cdot}01$) than their late-maturing counterparts. The 15 min observation of aggressive behaviour following re-mating predicted neither impregnation of the female nor death of the male in any group. For example, 44% of the 18 males fighting with late-maturing females during the 15 min observation period and 55% of the nine males not fighting with late-maturing females during the 15 min observation period died during the subsequent 3 weeks. Fighting immediately following pairing was not a predictor of subsequent mortality in males; age of female at vaginal introitus was. Thus, male stress and mortality resulting from standard laboratory practice, pairing for mating, can be substantially reduced, at the same time as breeding efficiency is enhanced, by using early-maturing gerbil females for breeding purposes.

It was also of some interest to find (Table 2) that both early- and late-maturing females that fought with unfamiliar males during the 15 min test period came from relatively male-biased litters, while the probability that males would perish in the 3 weeks following pairing was independent of the sex ratio of the natal litters of their mates. These data further suggest that fighting within pairs and death of males are independent events.

Table 2. Mean sex ratios of natal litters of females

	Number of males in the natal litters (%)
Early-maturing females:	
fight ($n = 17$)	$51{\cdot}6 \pm 4{\cdot}8^a$
not fight ($n = 10$)	$32{\cdot}3 \pm 5{\cdot}5^a$
males die ($n = 13$)	$40{\cdot}0 \pm 6{\cdot}0$
males not die ($n = 14$)	$46{\cdot}9 \pm 5{\cdot}3$
Late-maturing females:	
fight ($n = 4$)	$59{\cdot}6 \pm 8{\cdot}8^b$
not fight ($n = 25$)	$38{\cdot}1 \pm 4{\cdot}1^b$
males die ($n = 2$)	$66{\cdot}6 \pm 16{\cdot}7$
males not die ($n = 27$)	$39{\cdot}2 \pm 3{\cdot}9$

[a,b]Entries with the same superscript differed significantly by the median test (a, $\chi^2 = 6{\cdot}5$, $P < 0{\cdot}025$; b, $\chi^2 = 4{\cdot}9$, $P < 0{\cdot}05$).

Discussion

The present results indicate that early-maturing females, in addition to being more fecund than their late-maturing sisters, can be re-mated with a high probability of success and little risk to the unfamiliar males with which they are paired. In contrast, late-maturing females were likely to attack or kill unfamiliar males introduced into their cages and unlikely to be impregnated by them.

Those familiar with the published literature on age at vaginal opening in Mongolian gerbils will find surprising the fact that 40% of our females exhibited vaginal introitus before reaching 25 days of age. Norris and Adams (1979), for example, have reported vaginal opening in females in their colony at a mean age of 41 days (33–53 days) with first *conception* at 75 days postpartum. In our colony, vaginal opening is observed in females at a mean age of 26·8 days (13–43 days), with first *parturition* as early as 62 days of age.

Intercolony variation in both age at vaginal introitus and age at first reproduction probably reflects the immense difference in rates of growth of females (Bronson & Rissman, in the press) in our colony and in the colony of Norris and Adams. Our females weigh on average 23·8 g (± 1·4 g) at vaginal opening on day 27 postpartum; Norris and Adams' females weighed only 27·7 g (± 0·4 g) at vaginal opening on day 41 postpartum. By 40 days of age our females weigh 45·4 g (± 1·1 g).

We have not selected our breeding stock for either rapid growth or early sexual maturation. We find similar rates of growth and ages at vaginal opening in young born to females received directly from the major North American supplier, Tumblebrook Farms. Either there are genetic differences between North American and British strains of domesticated gerbils (although Norris and Adams' (1972) colony is of North American origin) or differences in present conditions of maintenance have produced a marked divergence in rates of gerbil growth and development in North America and Great Britain.

By examining female gerbils for vaginal opening at weaning at 25 days of age and using only early-maturing females as breeding stock, a colony can be established in which fecundity is high and in which the females failing to breed following a first pairing can be successfully bred with a second male. Successful breeding following one or, if necessary, two pairings by more than 90% of early-maturing females can be anticipated, compared with less than 75% of late-maturing females.

Robinson (1975) has suggested discarding female gerbils if a first mating is unsuccessful. Cheal (1983) has recommended attempting second matings only during a female's period of behavioural oestrus. The results of the present study indicate that in breeding colonies of early-maturing females neither discarding of failed breeders nor behavioural measures of reproductive state are necessary. Early-maturing females can be introduced to unfamiliar males with little danger to the male and a high probability of successful reproduction.

Acknowledgements

This research was supported by grants to B. G. G., Jr, from the National Sciences and Engineering Research Council of Canada and the McMaster University Research Board. Requests for reprints should be sent to Dr Mertice M. Clark, Department of Psychology, McMaster University, Hamilton, Ontario L8S 4K1, Canada.

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Laboratory Animals (1986) 20, 316–320.

Spontaneous endometrial tumours of 'Sabra' rats

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Summary

In a 2 year study of 171 female (101 virgin; 70 multiparous) 'Sabra' rats, spontaneous endometrial tumours were found in 69% of 2 year old animals. Tumour development appeared to be age related, and only virgin females showed tumours before 18 months of age. Polyps were the most prevalent tumour type, followed by adenocarcinomas. The Sabra rat can be included among those rat strains having a high incidence of spontaneous endometrial neoplasia.

Keywords: Rats, inbred strains; Uterine neoplasms; Polyps

Spontaneous tumours have frequently been reported among laboratory rats (Bullock & Curtis, 1930), and their incidence may vary greatly according to the breed or strain of the host (Snell, 1965). The 'Sabra' rat, a random-bred albino strain probably of Wistar origin, has been used by biomedical researchers, primarily in Israel but also abroad, for more than 60 years. In the past decade, two inbred substrains derived from the Sabra strain have received international acceptance for use in studies of type II diabetes (Cohen *et al.*, 1974) and hypertension (Ben-Ishay *et al.*, 1981). Despite wide use, little information concerning the incidence of neoplastic disease in the random-bred Sabra rat strain has been reported.

Herewith we report the occurrence of a large number of spontaneous uterine tumours seen in the course of a 2 year study designed to characterize the Sabra rat (Lutsky, Aizer & Mor, 1984).

Materials and methods

The 2 year study included 171 conventional female Sabra rats: 101 virgins and 70 multiparous breeders obtained from the Animal Breeding Unit of the Hebrew University Hadassah Medical School. Animals were housed in an air-conditioned light-controlled room, in solid-bottom stainless steel cages of the 'shoebox' type, 24 cm × 34 cm × 15 cm in size, with wire mesh lids. Bedding consisted of steam-sterilized soft wood shavings changed weekly.

Received 6 December 1985. Accepted 23 May 1986.

The number of rats per cage varied with their age and weight. All animals received a complete pelleted laboratory-animal diet containing a minimum of 18% protein and 3% fat, and a maximum of 5% fibre, *ad libitum*. Water in glass bottles, changed daily, was also available *ad libitum*. Cages and ancillary equipment were sanitized and replaced each week.

Animals were killed at regular intervals by intraperitoneal injection of sodium pentobarbital, 40 mg/kg bodyweight. At necropsy, liver, lungs, uterus, spleen, kidneys, adrenals, thyroid, ovaries and all tissues with visible abnormalities were routinely excised and fixed in Bouin's fluid or 10% formalin. Sections for light microscopy were stained with haematoxylin and eosin (H&E). The incidence rates of tumours were compared by the quadratic normal approximation (Brownlee, 1965).

Results

The distribution of tumours by age and type is summarized in Table 1. Endometrial tumours were noted to have developed at as early as 4 months of age in virgin rats but not earlier than 18 months in breeders. At 24 months, the prevalence of endometrial tumours did not differ significantly between the two groups ($P > 0{\cdot}05$).

On gross examination, affected uteri were enlarged and usually contained serous or purulent fluid, in which calcified material was occasionally seen. Uterine tumours were both unilateral and bilateral in distribution. The endometrial surface revealed a thickened and occasionally red mucosa that exhibited polypoid elevations. In 31 animals of all ages, the most frequently encountered tumours were stromal and adenomatous polyps. Of these, nine were adenomatous, 15 were stromal and seven had a mixed adenomatous and stromal composition. The polyps appeared as masses projecting into the uterine lumen. Single polyps occurred most frequently but multiple polyps were occasionally found; these were mostly stromal. Polyps varied in size from 1 to 10 mm in diameter (Fig. 1) and in colour from white to deep red. Small tumours were usually stromal, while the larger tumours were most often adenomatous. Upon incising the tumours, a central cyst was sometimes seen on the cut surface.