in the execution step of apoptosis, and TUNEL staining, an indicator of DNA fragmentation, increased within six hours of injury. Furthermore they found that $p75^{NTR}$, the proapoptotic neurotrophin receptor, also increased by 48 h after injury. Estradiol treatment attenuated these markers of apoptosis, strongly suggesting that it exerts its neuroprotective actions by suppressing factors that mediate apoptotic pathways of cell death. These studies complement others that suggest that estradiol acts on multiple steps of the apoptotic pathway to tip the balance in favor of cell survival and to decrease the chances of cell death.

An important aspect of the current study is that it was performed on male gerbils. This confirms the findings of others that estradiol is protective in both sexes [2–4]. The data suggest that if estrogens could be designed so that they did not feminize but exerted protective actions, they would be useful therapies for men as well as women.

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Meeting Report

# Early encounters, lifetime effects: hormones in the intrauterine environment

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**A multidisciplinary meeting convened by the Society for Women's Health Research and entitled 'Sex Begins in the Womb' was held at Crowne Plaza Cabana, Palo Alto, CA, USA on 1 March 2002.**

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Subtle alterations in the prenatal hormonal milieu can have lifetime effects on morphology, physiology and behavior.

## Wombmates: how intrauterine position affects development

The interplay of natural maternal hormone production, endocrine-disrupting chemicals from maternal circulation and/or hormonal transfer from adjacent fetuses of the opposite sex can alter development of the fetus. Developmental effects on the brain might not be detected until puberty or later in adulthood. John G. Vandenbergh (North Carolina State University, Raleigh, NC, USA) presented data on how intrauterine position in litter-bearing animals can affect morphology, physiology and behavior as hormones move across the fetal membranes.

He reported a study of female mice exposed to testosterone from adjacent males. Females were classified according to Frederick vom Saal's (University of Missouri, Columbia, USA) code [1]: developing between two males (2M); with a male on one side (1M); or between two females (0M). These naturally occurring testosterone exposure differentials produce marked anatomical and behavioral variations. Ano-genital distance is longer in 2M females than in 0M females, but shorter than in males. The 2M females are leaner than 0M females, with 6–7% body fat, whereas 0M females have 15–16% body fat. The sexually dimorphic nucleus of the preoptic area of the hypothalamus is larger in 2M females than in 0M females, but smaller than in normal males. Reproductive characteristics are masculinized in 2M females, including later onset of puberty than 0M females and irregular ovarian cycles. When pregnancy does occur, 2M females produce more males (~60% versus 40% in 0M females). The 2M females also exhibit increased mounting of other females, as well as delayed and less robust lordosis, the typical female mating posture. In addition, 2M females are more aggressive, adventurous and more likely to defend food sites.

## Total estrogen exposure matters

Frederick S. vom Saal reminded participants that the world is full of estrogens – natural estrogen, estradiol, estrogenic chemicals in plants that we eat, estrogenic drugs – and all of them can enter the fragile world of the developing fetus. His early work on intrauterine position effects revealed the exquisite sensitivity of the developing fetus to the smallest conceivable change in regulatory hormones. In mice, for example, 2F males experience biologically active estradiol levels of $0.05 \times 10^{-12}$ g, and have enlarged prostates, bigger than those of 0F males. vom Saal focused discussion on the effects of fetal exposure to exogenous estrogens – synthetic chemicals that invade the intrauterine environment and disrupt normal development. Endocrine-disrupting chemicals interact additively with background levels of estradiol, and it is the total estrogenic activity that matters most.

The fetus of a woman who becomes pregnant while using oral contraceptives is exposed to 17α-ethinyl estradiol. Studies in male mice show that ethinyl estradiol at one-fifth the equivalent dosage of oral contraceptives can have the same effects on the reproductive system as does diethylstilbestrol [2]. The prostate is enlarged, the urethra and seminal vesicles are smaller, and daily sperm production declines.

Another endocrine disruptor with estrogenic effects is bisphenol A (BPA), used in producing polycarbonate plastic. BPA is used to line food cans, and is in dental sealants, baby bottles and other products.

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**Key conference outcomes**

- The fetus develops in a complex hormonal environment where natural maternal hormones interact with endocrine-disrupting chemicals from maternal circulation and/or hormonal transfer from adjacent fetuses of the opposite sex.
- Intrauterine position in litter-bearing animals can affect morphology, physiology and behavior because of hormonal transfer through fetal membranes.
- Female mice exposed to testosterone *in utero* exhibit masculinized sexual characteristics, body types and behaviors. Male mice exposed to estrogen undergo feminization.
- Bisphenol A (BPA) is an endocrine disruptor ubiquitous in the environment. BPA has been detected in human umbilical cord blood at levels that, in animals, cause effects similar to those caused by diethylstilbestrol.
- Maternal stress and anxiety during pregnancy can alter cortisol levels in the fetus, leading to behavioral problems in offspring, for which males are at higher risk.
- Girls exposed to high levels of androgens *in utero* exhibit boy-typical behavior directly related to the degree of prenatal androgen excess.

An unstable polymer, BPA leaches into food or infant formula in concentrations that have major effects on mouse fetuses, and BPA is detectable in human umbilical cord blood. BPA does not bind to human sex hormone-binding globulin or α-fetoprotein in rodents, so the bioactive fraction that can move into cells is quite large [3]. BPA has the same effect as estradiol in some tissues, and its effects differ in males and females. Studies in snails, fish, frogs, reptiles, rats and mice have shown that BPA decreases daily sperm production [4]. The key factor is the free fraction of the steroid that is biologically active and hence clinically relevant.

Effects of BPA and other endocrine disruptors are visible only through animal dissection. vom Saal cautioned that the implications of these effects in humans merit attention. His results from animal studies suggest that endocrine disruptors could contribute to emerging trends in human populations, such as increasing incidence of hypospadias and prostate disease, decreasing sperm counts, increasing obesity and early onset of puberty.

### Prenatal stress and sexual differentiation

Animal studies have long shown that maternal stress during pregnancy can alter offspring behavior. Vivette Glover (Imperial College, London, UK) cited early studies in rats relating maternal stress during pregnancy to male offspring with reduced masculine-type behavior, increased feminine-type sexual and parenting behaviors, and reduced size of the sexually dimorphic nucleus of the preoptic area. Female offspring show reduced maternal behavior.

Primate studies show that maternal stress in the pregnant monkey leads to lower attention span, more anxiety and other behavioral problems in the offspring. These effects might be related to fetal/neonatal programming of the hypothalamo–pituitary–adrenal (HPA) axis: the hypothalamus releases corticotropin-releasing hormone, which acts on the pituitary, causing the adrenal to release cortisol. Altering HPA axis programming could increase vulnerability to subsequent behavioral problems. Glover and her colleagues' examination of human maternal and fetal blood samples found a highly significant correlation between maternal and fetal cortisol levels. Testosterone and cortisol levels are closely correlated in both male and female fetuses, which could mean that high cortisol levels lead to masculinization of female fetuses. Glover also reported on a longitudinal study begun in the early 1990s in which maternal anxiety was assessed at 18 and 32 weeks antenatally, and postnatally. Antenatal anxiety at 32 weeks, when corticoid receptors are developing, was shown to be a significant risk factor for behavioral problems in both boys and girls, with sons of anxious mothers having twice the risk for hyperactivity and attention disorders, compared with controls.

### Prenatal androgen exposure and childhood behavior

Girls with congenital adrenal hyperplasia (CAH) and their siblings offer a valuable opportunity to study behavioral effects of prenatal androgen exposure, as Sheri A. Berenbaum (Pennsylvania State University, University Park, PA, USA) reported [5]. CAH is caused by an enzymatic defect in 21-hydroxylase, which exposes females to elevated levels of androgens during gestation. Without prenatal diagnosis and treatment, girls with CAH are born with masculinized genitalia and the question is whether androgen also affects behavior and the brain.

Compared to their sisters, girls with CAH play more with boys' toys (transportation and building toys), have higher spatial ability, and show less interest in infants. Girls with the most severe type of CAH exhibit the most pronounced boy-typical play behavior, regardless of how well the disease was initially controlled. Berenbaum also described research by colleagues in Sweden who found that boy-typical play behavior is directly related to the degree of prenatal androgen excess (inferred from the severity of the genetic mutation), rather than parental response to a child with masculinized genitalia. Berenbaum has launched a prospective study of girls with CAH detected through newborn screening to explore these effects further.

Other presentations highlighted the effects of prenatal hormone exposure on brain architecture and function. A summary of the entire meeting will be available from the conference organizers (http://www.womens-health.org).

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