Physiology & Behavior, Vol. 42, pp. 15–18. Copyright © Pergamon Journals Ltd., 1988. Printed in the U.S.A. 0031-9384/88 $3.00 + .00

Effects of Uterine Position on Rate of Sexual Development in Female Mongolian Gerbils

MERTICE M. CLARK AND BENNETT G. GALEF, JR.

Department of Psychology, McMaster University
Hamilton, Ontario, L8S 4K1, Canada

Received 11 May 1987

CLARK, M. M. AND B. G. GALEF, JR. *Effects of uterine position on rate of sexual development in female Mongolian gerbils.* PHYSIOL BEHAV **42**(1) 15–18, 1988.—Examination of the rate of sexual maturation of 79 female gerbils from 32 Caesarean-delivered, foster-reared litters revealed that those females that, as fetuses, occupied uterine positions adjacent to one or two males (1M and 2M females) were less likely than those females occupying uterine positions not adjacent to males (0M females) to exhibit early vaginal opening. Our data further indicated that the uterine environment provided by late-maturing female gerbils biased their fetal daughters to themselves be late maturing to a greater extent than the daughters' uterine positions could explain. Daughters of late-maturing females in 1M and 2M uterine locations were more likely to be late-maturing than were daughters of early-maturing females in similar uterine locations. Because in female Mongolian gerbils age at vaginal opening is a powerful predictor of future reproductive strategy, the present results indicate that in female gerbils both prenatal maternal influence and uterine location are important determinants of future reproductive behaviors.

Uterine location Sexual development Sex ratio Puberty

AGE at vaginal opening is bimodally distributed in female domesticated Mongolian gerbils *(Meriones unguiculatus)* [1,3]. Some females, those that are early-maturing (E-M), exhibit vaginal opening prior to reaching 23 days of age (mean=15.9±0.5 days); their late-maturing (L-M) sibs fail to exhibit vaginal opening until they are more than 27 days of age (mean=35.2±0.7 days).

This bimodality in age at vaginal opening in Mongolian gerbils is of interest because in female gerbils [3], unlike female rats or mice [7, 10, 11], age at vaginal introitus predicts subsequent reproductive behaviors. Early-maturing female gerbils both give birth for the first time at an earlier mean age than do L-M female gerbils (86.1±2.9 vs. 108.5±5.0 days) and E-M females wean more than twice as many young during their reproductive lives as do L-M females (25.1±5.1 vs. 11.4±2.8 pups). Late-maturing females devote more time and energy to care of each litter than do E-M females [3]. Further, L-M females are both more likely than E-M females to attack and kill unfamiliar males with whom they are paired and less likely to become impregnated by unfamiliar males than are E-M females [2]. Thus, age at vaginal introitus is a convenient marker for distinguishing groups of female gerbils exhibiting quite divergent sexual, reproductive, and maternal profiles.

Age at vaginal introitus in female gerbils is correlated with both the sex-ratio and size of the litter in which a female was born. Early-maturing females tend to be born in large, female-biased litters, L-M females in small male-biased litters [3]. Further, there is concordance between mothers and daughters for age at vaginal introitus; E-M females tend to deliver large, female-biased litters with a high proportion of E-M daughters; L-M females tend to deliver small, male-biased litters with a high proportion of L-M daughters [3].

One necessary consequence of changes in the size and sex composition of litters is to alter the *a priori* probability that a female fetus will be located *in utero* adjacent to no males (0M females), one male (1M females) or 2 males (2M females). For example, as the proportion of males in a litter increases, the probability of a female fetus being located adjacent to one or two males fetuses should also increase. Further, as the size of the litter in which a female develops decreases, her probability of being located at one end of a uterine line of pups should increase. In consequence, a female's probability of being, for example, a 2M female should be decreased in small litters.

The experiment reported below was primarily concerned with the role of a female gerbil's uterine position in determining her subsequent probability of developing as an E-M or L-M female. It was secondarily concerned with whether differences in the age at vaginal opening of the daughters of E-M and L-M females could be explained in terms of correlated differences in the sex-ratios and sizes of the litters in which

TABLE 1

EFFECTS OF UTERINE POSITION ON PROBABILITY OF EARLY VAGINAL OPENING

	Female Uterine Position		
	0M	1M	2M
Vaginal Opening:			
Early	21	23	7
Late	1	17	10
Percentage of E-M females	95.5	57.5	41.1
Mean age at vaginal opening (days):	18.0 ± 1.1	25.5 ± 1.7	29.4 ± 2.7
E-M females	17.0 ± 0.5	16.2 ± 0.3	16.8 ± 0.5
L-M females	40.0	37.4 ± 0.5	38.1 ± 1.2

the daughters of E-M and L-M females matured.

METHOD

Subjects

Three-hundred and forty-nine Mongolian gerbil pups from 57 litters, each containing one or more females at weaning, served as subjects. These pups were born in the McMaster colony between May and November 1986 to multiparous females descended from stock acquired from Tumblebrook Farms (Brookfield, MA). Forty-four additional, recently-parturient, multiparous females from the same source served as foster mothers.

Thirty-two of the 57 litters, 19 born to E-M dams, 12 to L-M dams, and one to a dam of unknown age at vaginal opening, were assigned to the Caesarean-delivered, foster-reared, experimental group. Twenty-five control litters were vaginally delivered. Thirteen of these control litters were reared by their own dam, 12 by foster dams.

Procedure

Maintenance and breeding. Breeding pairs were individually housed in 35×30×15 cm polypropylene cages lidded with 1.3 cm (1/2 in.) hardware cloth and carpeted with a thin layer of wood-chip bedding (Betta-chip, Northeastern Products Corp., Warrensburg, NY). The colony was maintained on ad lib Purina Laboratory Rodent Chow and water on a 12:12 hr light:dark cycle (light onset 0700 hr) in a single temperature and humidity controlled colony room. The dates on which pairs copulated were noted with the expectation that a female would deliver 25 days following a copulatory episode. As each female entered the final week of her pregnancy, her mate was removed from her cage. Pups were weaned to separate cages on Day 25 postpartum (Day of birth = Day 1).

Surgical procedures. Twenty-four days following observed copulation (i.e., 1 day prior to anticipated vaginal delivery), each conspicuously pregnant female in the experimental group was anesthetized by ether inhalation, her abdomen opened, uterus externalized, and fetuses removed singly. The sex and uterine location [12] of each fetus was noted; each infant was toe-clipped for permanent identification, and each was placed in a chamber of an ice-cube tray floating in a constant temperature bath maintained at 31°C. Once all pups were removed from a dam, she was euthanized by injection of anesthetic.

Fostering Caesarean-delivered pups. Recently parturient Mongolian gerbils do not readily accept Caesarean-delivered (C-d) foster young. They generally neglect, attack, or cannibalize C-d pups introduced into their litters. The procedures for successful fostering of C-d pups described below were developed gradually during a period of trial and error.

Following delivery, each pup in a C-d litter was maintained in its individual compartment in the constant-temperature bath for 3–4 hr. Each pup in a litter was then rubbed with a piece of cotton batting that had first been rubbed on the ventral gland of the female to which that litter was to be fostered. Each foster mother had vaginally delivered a litter within 24 hr of the time of delivery of the C-d litter that foster mother was to rear.

Determining age at vaginal opening. Once fostering of C-d pups had been achieved, foster mothers and litters were left undisturbed until pups were 12 days of age. On Day 12, and on each day thereafter, females were examined to determine their ages at vaginal opening. Examination for vaginal introitus was accomplished by applying gentle pressure just below the vagina and was carried out daily until age at vaginal introitus of each female was established. Females exhibiting vaginal opening before reaching 25 days of age were considered early maturing, those exhibiting vaginal opening after reaching 25 days of age were considered late maturing [1–3].

RESULTS AND DISCUSSION

Effects of Uterine Position on Age at Vaginal Opening

The main results of the present experiment are shown in Table 1, which indicates the number and percentage of females in 0M, 1M and 2M uterine locations exhibiting early and late vaginal opening. As is clear from inspection of Table 1, uterine location of females relative to males significantly affected the age at vaginal opening of females. Ninety-five point five percent of 22 0M females were early-maturing, while only 52.6 percent of 57 1M and 2M females were early-maturing, $\chi^2(2)=14.08$, $p<0.001$. The proportion of E-M females in 1M locations did not differ significantly from the proportion of E-M females in 2M locations exhibiting early vaginal introitus, $\chi^2(1)=1.31$, $p=$n.s.

Females in 0M uterine locations tended to be from litters with a lower mean percentage of males at birth (29.0±3.9%) than did females in 1M (46.3±2.9%) or 2M (60.3±4.1%) uterine locations. Thus, it is possible that it was not uterine location per se, but overall proportion of males in a litter that influenced the probability of a female exhibiting early vaginal opening. Those females from litters containing less than the median proportion of males contained more than the median proportion of E-M females, $\chi^2(1)=4.84$, $0.02<p<0.05$.

Evidence that uterine location relative to males, in addition to proportion of males *in utero*, affected female sexual development was available from examination of litters containing females in more than one of the three uterine locations (0M, 1M or 2M). If uterine location relative to males, rather than proportion of males *in utero*, affected the probability of early female development, then one would expect, for example, that those females from 0M uterine locations would be more likely to be E-M females than would their littermates from 1M or 2M uterine locations.

Females were found in more than one uterine location relative to males in 19 Caesarean-delivered litters. In 10 of these litters, the percentages of females in 0M, 1M and 2M locations exhibiting early vaginal introitus were identical (i.e., 0 or 100 percent). In 8 of the 9 remaining litters, those

TABLE 2

CHARACTERISTICS OF LITTERS IN EXPERIMENTAL AND CONTROL CONDITIONS

	Experimental	Control	
	Caesarian-delivered	Foster-Reared	Undisturbed
Number of litters	32	13	12
Mean litter size:			
Birth	6.9 ± 0.3	6.6 ± 0.5	6.5 ± 0.5
Weaning	5.4 ± 0.4	5.9 ± 0.5	6.2 ± 0.5
Mean number dying between birth and weaning	1.6 ± 0.3	0.7 ± 0.3	0.5 ± 0.2
Percentage of males:			
Birth	51.5	48.1	53.6
Weaning	54.3	51.0	55.0
Percentage of E-M females	64.5	66.7	66.7
Percentage surviving to weaning:			
Males	83.0	96.2	93.3
Females	72.7	85.4	97.2

females in uterine locations adjacent to fewer males were more likely to mature early than were those females in uterine positions adjacent to greater numbers of males (Sign test, $x=1$, $p<0.04$). Thus, even with the sex-ratios of litters held constant, uterine location relative to males affected probability of early maturation in females.

Effects of Caesarean Delivery

Table 2 describes the composition of Caesarean-delivered (C-d) and control litters both at birth and at weaning on Day 25. As can be seen in Table 2, C-d litters did not differ from control litters in either proportion of male pups born or weaned or proportion of E-M females weaned (All χ^2s<0.5, dfs=1, ps=n.s.). Differences between number of pups per litter born to and weaned by C-d and control litters were also not significant (Kolmogorov-Smirnov Tests, both $\chi^2(1)<2$, p=n.s.).

Mortality between birth and weaning was higher among C-d litters than among control litters, possibly because of the trauma of surgical delivery. Twenty-one of 32 C-d litters lost pups between birth and weaning, while only 4 of 14 undisturbed and 5 of 12 foster-reared control litters did so, $\chi^2=6.2$, $p<0.05$. Although both C-d litters and foster-reared control litters were more likely to lose female than male pups, these differences did not approach significance (Both χ^2s<1.0, dfs=1, ps=n.s.).

In sum, although there were some effects of Caesarean delivery on pup survival to weaning, none of the measures in which we were most interested (litter size at birth, proportion of females at birth, and proportion of E-M females at weaning) was significantly affected by Caesarean delivery. It therefore seemed reasonable to use data from C-d pups to explain causes of early and late sexual maturation in vaginally-delivered pups.

Differences in Uterine Location of Females as an Explanation of Differences in the Proportion of E-M Daughters Born to E-M and L-M Mothers

As can be seen in Table 3, the proportion of daughters both at birth, $\chi^2(1)=8.2$, $p<0.01$, and at weaning, $\chi^2=7.4$, $p<0.01$, and the proportion of E-M daughters, $\chi^2(1)=5.5$, $p<0.02$, differed between litters born to E-M and L-M mothers. Early-maturing females had both female-dominated and E-M-female-dominated litters. It is therefore possible that the high proportion of E-M daughters found in litters born to E-M mothers was caused by the high proportion of daughters in the litters of E-M mothers reducing the probability that daughters of E-M mothers had developed in uterine locations adjacent to one or two males.

If differences in the proportion of E-M daughters born to E-M and L-M mothers resulted solely from the different probability of females in the litters of E-M and L-M females occupying 0M, 1M, and 2M uterine locations, then the probability of 0M, 1M, and 2M daughters from the litters of E-M and L-M dams developing to be E-M daughters should be equal. Table 4 shows the proportion of 0M, 1M, and 2M females born to E-M and L-M mothers that grew to be E-M daughters. As is evident from inspection of Table 4, 1M and 2M daughters born to E-M mothers were more likely to themselves be E-M females than were 1M or 2M daughters born to L-M mothers, $\chi^2(1)=4.64$, $p=0.05$. Thus, the difference in the proportion of E-M daughters born to E-M and L-M mothers cannot be attributed solely to the greater proportion of female fetuses carried by E-M mothers and the lower *a priori* probability daughters of E-M mothers had of developing in uterine locations adjacent to males.

CONCLUSION

The results of the present experiment show that the uterine position of a female gerbil fetus relative to male fetuses can affect her probability of exhibiting vaginal introitus at an early age. Female gerbils developing in uterine locations not immediately adjacent to a male were significantly more likely to exhibit precocial vaginal introitus than were female gerbils developing in uterine locations immediately adjacent to either one or two males.

Although the size and sex ratio of litters affects the probability of a female developing adjacent to 0, 1, or 2 males and uterine position relative to males affected the probability of early maturation in gerbil females, our observations indicated that these two effects were not sufficient to explain observed differences in the proportion of E-M daughters born to E-M and L-M dams. We found significant differences

TABLE 3

MEAN PERCENTAGE OF MALES AND E-M DAUGHTERS BORN TO E-M AND L-M DAMS

	Dams	
	Early-Maturing (n=19)	Late-Maturing (n=12)
Mean percentage of males/litter:		
Birth	43.1 ± 4.1	60.8 ± 6.2
Weaning	41.1 ± 5.5	64.1 ± 5.9
Mean percentage of E-M daughters	69.0 ± 7.7	31.9 ± 12.7

TABLE 4

OBSERVED PROBABILITY OF BEING AN E-M DAUGHTER AS A FUNCTION OF UTERINE POSITION AND DAMS' AGE AT VAGINAL OPENING

		Uterine Position of Daughter		
		0M	1M	2M
Dam	E-M	0.94 (17)	0.65 (26)	0.56 (9)
	L-M	1.00 (5)	0.38 (13)	0.25 (8)

Numbers in parentheses = number of pups in each condition.

in the proportion of daughters of E-M and L-M dams in 1M and 2M intrauterine locations developing into E-M females. Hence, there were differences in the uterine environments provided by E-M and L-M dams, unrelated to daughters' uterine positions relative to males, that influenced the rate of subsequent sexual maturation of daughters.

The uterine location of female rodents relative to males has been shown previously to affect many morphological and behavorial traits, particularly those traits influenced by perinatal exposure to androgen [4–6, 9, 12–15]. Of particular relevance to the present results McDermott, Gandelman and Reinisch [8] have reported that 0M female rats exhibit vaginal opening at an earlier mean age (34.2 days) than do 2M female rats (37.3 days). However, although statistically reliable, the effects of uterine location on age at vaginal opening in rats were small and age at vaginal opening has not been shown to predict subsequent reproductive life histories in female rats. The effects of uterine position on female gerbils reported here are far more profound: On average, 0M female gerbils exhibited vaginal opening at 18.0 days of age, 2M female gerbils at 29.4 days of age; 0M females were 1.7 to 4 times more likely to mature early than were 1M or 2M females. Early maturation predicts substantially elevated fecundity in female gerbils [3].

The results of the present experiment also suggest that uterine position is but one of a number of significant influences on age at vaginal opening and its reproductive sequalae in gerbils. Daughters of E-M females in 1M and 2M uterine positions were twice as likely to themselves be early maturing as were similarly-located daughters of L-M females. Prenatal [1], maternal effects acted independently of effects of uterine location to modify rates of development of female gerbils. Uterine position relative to males is but one of a number of factors playing an important role in modulating critical reproductive parameters in female Mongolian gerbils.

ACKNOWLEDGEMENTS

The research reported here was supported by grants from the Natural Sciences and Engineering Research Council of Canada and McMaster University Research Board to B.G.G. Jr. We thank Cheryl Spencer and Sylvie Malenfant for technical assistance and Martin Daly and Pauline Yahr for thoughtful critiques of earlier drafts.

REFERENCES

1. Clark, M. M. and B. G. Galef, Jr. Postnatal effects on reproduction and maternal care in early- and late-maturing gerbils. *Physiol Behav* **36:** 997–1003, 1986.
2. Clark, M. M., C. A. Spencer and B. G. Galef, Jr. Improving the productivity of breeding colonies of Mongolian gerbils (*Meriones unguiculatus*). *Lab Anim* **20:** 313–315, 1986.
3. Clark, M. M., C. A. Spencer and B. G. Galef, Jr. Reproductive life history correlates of early and late sexual maturation in female Mongolian gerbils (*Meriones unguiculatus*). *Anim Behav* **34:** 551–560, 1986.
4. Clemens, L. G., B. A. Gladue and L. P. Coniglio. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. *Horm Behav* **10:** 40–53, 1978.
5. Gandelman, R., F. S. vom Saal and J. Reinisch. Contiguity to male fetuses affects morphology and behavior of female rats. *Nature* **266:** 722–724, 1977.
6. Hauser, H. and R. Gandelman. Contiguity to males in utero affects avoidance responding in adult female mice. *Science* **220:** 437–438, 1983.
7. Kennedy, S. C. and J. Mitra. Body weight and food intake as initiating factors for puberty in the rat. *J Physiol* **166:** 408–418, 1963.
8. McDermott, N. J., R. Gandelman and J. M. Reinisch. Contiguity to male fetuses influences ano-genital distance and time of vaginal opening in mice. *Physiol Behav* **20:** 661–663, 1978.
9. Meisel, R. L. and I. L. Ward. Fetal female rats are masculinized by male littermates located caudally in the uterus. *Science* **213:** 239–242, 1981.
10. Vandenbergh, J. G. Effects of the presence of a male on the sexual maturation of female mice. *Endocrinology* **81:** 345–349, 1967.
11. Vandenbergh, J. G., L. C. Drickamer and D. R. Colby. Social and dietary factors in the sexual maturation of female mice. *J Reprod Fertil* **28:** 397–405, 1972.
12. vom Saal, F. S. Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. *J Reprod Fertil* **62:** 633–650, 1981.
13. vom Saal, F. S. Variation in infanticide and parental behavior in male mice due to prior intrauterine proximity to female fetuses: Elimination by prenatal stress. *Physiol Behav* **30:** 675–681, 1983.
14. vom Saal, F. S. and F. H. Bronson. Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. *Science* **208:** 597–599, 1980.
15. vom Saal, F. S. and C. L. Moyer. Prenatal effects on reproductive capacity during aging in female mice. *Biol Reprod* **32:** 1116–1126, 1985.