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INTRAUTERINE POSITIONING OF MALE AND FEMALE FETUSES INFLUENCES PRENATAL HORMONE TITERS AND ADULT BEHAVIOR

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Research concerning the relationship between gonadal hormone exposure during early life and adult behavior in rodents has influenced models concerning psychosexual differentiation in humans. Over the past 40 years, different models of the hormonal bases of sexual dimorphisms in behavior have prevailed (12). The intrauterine position phenomenon, the modification of the development of a fetus by gonadal steroids secreted by contiguous fetuses where multiple-uterine residence occurs, provides a unique model system for examining the role that variation in prenatal hormone titers plays in biasing development and later reproductive physiology and behavior without the necessity of pharmacological intervention. The results of experiments dealing with this phenomenon have led to a reevaluation of some traditional beliefs concerning sex-differences in behavior.

In humans, male fetuses have higher concentrations of circulating testosterone than female fetuses, with the largest difference occurring between Week 12-18 of gestation (1, 18). Human female fetuses have been reported to have higher blood concentrations of estradiol than male fetuses (17). During fetal life male mice have higher titers of testosterone than females, and females located between 2 male fetuses in utero (2M females) have higher amniotic fluid and blood titers of testosterone than females that do not develop contiguous to a male fetus (0M females; 14). Males do not differ in their amniotic fluid or blood titers of testosterone based on their proximity to other male fetuses. But, female mouse fetuses have higher concentrations of estradiol in their amniotic fluid than male fetuses (mean ± SEM: females = 33.6 ± 2.8 pg/fetus; males = 20.9 ± 1.5 pg/fetus; $p < .001$), and males that develop between female fetuses (0M males) have higher concentrations of estradiol in their amniotic fluid than 2M males, i.e., males that develop between other male fetuses (mean ± SEM: 0M males = 23.9 ± 0.9 pg/fetus; 2M males = 16.3 ± 1.1 pg/fetus; $p < .01$; unpublished).

It has been reported that in rats, the fetus rather than the placenta secretes estradiol into the maternal circulation (6). The above findings with mice suggest that female mouse fetuses secrete higher titers of estradiol than male fetuses, but the source of the estradiol within the fetus is, as yet, unknown. It is presumed that testosterone secreted by the testes of male fetuses and estradiol secreted by female fetuses passes across the amniotic and chorionic membranes surrounding each fetus via the amniotic fluid into the circulation of a contiguous fetus. A related observation is that during the last 4-5 days of gestation in mice (when sexual differenti-

ation is occurring (19), fetuses are tightly packed in the uterine horns. There is thus a large area of contact of the chorionic membranes of contiguous fetuses. In multiple birth (polytocous) species such as the mouse, unlike cattle in which freemartins are found due to anastomoses of chorionic vessels of male and female fetuses that develop together in utero (8), no evidence of vascular anastomoses between contiguous fetuses has been found (9, 11). It is possible that in any species, including humans, in which multiple-uterine residence occurs, intrauterine position effects similar to those found in rodents may be found based on the sex of the nearest womb-mate of a fetus.

Comparisons of 0M and 2M males and of 0M and 2M females have revealed that individual differences between males and between females in a broad range of behavioral and physiological characteristics correlate with differential exposure to gonadal hormones during fetal life based on prior intrauterine position. The positioning of fetuses in the uterus is determined by delivering the litters of time-mated females by cesarean section just prior to the expected time of parturition (11). All young are raised by foster mothers. Comparisons of 0M and 2M female mice have revealed that numerous sexually-related characteristics are influenced by the hormonal environment of a female during fetal life: 2M females (a) are highly aggressive toward other females but not toward males (11, 13), (b) enter puberty later (13), (c) have longer and more irregular estrous cycles (15, 16), (d) cease producing live young at a younger age (unpublished), and (e) are less attractive and sexually arousing to males (13) than are 0M females. While 2M female mice cease producing live litters at a younger age (about 2 months earlier) and thus produce fewer litters than 0M females, young adult 0M and 2M female mice do not differ in their capacity to produce and raise healthy young in an optimum laboratory environment (13).

Comparisons of 0M and 2M male mice that were castrated at birth have revealed that individual differences in the behavioral response to gonadal steroids correlate with prior intrauterine position: 2M males are more sensitive to testosterone (they have larger seminal vesicles and are more aggressive after adult testosterone treatment); 2M males are also less sensitive to estrogen and progesterone (0M but not 2M males elicit mounting and exhibit lordosis when they are injected with estradiol benzoate and progesterone and tested for the exhibition of female sex behavior when paired with a stud male; unpublished). These findings raise the intriguing possibility that exposure to elevated concentrations of estrogens during the fetal period of sexual differentiation has effects on some developing neural and peripheral tissues that are similar to those observed when males are exposed to androgen antagonists. In contrast, intact 0M male mice exhibit significantly more mounts and intromissions per minute than intact 2M males when paired with a sexually-receptive female, and intact 0M male rats exhibit more ejaculations to satiety than intact 2M male rats when paired with a sexually-receptive female (unpublished). Thus, one of the most interesting aspects of the intrauterine position phenomenon in male mice is that exposure to elevated titers of estradiol due to developing in utero next to female fetuses results in an enhancement of sexual performance (more mounts, intromissions and ejaculations) and a decrement in seminal vesicle growth

and the capacity to exhibit aggression in response to testosterone in adulthood.

During fetal life steroid hormones act as chemical inducers that regulate the course of development of numerous tissues, including specific "target" sites in the brain. One consequence of having sexual differentiation mediated by gonadal hormones is that variation is guaranteed, since no two animals secrete the same concentrations of hormones or are equally sensitive to their effects. These findings suggest that variation in hormone-mediated characteristics has been selected for in evolution. In the case of rodents, at least, variation in phenotype due to intrauterine position is a normal consequence of multiple-uterine residence.

Positioning of fetuses by sex in the uterus is a random process (11). At birth, 50% of the males and 50% of the females are classified as 1M (next to 1 male fetus), while 0M and 2M males each represent about 25% of the male population and 0M and 2M females each represent about 25% of the female population. The 1M males are intermediate in phenotype between 0M and 2M males, and 1M females are intermediate in phenotype between 0M and 2M females. If a trait is normally distributed within a population, there is a tendency to view individuals that differ significantly from the population mean as being atypical or even abnormal. But, when one examines any of the traits on which animals from known intrauterine positions have been found to differ, a trimodal distribution is observed (e.g., 2M female mice are significantly more aggressive than 1M females that are significantly more aggressive than 0M females). It is, of course, unreasonable to consider an animal from one intrauterine position as normal and animals from other intrauterine positions as abnormal. Instead, the emphasis should be on the biological significance of the evolution of a developmental system that guarantees that variation will occur.

One of the most interesting, and based on current models concerning sex differences in behavior unexpected, of the above findings is that female mice do fight and establish dominance hierarchies when placed together, and prior intrauterine position correlates with aggressiveness in female mice. There is also evidence that the aggressiveness of a female mouse may play an important role in determining her reproductive success (11). It is generally, but erroneously, assumed that female mice, as well as females of most mammalian species, are non-aggressive except during the postpartum period in defense of their young (12). Within the traditional view of aggressiveness as being a masculine trait and submissiveness as being a feminine trait, females that are observed to be highly aggressive would be labeled as having been masculinized. Aggression is elicited in male mice by cues emitted by other intact males, and cues emitted by females serve to inhibit attacks by males (4). 2M female mice are only highly aggressive toward other females, but not toward males. Thus, at least in terms of aggressiveness, it is inappropriate to label 2M females as masculinized, 1M females as partially masculinized, and 0M females as not masculinized, since aggression toward females is obviously a feminine and not a masculine trait in mice.

The implication of this finding for the study of sex differences in human behavior is that the traditional bias that "normal" females are passive has resulted in any female that is labeled as aggressive

being considered as "male-like" in her behavior. Aggression is used to describe a broad range of behaviors, and even young girls that are more physically active than the established "norm" for girls are labeled as tomboys (5). It may be just as inappropriate to refer to an active, assertive or even violent woman as being masculinized and thus abnormal as it is to consider 2M or 0M female mice as abnormal, even though 0M and 2M females both deviate significantly from the population mean (which represents the phenotype of 1M females). Furthermore, it is now quite apparent that the concept that "normal" males are active and aggressive and "normal" females are passive ignores the considerable evidence that exists to the contrary. An interesting example from research on non-human primates is the conclusion that the presenting or sex-soliciting posture exhibited by female monkeys and chimpanzees indicates that the female sex role is one of a passive sexual partner submitting to an active, sex-initiating, dominant male (7). This conclusion is based on the fact that the presenting posture resembles, at least superficially, the posture exhibited by a subordinate animal that is threatened by a more dominant animal. Evidence against such a view had previously been reported. The observation that if a sexually-experienced female chimpanzee presented to an inexperienced male and he failed to immediately mount her, the female would turn and attack him led Birch and Clark (3) to argue that the conclusion that females are normally passive represented "a projection of the Victorian idea of human sexuality into the realm of biological theory". There is now little argument that females play an active, initiating role in copulation in many mammals that have been studied (2).

CONCLUSION

Models concerning the etiology of sexual dimorphisms in behavior in humans have been influenced by research utilizing rodents. The findings from comparisons of both male and female mice from known intrauterine positions have led to a reevaluation of the traditional concept that aggressiveness is a masculine characteristic and that aggressive females should be labeled as "male-like" in their behavior. This label, of course, implies that such a female is abnormal, at least when viewed within the confines of traditionally accepted sex roles. In animal research there is a greater emphasis being placed on the adaptiveness of variation in sex-related characteristics. Perhaps this will result in a greater tolerance for individuals who differ in their behavior from the currently accepted norms of society.

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