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Reproductive Profiles of Adult Mongolian Gerbils Gestated as the Sole Fetus in a Uterine Horn

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CLARK, M. M., J. M. VONK AND B. G. GALEF, JR. *Reproductive profiles of adult Mongolian gerbils gestated as the sole fetus in a uterine horn.* PHYSIOL BEHAV 61(1) 77–81, 1997.—We compared reproductive profiles of Mongolian gerbils gestated alone in a uterine horn (Isolate males and Isolate females) with those of gerbils gestated in intrauterine positions between two male fetuses (2M males and 2M females) and two female fetuses (2F males and 2F females). We found that, when adult, the reproductive profiles of gerbils that had been gestated as isolates resembled that of gerbils that had been gestated as 2F fetuses: 1. Isolate females gestated litters containing both the same proportion of males as the litters of 2F females and a significantly smaller proportion of males than litters of 2M females. 2. Isolate males, like 2F males, were less likely to impregnate females than were 2M males, and 3. both 2F males and isolate males exhibited disturbed patterns of copulation and reduced levels of scent-marking relative to 2M males. Our results were entirely consistent with the view that intrauterine exposure to males, but not to females, was responsible for previously described differences in the reproductive profiles of 2M and 2F Mongolian gerbils. *Copyright © 1996 Elsevier Science Inc.*

Intrauterine position Reproductive success Sex ratios Copulatory patterns Scent marking Mongolian gerbils

ADULT rodents that, as fetuses, resided in intrauterine positions (IUPs) between two male fetuses (2M males and 2M females) differ in morphology, physiology, and behavior from adult rodents that were gestated in IUPs between two female fetuses (2F males and 2F females) [for review, see (7,18)]. Most important to the present experiments, fetal IUP can have profound effects on the reproductive life-history strategies of Mongolian gerbils (7): 1. adult 2F male Mongolian gerbils (*Meriones unguiculatus*) exhibit unusual patterns of copulatory behavior and are less likely than are adult 2M male Mongolian gerbils to inseminate females with which they are housed (10,11), and 2. adult 2M female Mongolian gerbils gestate and deliver litters containing a higher proportion of male fetuses than do their 2F sisters (6,9).

Often, effects of intrauterine position on adult morphology and behavior are attributed to differences in fetal exposure to testosterone that is secreted by male rodent fetuses during the last week of their gestation (19,22) and, at least in Norway rats, diffuses across fetal membranes (13,20) altering the internal hormonal milieu of co-residents of a uterine horn. Such naturally occurring variation in levels of exposure of fetuses to testosterone, correlated with IUP, is presumed to induce cascades of neurendocrine events (2,15,19,21) that have different reproductive life-history strategies as their endpoints (7,18). Although highly informative, discovery of a hormonal mechanism that mediates effects of exposure to male fetuses on their uterine neighbors has, generally, diverted attention from the possibility that female fetuses might also exert an influence on the developmental trajectories of their intrauterine neighbors.

However, as Gandelman and Graham (14) have indicated, the traditional method for analyzing effects of IUP on morphology, endocrinology, and behavior of adult rodents (comparison of adult phenotypes of individuals gestated between two male fetuses, two female fetuses or one male and one female fetus) cannot determine if intrauterine exposure to male neighbors is, in itself, sufficient to explain observed differences in the phenotypes of adult male and adult female rodents that matured in 2M and 2F IUPs. Gandelman and Graham (14) compared the aggressive behavior of ovariectomized 2F mice with that of ovariectomized female mice that had been surgically isolated in a uterine horn and found that, after implantation with testosterone, such surgically induced isolates were less aggressive than were 2F mice. The greater frequency of aggression exhibited by 2F than by Isolate females suggests that intrauterine exposure to females, like intrauterine exposure to males, might have important effects on adult phenotypes.

In the two experiments reported here, we compared reproductive behaviors of adult male and adult female Mongolian gerbils that, as fetuses, had resided alone in a uterine horn (isolate males and females) with the reproductive behaviors of adult 2M and 2F male and female gerbils.

Because female Mongolian gerbils deliver smaller litters than do either rats or mice, naturally occurring Isolate fetuses are more

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common in gerbils than in other species of laboratory rodent. Consequently, when gerbils are used as subjects, it is not necessary to intervene surgically to produce fetal isolates, as Gandelman and Graham had to do in mice.

Although it is fairly unusual to find a lone fetus in a uterine horn of a female gerbil following her delivery by cesarian section on the last day of her gestation, over the last 3 years we have delivered, and successfully reared to adulthood, 14 male and 9 female Isolate gerbil fetuses. As Gandelman and Graham's (14) work suggests, such isolate animals can provide a useful baseline for assessing effects on adult phenotypes of intrauterine exposure to both male and female fetuses.

Experiment 1

In the first experiment, we determined the effects of intrauterine exposure to male or female fetuses on the relatively low level of copulatory competence and reproductive success seen in 2F as compared with 2M male gerbils (11). If intrauterine exposure to fetal females affects the reproductive competence of their brothers, male isolates should differ from 2F male gerbils in reproductive success. On the other hand, if exposure to neighboring brothers is fully responsible for the greater reproductive competence of 2M than 2F male gerbils, the copulatory behavior and reproductive success of 2F males should be similar to that of isolate males.

We also examined the scent-marking behavior of our male subjects because frequency of scent marking in adult male Mongolian gerbils has been found to correlate with their circulating levels of testosterone (12). If we were to find differences in the frequency of scent marking by 2F and isolate male gerbils, it would suggest that differences in their reproductive behavior might be mediated by differences in their androgen titers in adulthood.

METHOD

Subjects

Forty-two male Mongolian gerbils delivered by cesarian section served as subjects in the first experiment. Fourteen of these males had been found alone in a uterine horn, 14 were found in 2M IUPs, and 14 in 2F IUPs. Because it took 3 years to collect an adequate sample of isolate males, we used, as subjects in Experiment 1, 1 2F male and 1 2M male delivered by cesarian section within 2 weeks of cesarian delivery of each isolate male. At delivery, some isolate males were abnormally small, but these underweight individuals never survived. Consequently, at sacrifice there were no significant differences in the mean body weights of isolate (Mean ± 1 SEM = 95.6 ± 3.9 g), 2M (96.1 ± 3.7 g), and 2F (98.9 ± 4.4 g) males [$F(2,39) = 0.20$, NS].

An additional 144 female gerbils served as mates for the 42 male subjects.

All animals used in Experiment 1 were third-generation descendants of breeding pairs acquired from Tumblebrook Farms (West Brookfield, MA), that were maintained in the vivarium of the McMaster University Department of Psychology. All were housed in 35 × 30 × 15 cm prolypropylene cages with ad lib access to Purina®; Rodent Laboratory Chow # 5001 and water. Cages were kept in a temperature- and humidity-controlled colony room illuminated on a 12:12 light-dark cycle.

Procedure

Delivery and maintenance of subjects. Twenty-four days after a stock female gerbil had been observed to copulate with her consort (i.e., on the day before her expected vaginal delivery), we delivered subjects by cesarian section. Immediately following delivery, we toe clipped each subject for permanent identification and fostered them with their litter mates to a gerbil dam that had delivered vaginally on the day of surgical delivery of her foster pups (4).

We weaned male subjects at 30 days of age and maintained them in groups of 2 or 3 brothers until they were 53 days old, when we placed each subject alone in a cage for 1 week.

Female gerbils participating in the experiment were weaned when 30 days of age and were then maintained in small groups of sisters until they were 60 to 70 days old.

Testing reproductive competence. We tested each male gerbil for reproductive competence by pairing him with a succession of 3 60- to 70-day-old virgin female gerbils. To begin a breeding test, we introduced a single female gerbil into a male's cage, but kept her separated from him by a hardware-cloth barrier for 24 h. We then removed the barrier separating male and female and left the couple undisturbed for 24 days, when we removed the male and placed him in a new cage where he remained alone for 6 to 10 days before we placed a new virgin female with him.

We checked each female's cage daily, both morning and late afternoon, to determine the number of pups in each vaginally delivered litter.

Testing copulatory behavior. Two weeks after we removed each male from the cage of the third female with which we paired him, we tested each of a subset of 6 2M males, 6 2F males, and 6 isolate males with a series of females that we had brought into induced estrus. In the first stage of the experiment, we had observed a high rate of reproductive failure in both 2F and isolate males. To ensure that all males whose copulatory behavior we examined were likely to achieve ejaculation, we used as subjects in our test of copulatory behavior only males that had impregnated 2 of the 3 females with which we had paired them to test their reproductive competence.

To induce estrous in the 18 60- to 70-day-old virgin females that we used to examine males' copulatory behavior, we implanted each female with a capsule of estradiol benzoate. Two weeks before a female was to be paired with a male, we anesthetized her by IP injection with 30 mg/kg of sodium pentobarbitol and implanted, under the skin at the base of her neck, a Silastic® capsule (Dow-Corning Silastic® tubing: 0.125 in o.d., 0.062 in i.d., Catalogue number: Dow 602-285, Dow-Corning Medical Products, Midland, MI) that we had closed at each end with Dow-Corning Silastic-Type® Adhesive. Each implant contained a 3 mm long (0.02 ml) column of estradiol benzoate and each was incubated in isotonic saline for at least 48 h before insertion into a subject.

To begin testing, we placed an implanted female in the cage of a male subject, but separated from him by a hardware-cloth partition. Eighteen h later, we removed the partition separating male and female and then recorded the sexual behavior of the male until he either achieved 2 ejaculations, 2 h had passed without him mounting the female, or ejaculation had failed to occur within 90 min of the first intromission. To ensure that we sampled each male's copulatory behavior adequately, we tested each male with 3 different stimulus females until he achieved 2 ejaculations with each. [Agren (1) has shown that 2 ejaculations are necessary for successful impregnation of female gerbils.] It was necessary to test a male for a maximum of 5 sessions to obtain measures of latency to 2 ejaculations.

During each test session, an experimenter, unaware of the IUP that a male had occupied as a fetus, recorded: 1. the number of mounts without intromission and number of intromissions preceding the ejaculations we observed in each male, 2. the latency

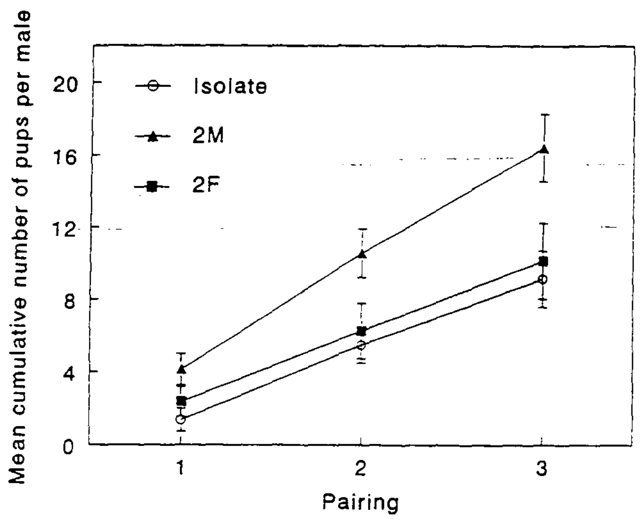

FIG 1. Mean cumulative number of pups delivered by females impregnated by isolate, 2M, and 2F male Mongolian gerbils. Bars = ± 1 SEM.

to ejaculation from the first mount in each ejaculatory series, and 3. the time from the first ejaculation to the next mount.

Scent-marking behavior. During the 2-week period between the end of testing of each male's reproductive competence and the start of testing of his copulatory behavior, we used a modified version of procedures developed by Thiessen et al. (16) [described in detail in (10)] to measure his frequency of scent marking. In brief, we placed individual subjects in an open field with a Plexiglas floor to which 9 small pegs had been attached. We counted the number of scent marks made by each subject during 5 5-min tests in the open field.

Data Analysis.

In cases of nonhomogeneity of variance, we used a log transform to equate variances. Data were analyzed using ANOVA and subsequent LSD tests for planned, post hoc comparisons. All reported p values are 2-tailed.

RESULTS AND DISCUSSION

Reproductive Competence

Figure 1 shows the mean cumulative number of offspring produced by isolate males and by males from 2M and 2F IUPs in their 3 opportunities to breed. As can be seen in Fig. 1, the IUP of males had a significant effect on their reproductive success ($F(2,39) = 4.55$, $p < 0.01$). Post hoc tests revealed that the reproductive success of males that developed in isolation was significantly lower than that of males from 2M IUPs ($p < 0.01$), but did not differ significantly from that of males from 2F IUPs.

Two factors, neither of which had statistically significant effects in isolation, acted in combination to produce the observed greater reproductive success of 2M males than of 2F and isolate males. Fewer females were inseminated by isolate males (Mean ± 1 SEM = 1.7 ± 0.4 females impregnated/male) and by 2F males (1.6 ± 0.4) than by 2M males (2.4 ± 0.3 females), and the mean number of pups in litters delivered by females impregnated by 2M males (Mean ± 1 SEM = 6.7 ± 0.3 pups/litter) was larger than was the mean number of pups in litters delivered by females impregnated by either 2F males (5.9 ± 0.5) or isolate males (5.4 ± 0.4). However, of the 6 LSD tests performed examining the effects of male IUP on either their probability of impregnating females or the size of litters produced by females they impregnated, only the size of litters produced by females impregnated by isolate and 2M males differed significantly.

Copulatory Behavior

Table 1 describes the copulatory patterns of isolate, 2M, and 2F males that had successfully impregnated at least 2 of the 3 females with which we paired them. We found significant effects of IUP on males' latencies to 2 ejaculations [$F(2,15) = 5.22$, $p < 0.02$] and on the total number of mounts without intromission that males exhibited en route to 2 ejaculations [$F(2,15) = 4.21$, $p < 0.04$]. This demonstration of effects of IUP on copulatory behavior is particularly convincing, given that we compared 2M males with only the more potent of our isolate and 2F males. We did not observe a significant effect of IUP on total number of intromissions to 2 ejaculations [$F(2,15) = 1.54$, NS].

As can also be seen in Table 1, isolate males and 2F males did not differ significantly on either measure of copulatory behavior that was affected by IUP, but did differ significantly from 2M males on both of those measures.

Scent Marking

Figure 2 illustrates the effects of IUP on scent-marking behavior. As can be seen in Fig. 2, there was a significant effect of IUP on scent-marking frequency [$F(2,39) = 4.94$, $p < 0.01$]. Post hoc tests revealed that isolate males scent marked significantly less often than did 2M males ($p < 0.01$), but did not differ from 2F males in their frequency of scent marking.

The data of Experiment 1 not only repeat our previous observations of significant differences in the reproductive success,

TABLE 1

MEAN (±1 SEM) NUMBER OF MOUNTS WITHOUT INTROMISSION, INTROMISSIONS, AND MIN TO 2 EJACULATIONS BY ISOLATE, 2M, AND 2F MALE GERBILS IN EXPERIMENT 1

Group	Isolate	2F	2M
n	6	6	6
Min to 2 ejaculations	50.85 ± 7.66[a]	48.50 ± 6.55[a]	28.18 ± 3.23[b]
Mounts to 2 ejaculations	23.8 ± 5.1[a]	34.5 ± 13.6[a]	9.9 ± 2.9[b]
Intromissions to 2 ejaculations	47.6 ± 4.7	61.4 ± 14.8	39.0 ± 3.2

Numbers in the same row with different alphabetic superscripts differed significantly by LSD tests.

copulatory behavior, and scent-marking behavior of 2M and 2F male Mongolian gerbils (2,10,11), but also indicate that the reproductive phenotype of isolate males is similar to that of 2F, not 2M, males.

These data are consistent with the view that differences in reproductive success and patterns of copulatory behavior of 2F and 2M male gerbils are a consequence of exposure to their male, not their female, intrauterine neighbors.

Experiment 2

As mentioned in the Introduction, IUP affects the reproductive performance of female Mongolian gerbils, as well as that of males. Female gerbils from 2M IUPs, like 2M female house mice (*Mus domesticus*), give birth to male- biased litters, and their 2F sisters give birth to female-biased litters (6–9,17). To determine whether effects of IUP on the sex ratios of litters gestated by 2M and 2F female gerbils are the result of intrauterine exposure of female fetuses to neighboring brothers or sisters, we compared the sex ratios of litters delivered by females that had developed alone in a uterine horn (isolate females) with the sex ratios of litters delivered by females from 2M and 2F IUPs.

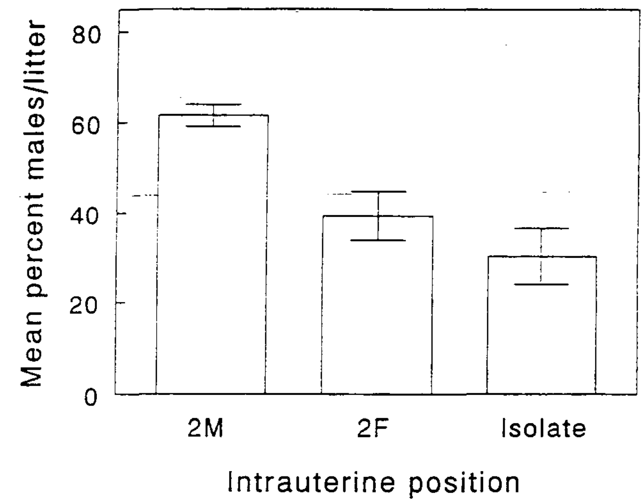

FIG. 3. Mean percentage of males per litter delivered by cesarian section from isolate, 2M, and 2F female gerbils. Bars = ± 1 SEM.

METHOD

Subjects

We used as subjects 27 female Mongolian gerbils delivered by cesarian section from females born and reared in the vivarium of the Department of Psychology of McMaster University. These source females were second- and third-generation descendants of breeding pairs acquired from Tumblebrook Farms.

Nine of the 27 subjects had been gestated alone in a uterine horn, 9 were from 2F IUPs, and 9 from 2M IUPs. As in Experiment 1, because it took 3 years to collect our Isolate females, we used as subjects 1 2F female and 1 2M female cesarian-delivered within 2 weeks of delivery of each isolate female subject.

Procedure

Delivery and maintenance of subjects. Each subject was delivered by cesarian section, foster reared, weaned and, until 60 days of age, maintained as were female gerbils that participated in Experiment 1.

Impregnation of subjects. When each of the 27 subjects was 60 days of age, we weighed her and placed her together with a sexually proven adult male. We noted the date on which each pair first copulated, with the expectation that a female would deliver vaginally 25 days after we had seen her mating. As each female entered the final week of her pregnancy, we removed her consort from her cage.

On the day before anticipated vaginal delivery, we weighed each female and then delivered by cesarian section each female that showed a weight gain consistent with her impregnation on the day of her observed copulation.

A trained observer, who was unaware of the IUP in which dams had matured, examined the anogenital distance of each fetus that we delivered by cesarian section and determined its sex (5).

Statistical Analyses

We used an arc-sine transform to normalize data describing the percentage of males in the litter of each female before performing ANOVAs, and we used LSD tests for post hoc comparisons. All reported p values are 2-tailed.

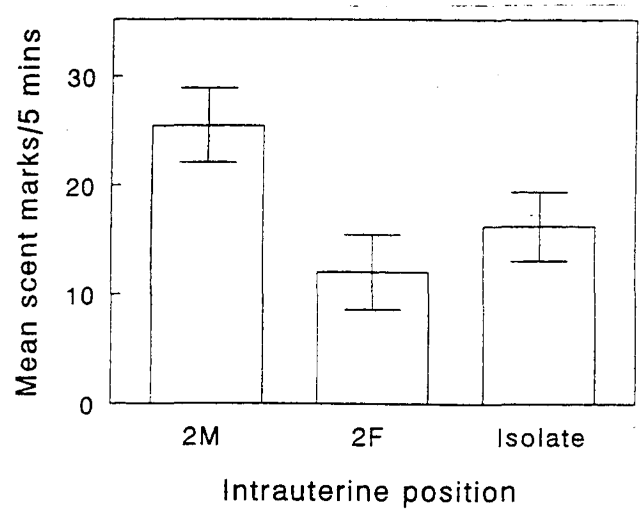

FIG. 2. Mean number of scent marks deposited by isolate, 2M, and 2F male gerbils. Bars = ± 1 SEM.

RESULTS AND DISCUSSION

The main results of Experiment 2 are presented in Fig. 3, which shows the sex ratios of litters delivered by cesarian section from isolate, 2M, and 2F females. As is evident from inspection of Fig. 3, we found a significant effect of IUP on the sex ratios of litters [$F(2, 24) = 8.30$, $p < 0.002$]. Post hoc tests revealed that litters of isolate females contained a significantly smaller proportion of males than did litters of 2M females ($p < 0.01$), but that the sex composition of litters of 2F and isolate females did not differ significantly.

Differences in the sex ratios of litters cannot be explained in terms of differences in litter size. Although the litters of isolate females (Mean ± 1 SEM = 6.3 ± 0.5) were slightly smaller than the litters of 2F (7.2 ± 0.5) and 2M (7.8 ± 0.6) females, there was no significant effect of IUP on litter size [$F(2,24) = 1.94$, NS], and no significant correlation between litter sizes and litter sex ratios in any of the 3 groups (Spearman $rs < 0.20$, NS).

The results of Experiment 2 are consistent with the view that observed effects of a gerbil dam's IUP on the sex ratio of her litter result from her intrauterine exposure to neighboring males, not neighboring females.

GENERAL DISCUSSION

Taken together, the results of the two experiments reported here do not parallel Gandelman and Graham's (14) finding of an effect of intrauterine exposure to neighboring female fetuses on the aggressive behavior of female house mice. We could find no effects of intrauterine exposure to female fetuses on reproductive profiles of either male or female Mongolian gerbils. Differences in reproductive performance of male and female gerbils from different IUPs that affect the population biology of the species appear to reflect androgenization of fetuses (3) that results from intrauterine association with male fetuses, not effects of intrauterine association with female fetuses.

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