Where

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Exposure to testosterone in the uterus has some unexpected effects on females.

By Mertice M. Clark and Bennett G. Galef Jr.

Ten years ago, we noticed some rather odd things about the females in our laboratory colony of Mongolian gerbils. All the animals—sand-colored rodents with jet black eyes, black toenails, and a black tuft of hair at the tip of an elegant tail—were living under apparently identical conditions, but some females were reaching sexual maturity when sixteen days old and still suckling, while others were experiencing their first estrus at twice that age, long after they had been weaned. The females that matured early went on to produce almost twice as many babies as did their late-maturing counterparts (females of both groups lived an average of eleven to twelve months).

Differences between the females also showed up in their behavior. Early-maturing mothers spent less time in the nest with their young and nursed them less often. They also rapidly became pregnant if we replaced their mate of many months with a new male. Late-maturing females were more likely to resist the advances of unfamiliar males—sometimes attacking and severely injuring them—and if they did accept them as mates, they took longer to do so.

Our curiosity was thoroughly piqued when we discovered that early-maturing females gave birth to more daughters than sons and that the opposite was the case with their late-maturing counterparts. Furthermore, to our surprise, the daughters tended to follow in their mother's developmental footsteps, maturing early if she had, and so on.

The divergent reproductive histories in these two groups of gerbils might have been genetically determined, but we suspected that something other than genes was at work. Our records revealed a striking pattern: the greater the proportion of males in a litter of gerbils, the greater the probability that any female in that litter would mature late. This correlation suggested to us that the development of female gerbils might be affected by the prenatal environment, a possibility we decided to pursue further.

If you were to look inside a pregnant gerbil, mouse, or just about any other small rodent, you would find her fetuses lined up rather like peas in a pod, with males and females distributed randomly within each of the two horns of her uterus. We knew from earlier research by Fred vom Saal, at the University of Missouri, and his colleagues that both the appearance and reproductive behavior of a female house mouse could be affected by the sex of her immediate neighbors in utero. Using radioactively labeled testosterone, vom Saal had shown that testosterone secreted by a male fetus in the last week of gestation enters the amniotic fluid and is absorbed by the fetuses on either side of him. Exposure to this hormone is known to have profound masculinizing effects on both males and females. Thus, at a critical period of development, fetuses developing next to males are exposed to more testosterone than are fetuses lying next to females. We suspected that similar things might be happening in the wombs of our gerbils and that they might be the cause of the differences in development and behavior we were seeing.

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Mouse embryos lined up in the uterus, above, each in its own amniotic sac. A huddle of Mongolian gerbils, right.

To find out, we started delivering gerbils by cesarean section—which enabled us to determine who had been lying next to whom in the womb—and we then documented the history of each female from birth. The results were clear: twenty-seven of twenty-eight females that matured late had been either next to a male or between two males; in contrast, twenty-one of twenty-two early-maturing females had developed in the womb without an immediate male neighbor. Further, as vom Saal's work had suggested, both male and female fetuses positioned between males in the womb had higher levels of testosterone.

the Males Are

Our next step was to determine what effect position in the womb, and thus testosterone level, had on male gerbils. We found that male gerbils that had been stuck between sisters grew up to be less attractive to females in estrus. Some of these testosterone-deprived males didn't appear interested in females; others exhibited apparently normal sexual behavior (pursuing females around the cage and attempting to mount them) but with relatively little success. Interestingly, when these males did mate, they proved to be good, helpful fathers—licking the pups, returning them to the nest when they strayed, and huddling over them in the same posture adopted by nursing females. All this attentiveness enabled their mates to produce a second litter sooner than females whose mates had developed in utero between males.

Other investigators have looked for evidence of effects of intrauterine position on the behavior of animals living in more natural circumstances. In one particularly ingenious study, John Vandenbergh and his colleagues at North Carolina State University used the circular grassy "islands" formed by the curving ramps of cloverleaf highway interchanges as giant mouse cages. Vandenbergh caught wild mice, bred them, delivered the babies by C-section, and then released the offspring—once fully mature—into the cloverleaf islands. The exit and entrance ramps enclosing each island acted as barriers, so each leaf of the interchange contained a separate population of mice. As the released mice went about the business of establishing a home range, Vandenbergh found that the females that roamed most widely were those that had developed between males as fetuses. This jibes well with what is known of wild house mice (and many other animals), in which high levels of testosterone are correlated with aggressive behavior and a tendency to hold a large home range or territory.

What effects might this sort of naturally occurring variation in prenatal exposure to testosterone have in our species? So far, studies are suggestive but inconclusive. Recently, for example, Edward Miller, of the University of New Orleans, reviewed a number of studies that compared fraternal twins of the same and opposite sex. In general, the studies found that women who had shared their mother's womb with a twin brother had more characteristics that the researchers labeled "malelike" than did women with a twin sister. Unfortunately, the determination of "malelike" behavior in these studies was based on a selective, sometimes even subjective, set of traits, ranging from a greater likelihood of using mind-altering drugs, of traveling, and of seeking adventure, to a susceptibility to boredom. Few of these studies looked at secondary sexual characteristics known to be indicative of high levels of testosterone (distribution of pubic hair, for example), which might have provided more useful, and certainly less controversial, evidence.

Our own work has recently focused on the connection between a female gerbil's position in the womb and the ratio of sons and daughters in her litters. The complete answer to this puzzle has so far eluded us, but we have made some interesting discoveries along the way. It turns out that male gerbil fetuses are more frequently

found in the right uterine horn than in the left. The reason for this lies in the ovaries, not the horns: eggs produced by the right ovary (even when surgically attached to the left horn of the uterus) are more likely to develop into males than are eggs from a left ovary. We haven't yet figured out why or whether the same process is responsible for the different sex ratios of litters produced by females from different intrauterine positions. Meanwhile, however, another study suggests that the finding may have relevance to other mammalian species, including our own.

Two years ago, Allan Pacey, a zoologist working in the Assisted Conception Unit at the University of Sheffield, in England, told us about some exciting findings in his laboratory. After reading about our discoveries in gerbils, Pacey had examined the medical records of hundreds of human births resulting from artificial insemination. He found that eggs taken from the right human ovary were roughly 30 percent more likely to produce sons than were eggs from the left ovary. Pacey looked at other possible determinants of offspring gender—including the mother's age and the timing of insemination—but none correlated with the sex of the babies.

The notion that the left and right sides of the human body and the gender of babies are somehow related is not new. More than twenty-five hundred years ago, the Greek mathematician Pythagoras asserted that maleness is associated with the right side of the body and femaleness with the left. Hippocrates thought the sex of a child was determined by its position on the left or right side of the womb. Aristotle vacillated, sometimes rejecting such ideas, sometimes accepting them. Early in the twentieth century, the discovery of sex chromosomes seemed to rule out any connection between side of the body and gender, but findings such as ours and Pacey's demand that we take another look. If the connection proves valid, it could potentially give parents-to-be more say in the gender of their babies. The ethical questions raised by such choices are another matter. □

Mother Knows Best

By Hubert Schwabl

Bird embryos develop outside the mother's body, inside a hard-shelled egg. Since hormones cannot travel from one egg to another or from the incubating mother to an egg, it might seem that bird embryos exist in hormonal isolation. As I discovered several years ago, however, a mother bird has a strong and direct hormonal influence on her offspring, delivering steroid hormones, such as testosterone and other androgens, into the yolk of the eggs before laying them. And, I was surprised to find, different eggs within a single clutch may contain different amounts of these maternal hormones. Cattle egret mothers, for example, generally lay clutches of three eggs and place twice as much androgen in the first two eggs as in the third. Canary mothers take the opposite approach: each egg in a clutch of four or five gets a little bit more testosterone than the one before, with the last egg containing three times as much as the first. What, if anything, are these mothers attempting to accomplish through these hormonal adjustments?

After hatching, nestlings of many species compete for food. Often, competition in the first few days is based on which nestling can beg most voraciously and stretch its neck closest to the food. As they grow, bigger nestlings may shove or aggressively peck at their smaller siblings. To the casual observer, the mother often appears indifferent to these squabbles, seldom attempting to make sure the "underbird" gets a fair share of the food. In fact, some of her actions—including the timing of egg laying and incubation and, perhaps, how much hormone she delivers to an egg—may even contribute to the inequality of her nestlings' competitive abilities.

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Competition among cattle egret nestlings can be fierce.

Many birds lay their eggs one or two days apart. If the mother begins to incubate before the last egg is laid, the first nestlings to hatch will have a significant head start developmentally. Cattle egret mothers generally start incubating after laying the first egg. The third nestling may not hatch until two or three days