Sexual Characteristics of Adult Female Mice Are Correlated with Their Blood Testosterone Levels During Prenatal Development

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Abstract. Mice produce litters containing many pups, and the female fetuses that develop between male fetuses have significantly higher concentrations of the male sex steroid testosterone in both their blood and amniotic fluid than do females that develop between other female fetuses. These two types of females differ during later life in many sexually related characteristics. Thus, individual variation in sexual characteristics of adult female mice may be traceable to differential exposure to testosterone during prenatal development because of intrauterine proximity to male fetuses.

Differentiation of mammalian fetuses into the masculine phenotype depends primarily on the secretion of androgens from the testes. The female phenotype is thought to occur if the fetus remains relatively free from the effects of androgens during the time of sexual differentiation. Inherent in this traditional concept of the “normal” development of a mammalian female is the assumption that females exposed to androgens during fetal life may be abnormal. Indeed, experiments in which female fetuses are exposed to increased concentrations of androgens, either by way of administration of hormones to the mother or because of a metabolic error that results in an increased production of androgens by the fetus, are often cited as evidence supporting this assumption.

Recent studies with rodents, which produce litters containing many pups, have shown that in both mice and rats there is considerable variability among adult females in terms of reproductive characteristics, and that part of this variability can be traced to the former intrauterine proximity of females to male fetuses during prenatal development. For example, female mice that developed in utero between two male fetuses (referred to as 2M females) were found to differ morphologically, physiologically, and behaviourally from females that did not develop next to a male fetus (0M females) (1). When these two types of females were compared, 2M females had larger anogenital spaces at birth and in adulthood, were more aggressive in a variety of test situations, marked their environment with urine at a higher rate, and had longer and more irregular adult estrous cycles than 0M females. The 0M females appeared to be more attractive and sexually arousing to males. Prior intrauterine position was also found to interact with housing density in terms of the time of onset of puberty in female mice (2). These findings suggest that in species that produce litters containing many pups, the reproductive characteristics of females may vary depending on their intrauterine proximity to male fetuses, and that such variation is normal in polytocous animals.

![Graph showing concentrations of testosterone, progesterone, and estradiol in the serum and amniotic fluid of 15-day-old 0M and 2M female fetuses.](image)

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It has been proposed (5) that the course of development of female fetuses that are contiguous to males in utero is altered by exposure of these females to increased concentrations of androgens, particularly testosterone. Presumably, androgens produced by male fetuses diffuse across the fetal membranes separating individual fetuses and into the amniotic fluid and blood of contiguous female fetuses. We designed the experiments described herein to investigate whether the differences in the reproductive characteristics of 0M and 2M females were related to differences in steroid hormone concentrations during fetal or adult life. Both blood and amniotic fluid were collected from 0M and 2M female fetuses and assayed for the presence of the sex steroids testosterone, 17β-estradiol, and progesterone. Other 0M and 2M females were raised to adulthood at which time the concentrations of testosterone in their blood and their attractiveness to males were compared. We found that the 2M female fetuses had significantly higher concentrations of testosterone both in their blood and in their amniotic fluid than did the 0M male fetuses; adult 0M and 2M females did not differ in their blood testosterone concentrations, but adult 0M females were significantly more attractive to male mice than were 2M females.

One group of timed-mated CF-1 female mice was killed by decapitation on day 17 of pregnancy (6) and their blood was collected for later hormone analyses. The pups were then removed from the uterine horns without rupturing the fetal membranes surrounding each individual fetus so that the amniotic fluid could be collected (7). The sex of each fetus, determined initially by examining the length of the anogenital space, was subsequently confirmed by autopsy. We collected blood and amniotic fluid from 125 0M and 125 2M female fetuses, and blood from 125 male fetuses. In each experiment we pooled 25 samples to obtain five replicates that we then subdivided for radioimmunoassay of testosterone, 17β-estradiol, and progesterone (8).

Blood testosterone concentrations differed significantly between male and female fetuses (mean ± standard error: males, 3.00 ± 0.14 ng/ml; females, 0.98 ± 0.05 ng/ml, P < 0.01, t-test). We found no sex differences in either blood 17β-estradiol (males, 0.18 ± 0.03 ng/ml; females, 0.23 ± 0.05 ng/ml, P > 0.5) or progesterone (males, 6.2 ± 0.3 ng/ml; females, 7.8 ± 0.9 ng/ml, P > 0.5).

The results of comparing hormone concentrations in the blood and amniotic fluid of 0M and 2M females are presented in Fig. 1 and reveal that only testosterone levels varied as a function of intrauterine position. The 2M females had significantly elevated levels of testosterone in both their blood (P < 0.05) and amniotic fluid (P < 0.01) relative to 0M females. Neither 17β-estradiol nor progesterone levels differed significantly (P > 0.5). Such differences could have been due to the fact that litters containing 2M females generally contain more male pups than do litters containing 0M females. Litters containing many males conceivably could increase the concentration of testosterone in the mother’s circulation and, in turn, increase blood concentrations of this hormone in the entire litter. To test for this possibility, we measured the sex steroids in blood collected from mothers that were killed on day 17 of pregnancy and were carrying either nine male and three female fetuses or three male and nine female fetuses (three mothers per group). Since we found no differences in the serum testosterone, 17β-estradiol, or progesterone concentrations between the two groups of mothers (Fig. 2), the differences in testosterone levels between 0M and 2M female fetuses appears not to be mediated by the maternal circulation.

From a second group of randomly chosen, timed-mated females we obtained cesarean section the 0M and 2M offspring on day 19 of pregnancy just prior to parturition. The pups were fostered to mothers that had just delivered naturally. These 0M and 2M females were raised to adulthood at which time we compared their serum testosterone levels and their relative attractiveness to male mice.

Ten 0M and ten 2M females in diestrus (as indicated by a vaginal smear and steroid weight subsequent to death) were killed when 120 days old, and blood levels of testosterone were determined. We found no significant differences in the serum concentrations of testosterone between these 0M and 2M females (mean ± standard error: 0M females, 29 ± 77 pg/ml; 2M females, 228 ± 73 pg/ml). Thus, behavioral differences between 0M and 2M females cannot be attributed to differential exposure to endogenously produced testosterone in adulthood. However, this experiment cannot rule out the possibility that adult 0M and 2M females differ in their sensitivity to testosterone (9). However, in previous experiments in which both physiological and behavioral measures were used, there was no difference in sensitivity to estradiol between adult 0M and 2M females (9).

The remaining adult 0M and 2M females were tested for their relative attractiveness to male mice. This experiment was conducted as a control measure, since it was deemed important to replicate one of the tests on which 0M and 2M females previously had been found to differ significantly. Using a procedure described elsewhere (3), we enclosed diestrous 0M and 2M females in wire-mesh cages that were placed in separate chambers of a test apparatus so that a male could jump from a platform into either the chamber containing a 2M female or the chamber containing a 0M female. Of the 24 adult males tested, 19 chose a 0M female (P > 0.05), thus replicating our previous finding (3).

The results presented here indicate that male fetuses have three times more circulating testosterone than female fetuses, and that 2M female fetuses have significantly higher concentrations of testosterone in both their blood and amniotic fluid than 0M females. Concentrations of testosterone in the mothers’ circulation did not appear to account for these differences. In adulthood, 0M and 2M females differed markedly in their attractiveness to males but not in their blood levels of testosterone. Taken together, these results support the hypothesis that the normal variation observed in some sexual characteristics of female mice is in part traceable to differential exposure to testosterone during prenatal.
life, which in turn is due to intraspecific proximity to male fetuses.

In both mice and rats there is evidence that exposure to high concentrations of testosterone shortly after birth can result in the complete loss of both estrous cyclicity and the capacity to ovulate (10). This period of maximum neural sensitivity to the depolarizing action of testosterone occurs after female pups have been removed from the influence of proestral male fetuses. Exposure of 2M females to higher concentrations of testosterone than 0M females in utero does not influ-
ence their capacity to ovulate, exhibit fe-
male sex behavior, or produce and raise normal offspring in an optimum laborato-
ry environment (5). But, variation in nu-
erous characteristics that could influence reproductive success is related to prior intrapartum status. We propose that under certain ecological conditions females with a particular set of char-
acteristics might be more likely to repro-
cude than other females. For example,
2M females might have a reproductive advantage over 0M females when popula-
tion density is high, because they are highly aggressive toward other females but not toward males, they fiercely de-
defend their young when lactating, and they enter puberty sooner than 0M fe-
male when housed in groups, in con-
trast, 0M females may be more likely to reproduce than 2M females when popula-
tion density is low, because 0M females are highly preferred by males and enter puberty sooner and have shorter estrous cycles than 2M females when housed in-
dividually. Thus, it appears that 0M fe-
male are neither more nor less "nor-
mal" than are 2M females, since intra-
uterine proximity to male fetuses does not influence a female's basic capacity to reproduce.

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6. During the 5% of gestation treatment whose course of development is influenced by estradiol, an differentiation. For instance, the proportion of females occurs in utero as well as in 2M females is at a maximum at this


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