BIOLOGY OF REPRODUCTION 32, 1116–1126 (1985)

Prenatal Effects on Reproductive Capacity during Aging in Female Mice[1]

FREDERICK S. VOM SAAL[2] and CAROL L. MOYER

Division of Biological Sciences
Department of Psychology
and
John M. Dalton Research Center
University of Missouri-Columbia
Columbia, Missouri 65211

ABSTRACT

The production of live young during successive pregnancies was investigated in female CF-1 house mice (*Mus musculus*) identified at cesarean delivery as having developed in utero between 2 male fetuses (2M females) or not next to a male fetus (0M females). 2M female mice have previously been found to be exposed to higher concentrations of testosterone than 0M females during fetal life, presumably as a result of the transport of steroids between contiguous fetuses. 0M and 2M females were paired with stud males. The males were removed prior to delivery of a litter and replaced by other males when the litter was weaned. This process was repeated until: 1) a female did not become pregnant within 2.5 mo or 2) two successive litters were produced in which all of the pups were dead. In Experiment 1 females were first mated when 25 days old, and 2M females ceased producing litters containing live pups at a younger age and after fewer litters than did 0M females; however, many females were terminated from the study as a result of producing 2 successive litters of dead young rather than failing to become pregnant during a 2.5-mo period. There was a gradual decline in the number of live young produced by 0M females as a function of age and parity, but 2M females abruptly ceased producing any live young after producing a litter of normal size. For the last live litter, there were thus significantly fewer live young produced by 0M females than by 2M females. None of these differences were observed in Experiment 2, in which 0M and 2M females were mated for the first time beginning at 7 mo of age. The 2M females in this experiment ceased producing live young at a significantly older age than did the 2M females first mated at puberty. In contrast, there was no effect of age at initial mating on the age at which 0M females ceased producing live young. This finding suggests that exposure of 2M females to elevated titers of testosterone during fetal life results in a reduction in reproductive life span if they first become pregnant during the pubertal period.

INTRODUCTION

Female mammals typically lose the capacity to successfully produce young during midlife. The decline in reproductive capacity in females during aging has been generally regarded as being multicausal in origin, with most research having emphasized changes in neuroendocrine function and in ovarian physiology (Finch, 1978; Butcher and Page, 1981; Finch et al., 1984). Circulating titers of gonadal steroids throughout life influence the rate of decline of reproductive capacity during aging (Finch et al., 1980). However, the sensitivity of target tissues to steroids is modulated by the concentrations of steroids that tissues are exposed to during "sensitive" periods in early development.

Pharmacologic studies involving injections of testosterone into female mice and rats during the neonatal period have revealed that high doses of testosterone render females permanently sterile; androgen-sterilized females are anovulatory because of the inability to exhibit surges in luteinizing hormone (LH). These females have polycystic ovaries and exhibit persistent vaginal cornification in adulthood. Treatment with a low dose of testosterone shortly after birth influences the timing of puberty and also results in a greatly reduced

Accepted January 28, 1985.
Received July 19, 1984.
[1] This research was supported by a grant from the National Science Foundation (BNS 8203714) and University of Missouri Biomedical Research Support Grant RR 07053 from NIH.
[2] Correspondence: Dr. Frederick S. vom Saal, 200 Lefevre Hall, Division of Biological Sciences, University of Missouri, Columbia, MO 65211.

period of fertility; such females cease ovulating in young adulthood. This is referred to as the delayed anovulatory syndrome (Gorski, 1979).

A naturally occurring developmental event in rodents, the intrauterine positioning of male and female fetuses within a uterine horn, provides a unique method of examining the relationship between fetal hormone concentrations and reproductive aging without pharmacologic intervention. During the latter part of fetal life in house mice (*Mus musculus*), male fetuses have high circulating concentrations of testosterone, and female fetuses that are located between 2 male fetuses within a uterine horn (termed 2M females) have higher blood and amniotic fluid concentrations of testosterone than do females not located next to male fetuses (termed 0M females; vom Saal and Bronson, 1980a). Since positioning by sex is a random developmental event in mice (vom Saal, 1981), 0M and 2M females are presumed to not differ systematically in genotype. The use of house mice (CF-1) that are derived from an inbred strain (but are random-bred in our laboratory) thus provides a powerful model system for examining the effects on adult reproductive function of exposure to different concentrations of hormones during fetal life due to an environmental event.

Previous experiments have revealed that 0M and 2M female mice and rats differ in a broad spectrum of morphologic, physiologic, and behavioral parameters relating to reproduction (vom Saal, 1981, 1983b). In the experiments reported here 0M and 2M female mice were compared for their capacity to produce and raise young during repeated pregnancies. In the first experiment the females were repeatedly mated beginning at puberty (25 days old), and in the second experiment the females were repeatedly mated beginning at 7 mo of age.

MATERIALS AND METHODS

CF-1 (Crl:CF1BR) albino mice (*Mus musculus*) were purchased from Charles River Breeding Labs. (Wilmington, MA) in 1979 and were random-bred in a closed colony. Animal rooms were maintained on a 14L:10D cycle with lights on at 0600 h. Room temperature was maintained at 23 ± 2°C with 50% humidity. During the experiments animals were individually housed in 18 × 29 × 13-cm polypropylene cages with food and water always available. Bedding was changed weekly unless a female was just about to deliver or had just delivered a litter. In such cases, the bedding was not changed until the young were at least 5 days old.

To produce young from known intrauterine positions, 70–90-day-old females were time-mated with males between 0800 and 1200 h on consecutive days, and the females were examined for vaginal plugs (Day 0 of pregnancy). Inseminated females were housed in groups of 3 females/cage until just prior to normal parturition on Day 19, at which time they were killed by cervical dislocation. The fetuses were removed from the uterine horns, and their positions were recorded. All young were placed with foster mothers that had delivered naturally within the prior 48 h. Foster-litters comprised of 10 females were created: 5 females that had developed between 2 male fetuses within a uterine horn (2M females) were placed with 5 females that had developed between a male and a female fetus (1M females), and 5 females that had not developed contiguous to a male fetus (0M females) were placed with 5 1M females. The animals were weaned when they were 23 days old, and the foster litters were divided such that there were 5 females from the same intrauterine position per cage.

RESULTS

Experiment 1: Reproductive Capacity during Aging in 0M and 2M Females First Mated at Puberty

The finding that administering a low dose of testosterone to newborn female mice decreases their reproductive life span led us to hypothesize that 2M females would cease becoming pregnant at an earlier age than 0M females, since 2M females are exposed to higher concentrations of testosterone during fetal life than are 0M females (vom Saal and Bronson, 1980a). Thirty 0M and 30 2M female mice were initially paired with adult stud males (3–6 mo old) when the females were 25 days of age (date of birth: 12/20/80). The females were observed daily without touching the cage. When a female was visibly pregnant, the male was removed. The date of delivery was recorded, and the number of male and female pups, as well as their body weights, were determined at birth and at 21 days of age, at which time the young were weaned. This procedure was repeated with a different stud male being introduced into a female's cage on the day the previous litter was weaned. Females were terminated from the study when 1) no litter was produced within 2.5 mo of a litter being weaned (a different male was placed into the female's cage after 1 mo if the female was not visibly pregnant, this male was removed from a female's cage after 1 mo, and the female was observed for another 2 wk prior to being terminated from the study), or 2) two successive litters of dead young were produced. When a female produced a litter in

which there were no live young, 6 newborn mice (produced in a colony of young females) were fostered to her to determine whether the female would exhibit maternal behavior and lactate. This procedure allowed us to examine whether the young within a litter might have died because of a deficit in maternal behavior and/or lactation.

One 0M and 3 2M females died of unknown causes during the experiment, so the groups consisted of 29 0M females and 27 2M females. Disregarding the reason for termination from the study, the data presented in Fig. 1A reveal that the 2M females ceased producing live young at a significantly younger age than did the 0M females ($F_{1,54}$ = 5.8, P<0.02). The 2M females also produced significantly fewer litters than did the 0M females ($F_{1,54}$ = 4.5, P<0.05; Fig. 1B).

Eight 0M females (mean ± SEM, 7.5 ± 0.6 live litters produced; 351 ± 29 days old at birth of last live litter) and 2 2M females (each produced 6 live litters and were 301 and 309 days old at birth of last live litter) were terminated due to a failure to produce young within a 2.5-mo period after delivering a live litter. This difference between 0M and 2M females in the proportion of females that ceased reproducing as a result of a failure to successfully deliver a litter within a 2.5-mo period was statistically significant [χ^2_1 = 4.3, P<0.05]. Eight 0M females (7.6 ± 0.3 live litters produced; 362 ± 12 days old at birth of last live litter) and 10 2M females (7.9 ± 0.9 live litters produced; 363 ± 25 days old at birth of last live litter) first delivered a litter in which all young were dead and then did not deliver a litter within a 2.5-mo period of time. These 0M and 2M females did not differ in the number of live litters produced or in their ages at delivery of the last live litter.

The remaining 13 0M females (6.3 ± 0.3 live litters produced; 304 ± 13 days old at birth of last live litter) and 15 2M females (4.6 ± 0.5 live litters produced; 219 ± 19 days old at birth of last live litter) were terminated from the study after producing two consecutive litters in which all young were found to be dead on the day of birth; these differences were statistically significant (number of live litters: $F_{1,26}$ = 7.8, P<0.01; age at last live litter: $F_{1,26}$ = 11.0, P<0.01). Thus, there was a significant effect of prior intrauterine position on the age and parity of these female mice at the time that they began producing successive litters of dead young. In addition, the 0M and 2M females that were terminated from the study after producing two

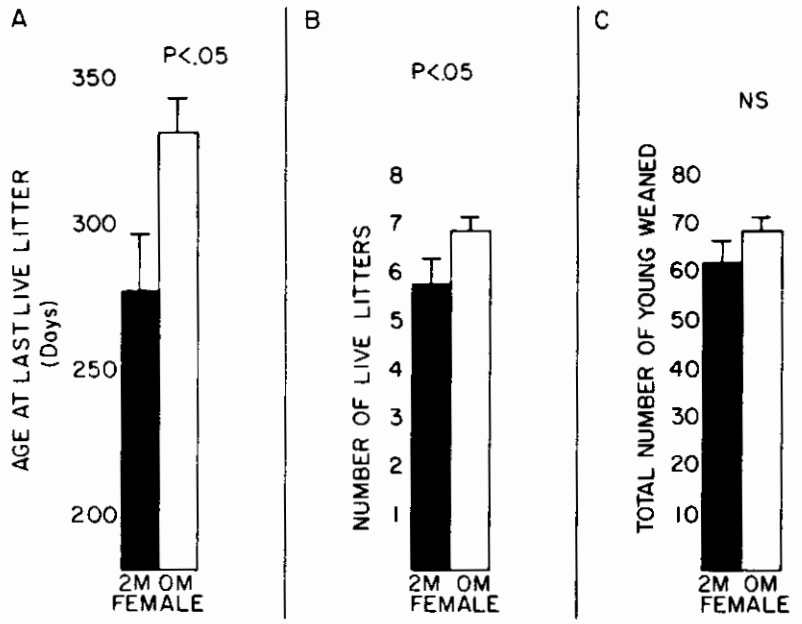

FIG. 1. For the 0M and 2M females in Experiment 1: *A)* mean (+ SEM) age (in days) at delivery of the last litter containing live young; *B)* number of litters produced that contained live young; and *C)* total number of live young weaned for all litters produced.

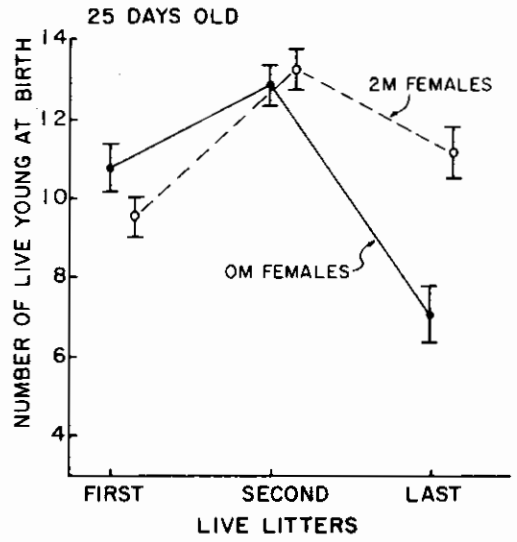

FIG. 2. Number of young found alive on the day of birth for the first, second, and last litters produced that contained live young for 0M and 2M females in Experiment 1.

consecutive litters of dead young delivered fewer live litters and ceased producing live young at a younger age than did the remaining 0M and 2M females described above. These 13 0M and 15 2M females were subsequently time-mated, and the length of pregnancy, as well as the proportion of animals that became pregnant, was determined (see below).

A surprising finding was that 0M and 2M females did not differ in the total number of live young found at birth or at weaning over all litters (see Fig. 1C). This prompted an analysis of the number of live young born and weaned in the first, second, and last live litters produced. The results, presented in Fig. 2, reveal that the last live litter produced by 0M females was significantly smaller than that produced by 2M females ($F_{1,54}$ = 18.0, P<0.001). There was also an increase from Litter 1 to Litter 2 in the number of young born for both 0M and 2M females. The 0M females exhibited a gradual decrease in the number of live young found on the day of birth, since the second-to-last live litter produced contained 8.9 ± 0.9 live young, which was 4 fewer live young than were found in the second live litter but 3 more than were found in the last live litter (see Fig. 2). The basis for only comparing the data from the first, second, and last litters produced was that the 0M and 2M females did not produce the same number of live litters (7 2M females, but only 1 0M female, produced fewer than 5 live litters).

There were no significant differences between 0M and 2M females in postnatal mortality throughout the study, and data obtained at weaning were virtually identical to those obtained at birth for all variables measured (see (Table 1). There were also no differences in the sex ratio (which was about 1) at birth or at weaning for any litter based on the intrauterine position of the mother.

For the first litter, the young produced by the 2M females tended to be heavier at birth (but not at weaning) than the young produced by the 0M females ($F_{1,54}$ = 3.8, P=0.06). Com-

TABLE 1. Number of young weaned and body weight per pup at weaning at 21 days of age for the first, second, and last litters produced by 0M females (n=29) and 2M females (n=27) in Experiment 1.

	Litters produced		
	First	Second	Last
Number of young			
0M females	10.0 ± 0.4*	12.3 ± 0.5	6.6 ± 0.8[a]
2M females	9.1 ± 0.5	13.2 ± 0.5	10.6 ± 0.6
Body weight			
0M females	8.9 ± 0.4	9.7 ± 0.3[b]	12.3 ± 0.3[c]
2M females	9.2 ± 0.4	8.8 ± 0.3	10.3 ± 0.4

*Mean ± SEM.

[a]Significantly different from 2M females for last litter (t-test, P<0.001).

[b]Not significantly different from 2M females for second litter (t-test, P=0.09).

[c]Significantly different from 2M females for last litter (t-test, P<0.001).

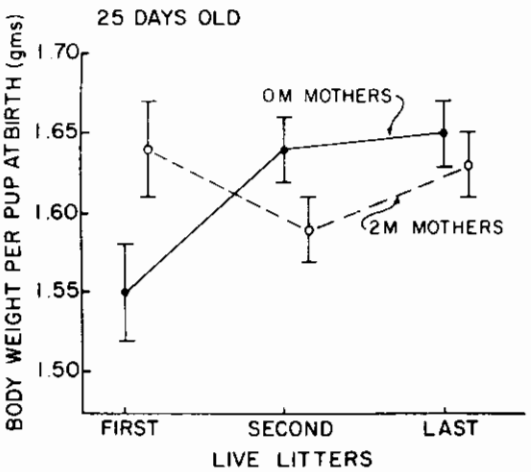

FIG. 3. Mean (± SEM) body weight (in g) per pup at birth for the first, second, and last litters produced that contained live young for 0M and 2M females in Experiment 1.

parisons of the young produced in subsequent litters by the 0M and 2M females revealed that they did not differ significantly in body weight at birth or at weaning (see Fig. 3 and Table 1). While the weight of pups at birth in successive litters did not change significantly for the 2M females, the young produced by the 0M females in the second litter and last live litter were significantly heavier than were the young produced in the first litter ($F_{2,84} = 4.6$, $P<0.02$).

In all cases in which the 0M and 2M females produced an entire litter of dead young, the dead young were removed and replaced with 6 newborn foster young that were produced by a young colony female. Only 1 0M female killed these foster young. In all other cases the 6 foster young were found to be of normal size and weight at weaning. CF-1 females thus did not seem to behave differently toward foster young and their own offspring regardless of prior intrauterine position, and the failure to produce live young seemed to be due to prenatal mortality of the young rather than to a failure of maternal behavior or lactation.

During this experiment cages were marked when a female was observed to be visibly pregnant. CF-1 female mice are easily identified as being pregnant 10–12 days after mating. However, we noticed that the females were delivering litters as long as 12–15 days after being labeled as pregnant, suggesting that parturition was not occurring at the normal time 19 days after insemination (mating is considered as Day 0). In addition, the few dead young that were found intact (i.e., that were not partially eaten) in such cases often weighed over 2 g rather than the normal 1.6 g. Litter size decreases during aging, and fetal size varies as a function of the number of fetuses within a uterine horn (Healy et al., 1960; McLaren and Michie, 1960). The large size of the dead fetuses could thus have been due to a reduction in intrauterine survival during aging (see Gosden et al., 1981). However, the 0M females produced an average of 7.1 young per litter for the last live litter compared to an average of 12.9 young in the second live litter produced, yet no difference in mean body weight of young at birth was observed. In addition, the hypothesis that the large size of the dead fetuses was due to a reduction in intrauterine servival during aging does not explain why the fetuses appeared fully developed but were dead, since all but one of the mothers that produced litters in which all of the pups were dead successfully raised foster litters. We therefore hypothesized that fetal death may have occurred as a result of a breakdown in the mechanisms controlling parturition. Previously, Holinka et al. (1978) reported that, when parturition occurred more than 2 days late in 11–12-mo-old C57BL/6J female mice, all young were dead at birth.

To examine the length of pregnancy in these 0M and 2M females, the 13 0M and 15 2M females (all about 14-mo old) that had previously been terminated from the reproductive aging study as a result of producing 2 litters of dead young were paired with males and examined daily for vaginal plugs indicating that mating had occurred. Eleven 0M females and 6 2M females were found to have vaginal plugs, but only 4 0M females (length of pregnancy = 21, 21, 23, and 23 days) and 3 2M females (length of pregnancy = 22, 24, and 25 days) delivered young. One of the 0M females that delivered on Day 21 produced 2 live young; all other young were dead at delivery. These females were also given 6 foster young to raise on the day that parturition occurred, and all foster young were alive and of normal size at weaning.

In summary, there were no differences in the mean age at which the 0M and 2M females produced corresponding litters, only a difference in the number of live litters produced and, therefore, the mean age at which the last live litter was born (see Fig. 1). The difference between 0M and 2M females in the age at which the last

live litter was born (55 days) was roughly equal to: 1) the length of time that females were allowed to nurse a live litter (21 days); 2) the interval between weaning and ovulation (probably about 5 to 10 days); and 3) the length of the next pregnancy (about 19 to 25 days).

The number of days between weaning of a litter and the day of delivery of the next litter was calculated. The above findings, as well as findings from other studies, demonstrate that the duration of pregnancy increases as a function of age. Thus, these data cannot be utilized to accurately assess the interval between mating and ovulation, since the length of pregnancy could have been between 19 and 25 days in aged females. Furthermore, there might also have been differences between 0M and 2M females in the degree to which pregnancy was prolonged as a function of aging. However, there were no significant differences between 0M and 2M females in the length of time between weaning of a litter and delivery of the next litter for the interval between the first and second live litters (0M females = 24.0 ± 0.8 days; 2M females = 23.2 ± 0.6 days), the second and third live litters (0M females = 24.4 ± 0.8 days; 2M females = 26.6 ± 2.0 days), or the next to last and last live litters (0M females = 28.5 ± 1.5 days; 2M females = 29.1 ± 1.9 days).

Experiment 2: Reproductive Capacity during Aging in 0M and 2M Females First Mated When 7 Months Old

In this experiment the 0M and 2M females were housed in groups of 5 females per cage from weaning until 7 mo of age, at which time they were paired with males. There were two objectives of this experiment: 1) to determine whether the previously observed difference in the decline of reproductive capacity during aging in 0M and 2M females that were repeatedly mated beginning at puberty would also be observed in 0M and 2M females first mated in mid-adulthood; and 2) to determine the length of gestation during successive pregnancies by examining the females daily and recording the day of insemination and delivery.

Twenty 0M and 18 2M females, 7 mo old at the beginning of the experiment, were utilized (date of birth: 2/20/82). One 0M female died of unknown causes and another 0M female produced a litter of dead young and then never became pregnant again. One 2M female never became pregnant and another 2M female became pregnant and delivered young six times prior to ceasing to become pregnant, but this female never successfully weaned any live young. Data from 18 0M and 16 2M females were thus utilized in the following analyses.

The females were paired with 3- to 6-mo-old stud males and examined daily at 0900 h for the presence of a vaginal plug by lifting the female's tail but not removing her from her cage. On the day that a plug was observed (Day 0 of pregnancy), the male was removed. The females were not disturbed during the next 2 wk (bedding was not changed during this time) to minimize the possibility of abortion due to stress during early pregnancy (see Chipman and Fox, 1966). The day of delivery was recorded, and the young were counted and weighed. The young were also counted and weighed at weaning at 21 days of age, and the mother was immediately rehoused with a male. This process was continued until 2 successive litters of dead young were produced or 2.5 mo passed without the female becoming pregnant, as described in the previous experiment.

The major finding from this experiment is that the 0M and 2M females did not differ in either the mean number of litters produced or the mean age at which they ceased producing live litters. What is most interesting is that the mean age at termination from the study for both the 0M females (358 ± 16 days old; 3.8 ± 0.4 live litters) and 2M females (354 ± 16 days old; 3.9 ± 0.3 live litters) examined in this experiment was similar to the mean age at termination for the 0M females that were repeatedly mated beginning at puberty (333 ± 12 days old; see Fig. 1A). However, the 2M females first mated at 7 mo of age were significantly older (354 ± 16 days old) at termination from the study than were the 2M females first mated at puberty (279 ± 19 days old; $F_{1,41}$ = 6.97, $P<0.02$).

There were no differences between 0M and 2M females in the number of live young at birth or at weaning for either the first, second, or last live litters produced (see Fig. 4 and Table 2). However, there was a decrease in the number of live young found at birth and at weaning for the last live litter relative to the first or second live litters for both 0M and 2M females (similar to the data for the 0M females in Experiment 1). In contrast to the previous experiment, the average weight per pup at birth was greater for the last live litter than for the second live litter

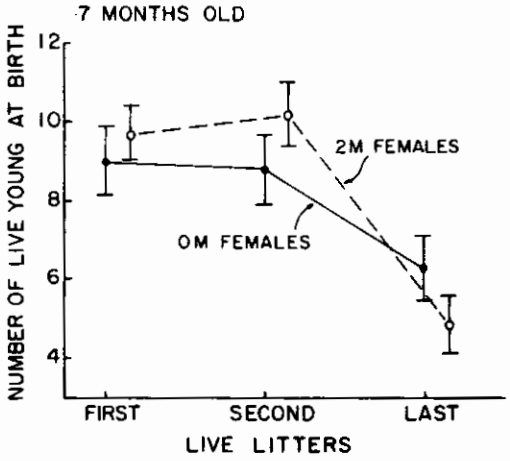

FIG. 4. Number of young found alive on the day of birth for the first, second, and last litters produced that contained live young for 0M and 2M females in Experiment 2.

produced for the 2M females (see Fig. 5 and Table 2).

Nine 0M and 7 2M females were terminated from the study (after having produced at least 1 live litter) as a result of not mating and becoming pregnant for 2.5 mo. Some of these females (3 0M and 5 2M) mated with the male partner after weaning a litter (they were observed to have vaginal plugs) but did not exhibit any signs of pregnancy and did not deliver any young. These females were therefore remated with other males 3 wk after having mated (when they should have delivered young). Again, vaginal plugs were found, indicating that the females had mated, but none of these females delivered young. This procedure was repeated with the same results until the females were terminated from the study at about 16 mo of age. The remaining 6 0M and 2 2M females were never found to have vaginal plugs and were terminated from the study after having cohabited with a male for 2 mo.

Nine 0M females and 9 2M females produced 2 litters of dead young. The mean duration of pregnancy for the first litter of dead young produced was: 0M females = 21.6 ± 0.2 days; 2M females = 21.7 ± 0.4 days. The mean duration of pregnancy for the last live litter produced by these same females was: 0M females = 20.1 ± 0.1 days; 2M females = 20.1 ± 0.1 days. Thus, in this study, litters that were produced more than 2 days late were found to contain only dead young. These same 9 0M and 9 2M females were then time-mated one more time. Only 3 0M and 3 2M females subsequently became pregnant. The 0M and 2M females that did not become pregnant never were observed to have vaginal plugs during 2 mo of cohabiting with a male. The pregnant 0M and 2M females were killed on Day 19 of pregnancy, and the young were removed from the uterus. The number of live and dead fetuses found on Day 19 of pregnancy for the 3 0M and 3 2M females was: 0M female 1—a total of 6 fetuses were found in both uterine horns, 4 alive and 2 dead; 0M female 2—one fetus was found alive in one uterine horn, none were dead; 0M female 3—five fetuses were found alive in both uterine horns, none were dead; 2M female 1—four fetuses were found in one uterine horn, 1 alive and 3 dead; 2M female 2—four fetuses were found in one uterine horn, 2 alive and 2 dead; and 2M female 3—five fetuses were found in one uterine horn, 4 alive and 1 dead. In 5 out of 6 cases foster mothers successfully raised these cesarean-delivered young (one foster mother

TABLE 2. Number of young weaned and body weight per pup at weaning at 21 days of age for the first, second, and last litters produced by 0M females (n=18) and 2M females (n=16) in Experiment 2.

	Litters produced		
	First	Second	Last
Number of young			
0M females	8.4 ± 0.9*	8.6 ± 0.9	5.6 ± 0.7
2M females	8.9 ± 0.6	9.2 ± 0.8	4.6 ± 0.8
Body weight			
0M females	11.6 ± 0.8	11.9 ± 0.5	13.1 ± 0.6
2M females	11.5 ± 0.5	12.3 ± 0.5	13.0 ± 0.9

*Mean ± SEM.

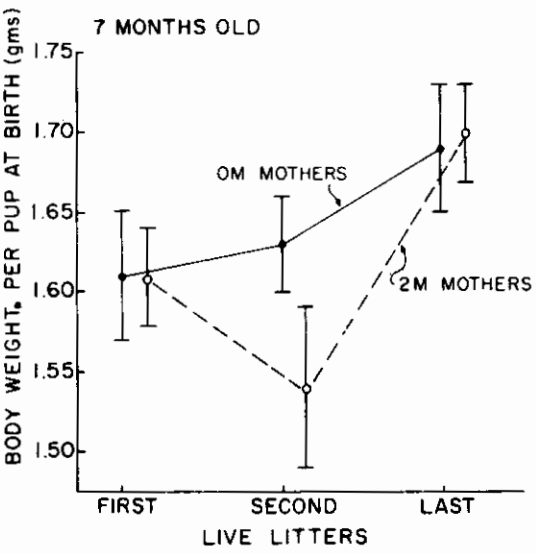

FIG. 5. Mean (± SEM) body weight (in g) per pup at birth for the first, second, and last litters produced that contained live young for 0M and 2M females in Experiment 2.

was not maternal). All foster litters contained 6 young; newborn young obtained from the colony were utilized to equalize foster-litter sizes.

DISCUSSION

The major finding from the first experiment was that 2M females ceased producing live young at an earlier age and after fewer litters than did 0M females. However, the basis of this difference between 0M and 2M females was quite different from what had been expected. Many of the females produced 2 litters of dead young and were then terminated from the study, and 2M females began producing litters of dead young at a younger age and after fewer litters than did 0M females. We had predicted that, because 2M female mice were exposed to higher titers of testosterone during fetal life than were 0M females, 2M females would cease ovulating at an earlier age than would 0M females, i.e., that 2M females would be similar to female mice with the delayed anovulatory syndrome (Gorski, 1979). However, in the first experiment, after having produced litters of live young, significantly more 0M females (28%) than 2M females (7%) were terminated from the study due to a failure to deliver young during a 2.5-mo period of cohabitation with a stud male. Since these animals were not examined daily for evidence of mating (vaginal plugs), whether the inability to deliver young was due to a failure to ovulate and mate or a failure to sustain pregnancy to term remains unresolved.

In the first experiment 0M and 2M females did not differ in the total number of live young produced over all litters. An analysis of the number of live young produced in the first, second, next to last, and last live litters revealed that 0M females exhibited a gradual decline in the production of live young during aging, whereas 2M females seemed to abruptly cease producing litters containing any live young after producing a normal-size litter of live young. Again, this cessation of reproduction occurred at a younger age for 2M females than for 0M females. Thus, 0M females had significantly fewer live young present in the last litter produced than did 2M females. In contrast, both 0M and 2M females produced more live young in the second litter than the first litter. There is a well-documented increase in intrauterine mortality during aging in rodents, but a decrease in litter size between the second and last live litters was not observed in 2M females.

We propose that the length of pregnancy may have gradually increased during successive pregnancies in 0M females, such that only a few fetuses were able to survive when delivered 1–2 days late. In a separate study utilizing time-mated, 14-mo-old CF-1 female mice that were mated for the first time, we observed that, if pregnancy extended beyond the morning of the twenty-first day of pregnancy (more than 2 days beyond the time of parturition in young females), all young were born dead (F. vom Saal and C. Moyer, unpublished observation). In Experiment 2 reported here, females that delivered young more than 2 days late also produced dead young. Holinka et al. (1978) reported similar findings concerning the effect of gestation length on fetal survival in C57BL/6J mice.

When the 0M and 2M females that had previously been terminated from the first experiment because they produced 2 successive litters of dead young were time-mated, the 2M females seemed to have longer delays in the onset of parturition than did the 0M females, although too few females became pregnant to allow for any firm conclusions to be drawn from the data. However, it is possible that the abrupt cessation of the production of any live young

by 2M females, after having just produced a litter of normal size, might have been due to pregnancy abruptly extending to greater than 21 days, with the result that all fetuses were born dead. Thus, the timing of parturition may have gradually broken down in 0M females, such that fewer and fewer live young were produced, whereas in 2M females there may have been an abrupt loss of the capacity to deliver young within 21 days after mating. Since CF-1 females usually cannibalize dead or deformed pups at birth, there was no way to tell in these experiments whether the decline in the number of live young found on the day of birth reflected a decrease in ovulation rate, an increase in embryonic mortality prior to the normal time of parturition, or fetal death due to an increase in the length of gestation.

In contrast to the first experiment, in which females were mated beginning at puberty, when 0M and 2M females were repeatedly mated beginning at 7 mo of age, no difference in the pattern of decline in reproductive capacity with aging was observed. In particular, the 2M females did not exhibit the premature decline in reproductive capacity observed when mating was first begun at puberty.

During early life steroids have generally been presumed to have permanent "organizational" effects, whereas by the time of weaning steroids have been thought to exert only transient "activational" effects on tissues (Gorski, 1979; vom Saal, 1983a). In fact, it does seem that the period of greatest sensitivity to the organizing effect of steroids is the perinatal period, and the farther in time that one gets from this period, the less sensitive are tissues to the organizing effects of steroids. However, animals remain sensitive to the organizational effects of steroids throughout adolescence and into young adulthood. There are data from both behavioral (see Edwards, 1970; Barkley and Goldman, 1977) and physiologic (Brawer et al., 1983; Mobbs et al., 1984) studies indicating that exposure to testosterone or estradiol during adolescence or young adulthood results in permanent changes in the responsivity to steroids during later life in female mice. For example, it is well documented that exposure to steroids (endogenous or exogenous) in young adulthood can accelerate the decline of reproductive capacity with aging (for reviews see Finch et al., 1980, 1984). These findings have thus led to a reevaluation of the proposition that only during a defined critical period in early life are there permanent effects of steroids on tissues (vom Saal, 1983a; Rines and vom Saal, 1984).

Swanson and Van der Werff ten Bosch (1964) injected pregnant rats with testosterone propionate within the last 4 days of pregnancy without causing sterility in the female offspring, whereas treatment of female rat pups with varying doses of testosterone propionate during the first 5 days after birth did result in sterility in adulthood (the time of onset of sterility was dependent on the dose and postnatal day of treatment). Other reports that rats (Pfeiffer, 1936) and mice (Barraclough and Leathem, 1954) were sterilized by postnatal exposure to androgen led to the general assumption that the process of masculinization/defeminization of behavioral and neuroendocrine systems in rats and mice began postnatally. However, male and female internal and external genitalia are organized prenatally in rats and mice (Schlegel et al., 1967; Rugh, 1968), and the volume of the sexually dimorphic nucleus of the preoptic area of the brain (SDN-POA) is larger in males than in females at birth (Jacobson et al., 1980) and differs significantly in males from different intrauterine positions (these males also differed in their sexual behavior; vom Saal et al., 1984). This assumption is thus obviously incorrect.

Further evidence that exposure to different concentrations of steroids during prenatal life influences adult reproductive characteristics is provided by other comparisons of mice and rats from known intrauterine positions. 0M and 2M female mice and rats have been found to differ in the timing of puberty and length of postpubertal estrous cycles. For example, in Sprague-Dawley rats, the first vaginal estrus (which serves as an indirect measure of the timing of puberty) is delayed in 2M females relative to 0M females, and 0M females have significantly shorter (predominantly 4–5 day) estrous cycles than do 2M females (predominantly 5–6 day estrous cycles; vom Saal, 1981). Similar findings have been reported in comparisons of 0M and 2M female mice, although the studies involving the timing of puberty in mice are complicated by the fact that pheromones modulate the timing of puberty, and 0M and 2M females seem to differ in the emission of as well as the response to these cues (vom Saal and Bronson, 1978, 1980b; vom Saal, 1981; vom Saal et al., 1981; F. vom Saal, unpublished observation). Taken together, however, these findings reveal that 2M

females have a reduced reproductive life span relative to 0M females. Again, what is intriguing about the present data is that they suggest that the cessation of reproduction in 2M females at about 9 mo of age may be due to a breakdown in the mechanisms regulating parturition relative to the status of these mechanisms in 0M females, rather than to a difference in the capacity to ovulate. In summary, it seems that the period during which exposure to elevated concentrations of testosterone can interfere with the capacity for females to ovulate in adulthood in rats and mice begins around the time of birth. Exposure of 2M females to increased concentrations of testosterone during prenatal life delays puberty, increases the length of estrous cycles, and accelerates the decline of reproductive function during aging (and also influences other morphologic and behavioral traits; vom Saal, 1981, 1983b), but does not seem to result in the delayed anovulatory syndrome.

Concerning the present findings, when females are exposed to high concentrations of steroids after puberty, there are permanent effects on reproductive function during aging. However, as mentioned previously, exposure to different concentrations of steroids during fetal life influences the sensitivity of target organs to steroids during later life. 2M female mice are exposed to higher titers of testosterone than are 0M females during fetal life, and numerous experiments have revealed that in adulthood 2M females are more sensitive to testosterone than are 0M females, whereas 0M females are more sensitive to estrogen and progesterone than are 2M females (for a review see vom Saal, 1983b). Since 2M females that were mated beginning at puberty exhibited a loss of reproductive capacity at a relatively young age, we propose that exposure to high concentrations of testosterone during fetal life rendered 2M females more sensitive to the permanent "organizational" effects of high concentrations of testosterone that females are exposed to during pregnancy. In particular, 2M females may be most sensitive to exposure to high concentrations of testosterone during a pregnancy that occurs at a very young age, i.e., shortly after puberty. Females rendered sensitive to testosterone by exposure to high concentrations of testosterone during fetal life may thus represent a subpopulation of females that are at risk in terms of long-term negative effects of a pregnancy during early adolescence.

We have recently examined blood concentrations of testosterone throughout pregnancy in young adult CF-1 mice. Serum testosterone concentrations in pregnant female mice range from 1.5 to 2.2 ng/ml between Days 11 and 19 of pregnancy, and there is a dramatic elevation of blood testosterone up to 4.0 ng/ml on Days 9 and 10 of pregnancy. Before Day 9 and on the day after birth, serum testosterone concentrations are approximately 0.15–0.40 ng/ml (vom Saal et al., 1985). What proportion of testosterone is in the free versus bound compartments during pregnancy in CF-1 mice remains to be determined.

In a previous experiment in which 0M and 2M female CF-1 mice were compared for their capacity to produce and raise young for 2 successive litters beginning at 60 days of age using the same procedures described above, no differences between 0M and 2M females in the number of young at birth or at weaning were observed for either the first or second litters. It had previously been concluded, therefore, that young adult 0M and 2M females did not differ significantly in any measure of gross reproductive performance when the females were mated individually with a male in an optimum laboratory environment (vom Saal and Bronson, 1978). It thus seems that, for differences in reproductive capacity due to prior intrauterine position to be observed, the females need to become pregnant in early adolescence, with the differences becoming apparent only after the production of 3 or more litters.

ACKNOWLEDGMENTS

We thank C. E. Finch for his comments during the preparation of this manuscript.

REFERENCES

Barkley, M. and Goldman, B. (1977). Testosterone-induced aggression in adult female mice. Horm. Behav. 8:208–218.

Barraclough, C. and Leathem, J. (1954). Infertility induced in mice by a single injection of testosterone propionate. Proc. Soc. Exp. Biol. Med. 85: 673–674.

Brawer, J., Schipper, H. and Robaire, B. (1983). Effects of long term androgen and estradiol exposure on the hypothalamus. Endocrinology 112:194–199.

Butcher, R. and Page, R. (1981). Role of the aging ovary in cessation of reproduction. In: Dynamics of Ovarian Function (N. Schwartz and M. Hunzicker-Dunn, eds.). Raven Press, New York, pp. 253–271.

Chipman, R. and Fox, K. (1966). Oestrous synchronization and pregnancy blocking in wild house mice (Mus musculus). J. Reprod. Fertil. 12: 233–236.

Edwards, D. (1970). Post-neonatal androgenization and adult aggressive behavior in female mice. Physiol. Behav. 5:465–467.

Finch, C. (1978). Reproductive senescence in rodents: factors in the decline of fertility and loss of regular estrous cycles. In: The Aging Reproductive System (E. Schneider, ed.). Raven Press, New York, pp. 193–212.

Finch, C., Felicio, L., Flurkey, K., Gee, D., Mobbs, D., Nelson, J. and Osterburg, H. (1980). Studies on ovarian-hypothalamic-pituitary interactions during reproductive aging in C57BL/6J mice. Peptides 1(Suppl. 1):163–175.

Finch, D., Felicio, L., Mobbs, C. and Nelson, J. (1984). Ovarian and steroidal influences on neuroendocrine aging processes in female rodents. Endocr. Rev. 5:467–497.

Gorski, R. (1979). The neuroendocrinology of reproduction: an overview. Biol. Reprod. 20:111–127.

Gosden, R., Holinka, C. and Finch, C. (1981). The distribution of fetal mortality in ageing C57BL/6J mice: a statistical analysis. Exp. Gerontol. 16: 127–130.

Healy, M., McLaren, A. and Michie, D. (1960). Foetal growth in the mouse. Proc. Royal Soc. Lond. 153:367–379.

Holinka, C. and Finch, C. (1981). Efficiency of mating in C57BL/6J female mice as a function of age and previous parity. Exp. Gerontol. 16:393–398.

Holinka, C., Tseng, Y. and Finch, C. (1978). Prolonged gestation, elevated preparturitional plasma progesterone and reproductive aging in C57BL/6J mice. Biol. Reprod. 19:807–816.

Jacobson, C., Shryne, J., Shapiro, F. and Gorski, R. (1980). Ontogeny of the sexually dimorphic nucleus of the preoptic area. J. Comp. Neurol. 193:541–548.

McLaren, A. and Michie, D. (1960). Control of prenatal growth in mammals. Nature 187:363–365.

Mobbs, C., Flurkey, K., Gee, D., Yamamoto, K., Sinha, Y. and Finch, C. (1984). Estradiol-induced anovulatory syndrome in female C57BL/6J mice: age-like neuroendocrine, but not ovarian, impairments. Biol. Reprod. 30:556–563.

Pfeiffer, C. (1936). Sexual differences of the hypophyses and their determination by the gonads. Am. J. Anat. 58:195–225.

Rines, J. and vom Saal, F. (1984). Fetal effects on sexual behavior and aggression in young and old female mice treated with estrogen and testosterone. Horm. Behav. 18:117–129.

Rugh, R. (1968). The Mouse: Its Reproduction and Development. Burgess Pub. Co., Minneapolis.

Schlegel, R., Farias, E., Russo, N., Moore, J. and Gardner, L. (1967). Structural changes in the fetal gonads and gonaducts during maturation of an enzyme, steroid 3β-ol-dehydrogenase, in the gonads, adrenal cortex and placenta of fetal rats. Endocrinology 81:565–572.

Swanson, H. and Van der Werff ten Bosch, J. (1964). The early androgen syndrome; differences in response to pre-natal and post-natal administration of various doses of testosterone propionate in female and male rats. Acta Endocrinol. 47: 37–50.

vom Saal, F. (1981). Variation in phenotype due to random intrauterine positioning of male and female fetuses in rodents. J. Reprod. Fertil. 62: 633–650.

vom Saal, F. (1983a). Models of early hormonal effects on intrasex aggression in mice. In: Hormones and Aggressive Behavior (B. Suare, ed.). Plenum Press, New York, pp. 197–222.

vom Saal, F. (1983b). The interaction of circulating estrogens and androgens in regulating mammalian sexual differentiation. In: Hormones and Behavior in Higher Vertebrates (J. Balthazart, E. Prove and R. Giles, eds.). Springer-Verlag, Berlin, pp. 159–177.

vom Saal, F. and Bronson, F. (1978). In utero proximity of female mouse fetuses to males: effect on reproductive performance during later life. Biol. Reprod. 19:842–853.

vom Saal, F. and Bronson, F. (1980a). Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208:597–599.

vom Saal, F. and Bronson, F. (1980b). Variation in length of the estrous cycle in mice due to former intrauterine proximity to male fetuses. Biol. Reprod. 22:777–780.

vom Saal, F., Pryor, S. and Bronson, F. (1981). Change in oestrous cycle length during adolescence in mice is influence by prior intrauterine position and housing. J. Reprod. Fertil. 62:33–37.

vom Saal, F., Coquelin, A., Schoonmaker, J., Shryne, J. and Gorski, R. (1984). Sexual activity and sexually dimorphic nucleus volume in male rats are correlated with prior intrauterine position. Paper presented at Society for Neuroscience meeting, Anaheim, CA.

vom Saal, F., Even, M., Khan, S. and Ganjan, V. (1985). Maternal stress increases blood testosterone titers in male and female mouse fetuses. Biol. Reprod. 32(suppl. 1), abstr. 243 (in press).