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# Effects of Maternal Stress on Puberty, Fertility and Aggressive Behavior of Female Mice From Different Intrauterine Positions

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VOM SAAL, F. S., M. D. EVEN AND D. M. QUADAGNO. *Effects of maternal stress on puberty, fertility and aggressive behavior of female mice from different intrauterine positions.* PHYSIOL BEHAV **49**(6) 1073–1078, 1991.—We examined the effects of maternal stress (bright light and heat) during the last third of pregnancy on subsequent reproductive and behavioral characteristics of female mice from different intrauterine positions. Female mice that develop in utero between two male fetuses (2M females) differ from females that develop between two female fetuses (0M females) in their serum concentrations of both testosterone and estradiol during the fetal period of sexual differentiation. After birth, 0M and 2M females differ in a wide range of reproductive characteristics. We examined the effects of maternal stress on the response to social cues regulating the timing of first vaginal estrus and the length of the first postpubertal estrous cycle when 4 0M or 4 2M females were housed together next to an adult male. Maternal stress decreased the inhibitory effect of being housed with other females in terms of the length of the first postpubertal estrous cycle, but this only occurred in 0M females. We found no effect of maternal stress or intrauterine position on the capacity to mate and remain pregnant, regardless of whether 0M or 2M females were stressed or not stressed during early pregnancy prior to implantation. While there was no effect of prior intrauterine position on interfemale aggression or behavior toward young, maternal stress did tend to reduce the likelihood that females (in diestrus) would exhibit aggression toward other females.

Sexual differentiation Intrauterine position Maternal stress Puberty Fertility Aggression Infanticide Female mice

DURING the last third of pregnancy in rats and mice, differentiation of the gonads, reproductive tract and brain begins. Stressing pregnant females during this time reduces fertility and fecundity in female offspring in rats (6) but not mice (19). In both rats and mice, maternal stress also results in a delay in the age at vaginal opening and the lengthening of estrous cycles of female offspring (7, 19, 38). Maternal stress can also influence aggressive behavior in female mice, although the effect of maternal stress depends on the type of aggression being studied and the mouse strain that is used (9,10).

Another source of variation in all of the above traits in mice, rats and gerbils (30) and pigs (22) is the intrauterine position in which a female develops relative to other male and female fetuses. Specifically, female mice that developed in utero between two male fetuses (2M females) have been compared with females that did not develop next to a male fetus (0M females). 2M females were more aggressive than 0M females while virgins (20,32) as well as when pregnant and in defense of their young after parturition (12,32). 2M females also exhibited a deficit in sexual attractiveness and in sexual behavior (21,32). Prior intrauterine position also accounts for variation in the age at which fertility begins in mice, rats and gerbils (3, 26, 30–32, 37). These differences are presumed to be mediated by differential exposure to both testosterone (16, 25, 29) and estradiol during the fetal period of sexual differentiation as a result of developing between either two male or between two female fetuses (33,38).

In male mice, maternal stress only led to a change in behavior toward young in males positioned in utero between two female fetuses (27). Also, only 0M females showed postnatal changes in genital morphology and length of adult estrous cycles in response to maternal stress (38). Thus in no prior study has maternal stress been found to have any effect on either males or females positioned in utero between two male fetuses. In the studies reported here, we examined the effects of maternal stress on the timing of puberty, length of the first postpubertal estrous cycle, fertility and aggressive behavior of female mice from different intrauterine positions.

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## GENERAL METHOD

### *Animals and Housing Conditions*

Adult CF-1 mice (*Mus domesticus*) were initially purchased from Charles River Laboratories in 1979 and have been outbred in a closed colony since that time. Mice were housed in 18 × 29 × 13-cm polypropylene cages and maintained at 25 ± 1°C on a 12:12 light:dark cycle, with lights on at 1200 h. All work in the animal rooms during the dark phase of the cycle was conducted with a 25-W red light. Mouse breeder chow (Purina 5008) and water were available ad lib.

### *Intrauterine Position Classification Scheme*

The scheme that was used to designate the intrauterine positions of females takes into account the sex of fetuses that are directly adjacent to the fetus being classified. In the experiments described below, 2M females developed between 2 male fetuses, 1M females developed between a male and a female fetus, and 0M females developed between 2 female fetuses in utero. Intrauterine positioning is a random developmental event (4,26) and, using this classification scheme, we find, on average, 1 0M, 2 1M and 1 2M female fetuses per litter. Average litter size in CF-1 mice is 12 pups, and 33% of the pups (at the ends of the two uterine horns) cannot be classified using this scheme. Other schemes have been proposed for classifying animals for intrauterine position studies, but only this classification scheme accounts for a significant portion of the variance in reproductive traits in mice (36).

### *Mating and Delivery Procedure*

To obtain mouse fetuses from known intrauterine positions, adult CF-1 female mice were time-mated by being placed daily with a stud male beginning at 0800 h, and examination for a vaginal plug was 4 h later (Day 0 of pregnancy). Inseminated females were housed three per cage and not disturbed until Day 10 of pregnancy, at which time the cages were changed. Pregnant females were housed individually one day prior to delivery.

Pregnant females were killed by cervical dislocation beginning at 1030 h on Day 19 of pregnancy (the mean time of parturition is 1430 h for CF-1 mice on this light:dark cycle). Pups were fostered to females that had delivered normally within the preceding 24 h in groups consisting of 5 0M and 5 1M females or 5 2M and 5 1M females from the same prenatal treatment group. The animals were weaned at 24 days of age and were then housed with other foster-littermates from the same intrauterine position (4–5 females per cage) until being assigned to an experiment.

### *Prenatal Stress Procedure*

Randomly selected pregnant mice were stressed by being placed into Plexiglas mouse-restraining chambers (9 × 6.3 × 5 cm) under a bank of 150-W flood lights (350 footcandles; 38°C temperature inside the chamber) two times per day at 0900 and 1600 h. The first stress session occurred at 0900 h on Day 13 of pregnancy. The last stress session was at 1600 h on Day 18 of pregnancy. There was a total of 12 stress sessions, with each stress session lasting 45 min.

### *Data Analysis*

Data were analyzed by analysis of variance using the Statistical Analysis System (SAS), General Linear Model. Comparisons of differences between group means were made using the LS means test in SAS if the overall ANOVA showed a significant main effect or interaction. The criterion for rejecting the null hypothesis was $p<0.05$; when rejection was at a greater level of confidence, it is noted.

## EXPERIMENTAL METHOD AND RESULTS

### *Age at First Estrus and Length of First Postpubertal Estrous Cycle*

The objective of this experiment was to assess the effects of maternal stress on the timing of puberty and length of the first postpubertal estrous cycle in 0M and 2M female mice. In prior studies, we showed that 0M females (produced by nonstressed mothers) passed through puberty (ovulated and mated) at a younger age and subsequently exhibited shorter estrous cycles than did 2M females when each female was housed either together with or across a wire partition from a male. However, exactly the opposite occurred when the females were housed both with a male and with other females. In this situation, 0M females entered puberty later and exhibited prolonged estrous cycles during early adolescence, relative to 2M females. As in most comparisons, 1M females were intermediate between 0M and 2M females on these measures (31, 32, 34, 37).

The findings described above showed that, in addition to intrauterine position influencing the intrinsic timing of the pubertal ovulation and the length of subsequent estrous cycles, there was also an effect on the social cue(s) (presumed to be pheromonal) which influence the timing of these events in female mice. Studying the effects of intrauterine position and maternal stress on the timing of puberty and length of subsequent estrous cycles is thus complicated by the dramatic role that pheromonal cues produced by both males and females play in regulating the timing of these events in mice (24). The conclusion from recent studies (31) is that, relative to 2M females, 0M females produce more potent cues and are also more sensitive to the cues that regulate the timing of puberty and length of estrous cycles during early adolescence. However, the estrous cycle-prolonging effect due to the presence of other females which is observed in 0M but not 2M females only occurs during early adolescence. By adulthood (after 55–60 days of age), if a male is present, 0M females have shorter estrous cycles than 2M females, regardless of the presence or absence of other females (31,34).

In this experiment, first vaginal estrus was used as an indirect method of assessing the timing of the first (pubertal) ovulation. First vaginal estrus in CF-1 female mice that are housed across a wire partition from a male does not differ from the age at which females ovulate, mate and become pregnant when cohabiting with a male (31). The age at first vaginal estrus thus serves as a reliable method of assessing the age at which females experience the first ovulation signalling the completion of puberty. Without the presence of a male, individually housed juvenile female mice cannot reliably be assessed as ovulating based on the presence of cornified epithelial tissue in the vagina, and individually housed female mice do not exhibit regular estrous cycles prior to around 150 days of age (18, 31, 34).

*Method.* The age at first vaginal estrus and the length of the first postpubertal estrous cycle were examined in 0M and 2M prenatally stressed and control female mice (n = 28/group). 1M females were not examined. The females were housed in groups of 4 females from the same intrauterine position (7 replicates/intrauterine position) in 30 × 30 × 15-cm cages divided into two compartments of equal size (15 × 15 × 15 cm) by a wire-mesh partition. An adult 1M male (saved from control litters) was housed across the wire partition from the females.

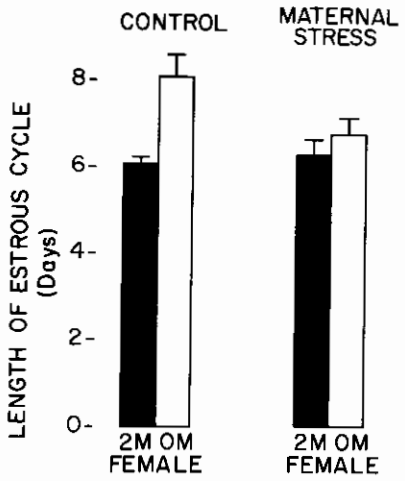


FIG. 1. The mean (±SEM) length (in days) of the first complete estrous cycle exhibited in pubertal 0M and 2M females mice delivered by cesarean section from control and stressed mothers. Vaginal smears were obtained daily beginning at vaginal opening, and the day on which most cells were cornified was labeled as the first day of the cycle. 0M control females differed significantly ($p<0.01$) from all other groups.

The females were 26 days old and weighed between 13–15 g at the start of the experiment. Heavier or lighter females were not included, since body weight serves as a predictor of the age at puberty in mice (1). Beginning at vaginal opening, vaginal smears were obtained at 1600 h.

*Results.* The age at first vaginal estrus was slightly later in control 0M females (mean ± SEM age: 39.1 ± 1.7 days old) than in control 2M females (37.8 ± 1.1 days old; $p>0.1$). Body weights at first vaginal estrus did not differ significantly ($p>0.1$) between control 0M and 2M females. The mean length of the first postpubertal estrous cycle was significantly ($p<0.001$) longer for control 0M than control 2M females (Fig. 1).

A comparison of prenatally stressed 0M and 2M females revealed no significant difference in ages or body weights at first vaginal estrus [mean ± SEM age: 0M females = 37.4 ± 0.8 days old; 2M females = 37.9 ± 0.9 days old; ($p>0.1$)], although both prenatally stressed 0M and 2M females were more similar to control 2M females than control 0M females in terms of the age at first vaginal estrus. The overall ANOVA comparing prenatally stressed and control 0M and 2M females was not significant ($p>0.1$) for either body weight or age at first vaginal estrus.

The significant difference between control 0M and 2M females in the length of the first postpubertal estrous cycle was not observed in a comparison of prenatally stressed 0M and 2M females ($p>0.1$). However, there was a significant interaction between maternal condition (control vs. stressed) and intrauterine position ($p<0.01$). While the first postpubertal estrous cycle for all prenatally stressed 0M and 2M females was similar to that for control 2M females, post hoc analysis showed that all three of these groups differed significantly ($p<0.01$) from control 0M females (Fig. 1).

### *Fertility of Control and Prenatally Stressed 0M and 2M Females When Left Undisturbed or When Stressed During Early Pregnancy*

0M and 2M females produced by either control or stressed mothers were compared in adulthood for their capacities to produce litters when either 1) left undisturbed or 2) stressed during the first five days of pregnancy (prior to and during the time of implantation). The likelihood of female mice aborting due to stress during pregnancy decreases after implantation of the embryos (2). This appears to be due to inhibitory effects of adrenal steroids on implantation (8) and the fact that the placentae take over gonadotropic control of the secretion of progesterone by the maternal ovaries during the second week of pregnancy (15,17). We therefore only subjected females to stress during early pregnancy (Days 1–5), prior to and during implantation of the embryos, which occurs at the beginning of Day 4 after fertilization (unpublished observation). Stress during this time results in abortion in some female mice, while other females remain pregnant. Specifically, over many years of mating CF-1 mice and examining females for copulatory plugs to verify pregnancy, we have typically found that between 20–30% of females that had copulatory plugs never showed any evidence of becoming pregnant (unpublished observation). In CF-1 mice, there is thus a subgroup of females that appears to be likely to abort the entire litter due to even mild disturbance (picking up and examining the females) during early pregnancy. However, the proportion of females aborting due to stress prior to implantation depends on the mouse stock being examined (2).

In previous studies, young adult 0M and 2M CF-1 female mice were found to be virtually identical in their capacities to become pregnant and produce and raise healthy litters of young when paired with males under optimum conditions (32,35). However, after repeated pregnancies beginning at puberty, 2M female mice ceased producing live pups at a younger age than did 0M females (35). These findings show that prior intrauterine position is not the basis for individual differences in fertility or fecundity in young adult females. In this study, we examined whether the likelihood of female mice remaining pregnant and successfully producing normal litters of young when stressed during early pregnancy was related to prior intrauterine position and/or having, themselves, been produced by mothers that had been stressed during pregnancy. Our prediction was that control 0M females would be the most likely to abort when stressed during early pregnancy. This prediction is based on the hypothesis that the functioning of the adrenals is influenced by gonadal steroid levels during fetal life (28), with elevated testosterone (in control 2M female fetuses or all stressed female fetuses) leading to a dampening of the pituitary-adrenal response to stress during later life.

*Method and results.*

*Nondisturbed group.* Twenty-two control 0M and 26 control 2M females (i.e., produced by control mothers) as well as 21 prenatally stressed 0M females and 20 prenatally stressed 2M females were paired with males at 4 months of age. Females were examined each day for the presence of a copulatory plug 1 h after the onset of the light phase of the light:dark cycle. Examination was accomplished by gently lifting the tail of the female without picking her up. The male was transferred to another cage when a plug was found to minimize disturbance of the females. We recorded the proportion of females mating, remaining pregnant, and producing young, as well as the number of young alive on the morning following delivery.

All females that were paired with males were found to have a vaginal plug within one week of being paired with a male. For control females, of the 22 0M females, 19 (86%) delivered (mean ± SEM) 10.7 ± 0.5 live pups, while 22/26 (85%) of the 2M females delivered 12.8 ± 0.5 live pups (number of live pups; $p<0.01$). For the prenatally stressed females, 20/21 (95%) 0M females delivered 12.5 ± 0.2 live pups, and 19/20 (95%) 2M females delivered 10.9 ± 0.6 live pups (number of live pups; $p<0.05$). This latter finding is thus opposite to the results with

control females in which control 2M females produced significantly more pups than did control 0M females.

*Stressed during early-pregnancy group.* Thirty control 0M females and 23 control 2M females, as well as 12 prenatally stressed 0M females and 15 prenatally stressed 2M females (4 months old), were mated (Day 0 of pregnancy) by being placed with a stud male, as described above. These females were disturbed on Days 1–5 of pregnancy by being placed in mouse-restraining cages ($9 \times 6.3 \times 5$ cm) under 150-W lights (325 footcandles) at 0900 h and 1400 h each day; each session lasted 45 min. The proportion of females delivering young and the number of live young were again recorded.

0M and 2M control females showed no significant difference in the proportion of females delivering young or the number of young found alive on the day of birth: 17/30 (57%) of the mated control 0M females became pregnant and delivered (mean ± SEM) $11.4 \pm 0.8$ live pups, while 13/23 (56%) of the mated control 2M females became pregnant and delivered $11.3 \pm 0.7$ live pups (ANOVA for live pups, $p > 0.1$). For the prenatally stressed females, 4/12 (33%) mated 0M females delivered $9.3 \pm 1.1$ pups, while 7/15 (47%) mated 2M females delivered $10.4 \pm 0.7$ pups (ANOVA for live pups produced by 0M vs. 2M females, $p > 0.1$).

There were no significant differences between 0M and 2M females in any of the groups in the likelihood of remaining pregnant after mating ($\chi^2$, $p > 0.1$). Regardless of prior intrauterine position, there was a greater likelihood of aborting in females stressed during the first 5 days of pregnancy relative to nondisturbed females; this was true for control females ($\chi^2$, $p < 0.01$) and for prenatally-stressed females ($\chi^2$, $p < 0.001$). When the effects of being stressed during early pregnancy were compared in prenatally stressed vs. control females (regardless of prior intrauterine position), being disturbed during early pregnancy had a slightly greater ($\chi^2$, $p > 0.1$) effect on prenatally stressed females (11/27; 41% delivered litters) than on control females (30/53; 57% delivered litters).

### *Aggressive Behavior*

In a previous study, adult 2M females were found to be more likely to attack and establish dominance over 0M females when these two types of females were paired during the diestrous phase of the estrous cycle (32). Adult female mice not only show aggression toward other adults, but, under some circumstances, they will also attack nursing young [referred to as infanticide (14)]. Relative to wild mice, in which most females exhibit infanticide, few (10–20%) adult CF-1 female mice exhibit infanticide (14). However, in some domesticated stocks of mice, females are more likely to kill young during early adolescence than in adulthood (23).

In this experiment, we examined the behavior of 0M and 2M females toward young during early adolescence as well as aggression toward other females in adulthood. The objective was to determine whether prenatal stress elevated or decreased these two different types of aggression in 0M and 2M female CF-1 mice.

*Method.* Females were weaned at 24 days of age and maintained in groups of 5 females per cage until being housed individually at 30 days of age ($n = 20$/group). At 34 days of age, each female was tested for her behavior toward a newborn pup, which was quietly placed into a corner of the cage. The pup was immediately removed from the cage if an attack was observed, or females were observed for 30 min if the pup was not attacked. Females that did not attack the pup either exhibited parental behavior (the female retrieved the pup to the nest and crouched over it to keep it warm) or did not handle the pup.

At two months of age, these same females were housed 4 per cage ($30 \times 30 \times 15$ cm) that was divided into two equal compartments by a wire-mesh partition. An adult control 1M male was housed on the other side of the barrier to induce regular estrous cycles (27). Preliminary studies had indicated that being group-housed with other females in the presence of a male increased the incidence of interfemale aggression in CF-1 mice.

Each cage contained one female from each group: control 0M, control 2M, prenatally stressed 0M, prenatally stressed 2M (16 replicates). When three months old, a female was tested for interfemale aggression. Fifteen min prior to testing, the male and three of the four females were removed from each cage. The one female remaining in the cage was the resident, while an opponent female from the same experimental group but housed in another cage was assigned to be the intruder (8 pairs of females were observed for each experimental group, and each female was only used in one test). The intruder was placed into the resident's cage for 5 min. Biting and chasing (labeled as aggression) were recorded.

Vaginal smears were obtained so that all behavioral tests were conducted when females were in diestrus. Behavioral tests were conducted on the day after females showed a vaginal smear indicating diestrus-1. On the day of behavioral testing, vaginal smears were not obtained until after the behavioral test to minimize disturbance of the test females.

*Results.* There was no effect of prior intrauterine position or maternal stress on the proportion of 34-day-old females exhibiting infanticide (control 0M = 25%, control 2M = 20%, prenatally stressed 0M = 40%, prenatally stressed 2M = 25%). There was also no difference in the proportion of females in each group that did not handle the pup (50% of the females in each group) or that exhibited parental behavior.

When tested in adulthood, the proportion of females fighting (biting and chasing) did not differ between control 0M females (60%) and 2M females (67%) or between prenatally stressed 0M females (22%) and 2M females (40%). There were also no differences between females in any of the groups in the intensity of aggression. In virtually all cases, the resident female initiated the attacks, while the intruder female fought back. Disregarding prior intrauterine position, fewer pairs of prenatally stressed females (31%) than control females (63%) exhibited aggression, although the difference was not statistically significant ($\chi^2$, $p > 0.1$).

## DISCUSSION

Previous experiments have shown that, when housed with other females near or with an adult male, control 2M females exhibit first vaginal estrous (and also ovulate, mate and deliver litters) at a younger age than 0M females (31,32). We showed here that prenatally stressed 0M and 2M females were similar to control 2M females in the age at first vaginal estrus. In contrast, control 0M females showed first vaginal estrus at a slightly later age than any of the other females (Table 1). Similarly, the length of the first postpubertal estrous cycle was altered (shortened) in prenatally stressed 0M females relative to control 0M females, while prenatal stress had no effect on 2M females relative to control 2M females (Fig. 1). These findings suggest that differentiation of the pheromonal mechanisms which regulate both the timing of puberty and the length of estrous cycles during early adolescence is altered by maternal stress, but only in 0M female mice.

In a prior study, we examined the length of estrous cycles in adult female mice housed individually near a male; these animals were examined in adulthood without other females present to eliminate effects on estrous cycles of pheromonal cues pro-

TABLE 1

RESULTS OF COMPARISONS OF 0M AND 2M FEMALES PRODUCED BY MOTHERS WHO WERE STRESSED OR NOT STRESSED (CONTROL) BETWEEN DAY 13–18 OF PREGNANCY IN EXPERIMENTS 1–3

| Experiment | | Control | | Maternal Stress | |
|---|---|---|---|---|---|
| | | 0M Females | 2M Females | 0M Females | 2M Females |
| 1 | Age at first estrus (days) | 39.1 ± 1.7 | 37.8 ± 1.1 | 37.4 ± 0.8 | 37.9 ± 0.9 |
| 2 | % Delivering young | | | | |
| | nondisturbed | 86% | 85% | 95% | 95% |
| | Stressed | 57% | 56% | 33% | 47% |
| 3 | % Exhibiting infanticide | 25% | 20% | 40% | 25% |
| 3 | % Exhibiting interfemale aggression | 60% | 67% | 22% | 40% |

Results for Experiment 2 are the proportion of mated females that produced young when not disturbed throughout pregnancy or when stressed on Day 1-5 of pregnancy. None or these comparsions were statistically different ($p>0.1$).

duced by females when housed in groups. The length of estrous cycles was significantly increased in prenatally stressed 0M females relative to control 0M females, while prenatally stressed 2M females did not differ from control 2M females in estrous cycle length (38). This finding suggested that the intrinsic timing of estrous cycles had been influenced by maternal stress, but, again, only in 0M females. Our present findings concerning the timing of puberty and length of the first postpubertal estrous cycle are thus consistent with these prior findings in that maternal stress was found to influence the development of the social cuing system which modulates these events in 0M females, while no difference was observed in prenatally stressed 2M females relative to control 2M females.

In our previous study on maternal stress, we also examined the effects of maternal stress on serum concentrations of testosterone and estradiol in 0M, 1M and 2M female CF-1 mouse fetuses and on genital morphology at birth (38). Maternal stress only altered postnatal reproductive traits in 0M females, while no effect of maternal stress in 1M or 2M females was observed, similar to the findings in Experiment 1 reported here. Maternal stress resulted in a significant increase in serum testosterone in all female fetuses, with the magnitude of the increase being similar in females from each intrauterine position; there was no effect of maternal stress on serum concentrations of estradiol in female fetuses. The observation that maternal stress only altered reproductive traits in 0M but not 2M females was thus not related to differential effects of maternal stress on circulating testosterone or estradiol in 0M vs. 2M female fetuses. Our findings do not rule out the possibility that the mechanism by which maternal stress alters reproductive traits in rodents is via changes in testosterone (38), which is increased in female fetuses by maternal stress, but it appears likely that other components of the endocrine system, such as the endogenous opiate system (11,13), are involved.

In rats, there is also evidence that intrauterine position influences reproductive traits [reviewed in (30)]. Whether in rats maternal stress only alters the traits of 0M females, while 2M females are unaffected by maternal stress, remains to be examined. However, this is certainly possible, since there is evidence that maternal stress alters the traits of female offspring in a masculine direction in rats [(11,13); reviewed in (38)]. Maternal stress has also been reported to have dramatic effects on fertility in rats (6). However, our findings confirm those of Politch and Herrenkohl (19) that prenatal stress does not reduce fertility or fecundity in female mice. Specifically, we did not find differences between control 0M and 2M females or prenatally stressed 0M and 2M females in the likelihood of remaining pregnant and producing healthy young when they were subjected to heat and light stress during the first 5 days of pregnancy or left undisturbed throughout pregnancy. However, disturbance during the first 5 days of pregnancy did result in a reduction in the proportion of mated females from all groups that delivered young, replicating other findings in mice (Table 1) (39).

We found that the likelihood of observing aggression between adult females in diestrus tended to be reduced in prenatally stressed CF-1 females relative to control females, although the difference was not statistically significant; there were no differences in interfemale aggression due to intrauterine position. We also did not observe any effect of prenatal stress or prior intrauterine position on the behavior of female mice toward newborn pups, although most females did not exhibit infanticide [see also (12)].

Numerous experiments have demonstrated that increasing testosterone levels during both the prenatal and neonatal periods of sexual differentiation increases aggressiveness toward other adults but decreases aggression toward infants (16, 25, 29). The decrease in interfemale aggression observed in prenatally stressed 0M and 2M females was thus surprising, since maternal stress results in increases in serum testosterone concentrations in all female mouse fetuses, regardless of intrauterine position (38). An increase in aggression toward unfamiliar adult male mice both before and after parturition was observed in prenatally stressed female mice of one strain (C57BL/6J), while in another mouse strain (DBA/2J), postpartum aggression toward an unfamiliar male was reduced in prenatally stressed females (9). In an outbred stock of mice (R-S), maternal stress decreased aggressiveness during pregnancy but elevated maternal aggressiveness in defense of young after parturition (10). The basis for strain differences in the effects of maternal stress on various types of aggression remains to be examined. But these findings suggest that the physiological response to maternal stress differs even among mouse strains. The implications of the marked strain

and species differences in the effects of maternal stress have typically been ignored by those proposing a relationship (based on studies with rodents) between maternal stress and human sexual behavior (5).

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## REFERENCES

1. Bronson, F.; Desjardins, C. Circulating concentrations of FSH, LH, estradiol and progesterone associated with acute, male-induced puberty in female mice. Endocrinology 94:1658–1668; 1974.
2. Chipman, R.; Fox, K. Oestrous synchronization and pregnancy blocking in wild house mice (*Mus musculus*). J. Reprod. Fertil. 12: 233–236; 1966.
3. Clark, M.; Galef, B. Effect of uterine position on rate of sexual development in female Mongolian gerbils. Physiol. Behav. 42:15–18; 1988.
4. Clark, M.; vom Saal, F.; Galef, J. Nonrandom sex composition of gerbil, mouse and hamster litters before and after birth. Dev. Psychobiol., in press; 1991.
5. Dorner, G.; Docke, F.; Goyz, F.; Rohde, W.; Stahl, F.; Tonjes, R. Sexual differentiation of gonadotropin secretion, sexual orientation and gender role behavior. Steroid Biochem. 27:1081–1987; 1987.
6. Herrenkohl, L. Prenatal stress reduces fertility and fecundity in female offspring. Science 206:1097–1099; 1979.
7. Herrenkohl, L.; Politch, J. Effects of prenatal stress on the estrous cycle of female offspring as adults. Experientia 34:1240–1241; 1978.
8. Johnson, D.; Dey, S. Role of histamine in implantation: Dexamethasone inhibits estradiol-induced implantation in the rat. Biol. Reprod. 22:1136–1141; 1980.
9. Kinsley, C.; Svare, B. Genotype modulates prenatal stress effects on aggression in male and female mice. Behav. Neural Biol. 47: 138–150; 1987.
10. Kinsley, C.; Svare, B. Prenatal stress alters maternal aggression in mice. Physiol. Behav. 42:7–13; 1988.
11. Kinsley, C.; Insel, T.; Mann, P.; Bridges, R. Reduction in brain opiate receptors in prenatally stressed male and female rats. Soc. Neurosci. Abstr. 15:42.7; 1989.
12. Kinsley, C.; Konen, C.; Miele, J.; Giraldi, L.; Svare, B. Intrauterine position modulates maternal behaviors in female mice. Physiol. Behav. 36:793–799; 1986.
13. Kinsley, C.; Mann, P.; Bridges, R. Prenatal stress alters morphine- and stress-induced analgesia in male and female rats. Pharmacol. Biochem. Behav. 30:123–128; 1988.
14. McCarthy, M.; vom Saal, F. The influence of reproductive state on infanticide by wild female house mice. Physiol. Behav. 35:843–849; 1985.
15. MacLeod, K.; Smith, W.; Ogren, L.; Talamantes, F. Recombinant mouse placental lactogen-I binds to lactogen receptors in mouse liver and ovary: Partial characterization of the ovarian receptor. Endocrinology 125:2258–2266; 1989.
16. Mann, M.; Svare, B. Prenatal testosterone exposure elevates maternal aggression in mice. Physiol. Behav. 30:503–507; 1983.
17. Mednick, D.; Barkley, M.; Geschwind, I. Regulation of progesterone secretion by LH and prolactin during the first half of pregnancy in the mouse. J. Reprod. Fertil. 60:201–207; 1980.
18. Perrigo, G.; Bronson, F. Foraging effort, food intake, fat deposition and puberty in female mice. Biol. Reprod. 29:455–463; 1983.
19. Politch, J.; Herrenkohl, L. Effects of prenatal stress on reproduction in male and female mice. Physiol. Behav. 32:95–99; 1984.
20. Quadagno, D.; McQuitty, C.; McKee, J.; Koelliker, L.; Wolfe, G.; Johnson, D. The effects of intrauterine position on competition and behavior in the mouse. Physiol. Behav. 41:639–642; 1987.
21. Rines, J.; vom Saal, F. Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and progesterone. Horm. Behav. 18:117–129; 1984.
22. Rohde-Parfet, K.; Ganjam, V.; Lamberson, W.; Rieke, A.; vom Saal, F.; Day, B. Intrauterine position effects in female swine: Subsequent reproductive performance, and social and sexual behavior. Anim. Reprod. Behav. Sci. 26:349–362; 1990.
23. Svare, B.; Kinsley, C.; Mann, M.; Broida, J. Infanticide: Accounting for genetic variation in mice. Physiol. Behav. 33:137–152; 1984.
24. Vandenbergh, J.; Coppola, D. The physiology and ecology of puberty: Modulation by primer pheromones. In: Rosenblatt, J.; Beer, C.; Busskel, M., eds. Pheromones and reproduction in mammals. New York: Academic Press; 1986:95–100.
25. vom Saal, F. Prenatal exposure to androgen influences morphology and aggressive behavior of male and female mice. Horm. Behav. 12:1–11; 1979.
26. vom Saal, F. Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. J. Reprod. Fertil. 62:633–650; 1981.
27. vom Saal, F. 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.
28. vom Saal, F. The interaction of circulating estrogens and androgens in regulating mammalian sexual differentiation. In: Balthazart, J.; Prove, E.; Giles, R., eds. Hormones and behavior in higher vertebrates. Berlin: Springer-Verlag; 1983:159–177.
29. vom Saal, F. Perinatal testosterone exposure has opposite effects on intermale aggression and infanticide in mice. In: Brain, P.; Mainardi, D.; Parmigiani, S., eds. House mouse aggression. New York: Harwood Academic Pub.; 1988:179–204.
30. vom Saal, F. Sexual differentiation in litter bearing mammals: Influence of sex of adjacent fetuses in utero. J. Anim. Sci. 67:1824–1840; 1989.
31. vom Saal, F. The production of and sensitivity to cues that delay puberty and prolong subsequent oestrous cycles in female mice are influenced by prior intrauterine position. J. Reprod. Fertil. 36:457–471; 1989.
32. vom Saal, F.; Bronson, F. In utero proximity of female mouse fetuses to males: Effect on reproductive performance during later life. Biol. Reprod. 19:842–853; 1978.
33. vom Saal, F.; Bronson, F. Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208:597–599; 1980.
34. vom Saal, F.; Bronson, F. Variation in length of the estrous cycle in mice due to former intrauterine proximity to male fetuses. Biol. Reprod. 22:777–780; 1980.
35. vom Saal, F.; Moyer, C. Prenatal effects on reproductive capacity during aging in female mice. Biol. Reprod. 32:1116–1126; 1985.
36. vom Saal, F.; Even, M.; Dhar, M. The intrauterine position phenomenon: Role of uterine blood flow and intrauterine transport of steroids in pregnant rats. Conference on Reproductive Behavior. Saratoga Springs, NY, June, 1989 (abstract).
37. vom Saal, F.; Pryor, S.; Bronson, F. Change in oestrous cycle length during adolescence in mice is influenced by prior intrauterine position and housing. J. Reprod. Fertil. 62:33–37; 1981.
38. vom Saal, F.; Quadagno, D.; Even, M.; Keisler, L.; Keisler, D.; Khan, S. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine positions. Biol. Reprod. 43:751–761; 1990.
39. Wiebold, J.; Stanfield, P.; Becker, W.; Hillers, J. The effect of restraint stress in early pregnancy in mice. J. Reprod. Fertil. 78:185–192; 1986.