Endocrine Disruptors and Testis Development

Project Scope

Declines in human and animal male fertility have been observed in recent years by several researchers. Possibly related declines also have been observed in sperm concentrations and related indicators of semen quality. Rates of testicular cancer and congenital abnormalities such as cryptorchidism and hypospadias also appear to be increasing. The declines in semen quality vary regionally, suggesting an association with exposure to endocrine disrupting chemicals (EDCs), which can influence normal sex determination, testis development, and sperm viability. The objective of this grant was to develop a better understanding of how endocrine disruptors affect testis development and function in mammals.

The development of the testis requires the coordination of the growth and differentiation of several cell types. The testis is formed initially from an undifferentiated bipotential gonad. The first sign of male development is the differentiation of precursor Sertoli cells, which then aggregate with germ cells and undergo a transition from mesenchyme to epithelia to form testicular cords. Another characteristic of the development of the testis after the formation of cords is rapid growth compared to the analogous ovarian precursor. The peak of Sertoli cell proliferation occurs just before birth, and cell proliferation in the embryonic and early postnatal periods is crucial for establishing the mature adult size of the testis. In addition, this proliferation is necessary for reproductive function, in order to maintain a sufficient population of sperm-producing Sertoli cells during adulthood.

Preliminary research indicates that two families of paracrine growth factors directly influence testis development and function. Members of the transforming growth factor family (for example, TGF-α) appear to be critical for embryonic testis growth, and at least one neurotropin (neurotropin 3 or NT3) appears to have a critical role in the morphogenesis of testis development. Abnormal testis development and male infertility caused by endocrine disruptors may, in part, be mediated through pathways involving these factors.

Thus, the hypothess initially tested under this grant was that endocrine distruptors affect locally produced paracrine growth factors that are essential for testicular cell growth and differentiation during embryonic and postnatal development. The adverse developmental effects can later manifest themselves as reduced male fertility and sperm production. Experiments investigated the effects of endocrine disruptors on TGFs and neurotropins during testis development effects on male fertility. Environmental agents with estrogenic activity (i.e., methoxychlor [MXC], a general use organochlorine pesticide) and antiandrogenic activity (i.e., vinclozoline, a selective contact fungicide) were assessed using a rat embryonic testis organ culture system and a rat *in vivo* model.

Grant Title and Principal Investigator

Endocrine Disruptors and Testis Development

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Key Findings and Implications

- An embryonic testis organ culture system was developed to assess the effects of endocrine disruptors on organ morphogenesis and tissue function. Methods also were developed to assess transforming growth factor (TGF) and neurotropin (NT) gene expression and gene product levels.
- Endocrine disruptors (e.g., methoxychlor [MXC] and vinclozolin) were found to alter early embryonic testis development when maternal exposures occurred at the time of fetal sex determination and testis morpho-genesis.
- *In vivo* exposure of gestating rats to MXC demonstrated a heritable reduction in sperm function and increased spermatogenic cell apoptosis affecting males in generations F1 through F4. Studies show that this heritable effect may be associated with alteration of the DNA methylation patterns in male germ cell precursors.

Publications include ten peer reviewed articles.

Project Period: August 1999 to July 2002

Project Results and Implications

Treatment of gestating mothers had no major effect on fetal sex determination or gross testis histology at any time during development. However, animals exposed *in utero* between embryonic day 8 (E8) and E15 experienced pubertal and adult spermatogenic defects in the F1 generation. Treated animals also had an increase in spermatogenic cell apoptosis (programmed cell death). Similar results were observed at postnatal day 20 (P20) or P60 for MCX and vinclozoline. In addition to this decreased spermatogenic cell survival, sperm motility was impaired and morphology was abnormal. Interestingly, animals exposed *in utero* to the same dose of endocrine disruptor later in development (E25-E20) had no spermatogenic cell defects. This finding is consistent with the occurrence of critical processes such as germ cell DNA remethylation, cord formation, and sex determination during the E10-E15 period, which are complete after E15.

These studies has provided insight into the mechanisms by which endocrine disruptors influence testis growth and function. One key observation was the transgenerational effects of a transient *in utero* exposure to the endocrine disruptors MXC and vinclozolin on testis function and gametogenesis. In particular, these studies demonstrated the ability of MXC and vinclozolin to promote a transgenerational phenotype in spermatogenic cells

Additional experiments were conducted that demonstrated the effects of exposure of endocrine disruptors on the F1 offspring could be passed to subsequent generations. When the male rats of the F2 generation (derived from the animals discussed above) were examined, the same increased apoptosis rate in spermatogenesc cells was identified as the F1 generation. Specifically, a three- to five-fold increase in apoptosis was observed in F2 rats at maturity. Also, an approximately 20 percent decrease in sperm motility and forward movement also was observed. These experiments show that EDC exposures to a gestating dam at the critical period of fetal sex determination and testis morphogenesis (i.e., cord formation) appears to cause a germline effect, i.e., a decrease in spermatogenic capacity and sperm viability that seems to be heritable in the male. This experiment was repeated with four different gestating mothers, and similar results were observed through the F4 generation. An outcross of an affected treated male with a wild type untreated female slightly reduced the magnitude of the effect, but the basic feature of the phenotype (i.e., increased spermatogenic apoptosis) remained.

Researchers postulated that the transgenerational effect of maternal EDC exposures male spermatogenesis arises through disruption of DNA methylation in the male germline. This hypothesis was investigated by exposing gestating dams to 100 mg/kg MXC from E8-E15, and collecting testes for analysis on P6. DNA was isolated and methylation state determined with methylation-sensitive restriction enzymes and polymerase chain reaction (PCR) methods. A number of PCR products with altered methylation patterns were detected in MXC-treated cells. This observation further supports the proposal that the epigenetic effects of MXC on spermatogenesis are mediated by changes in DNA methylation.

Relevance to ORD's Multi-Year Research Plan

This project contributes to all three of ORD's Multi-Year Plan long-term goals (LTGs 1-3), in particular (1) providing a better understanding of the science underlying the effects and assessment of endocrine disruptors, (2) determining the extent of the impact of endocrine disruptors on humans and wildlife, and (3) supporting EPA's screening and testing program. More specifically, the results address the effects of early developmental exposure to endocrine disrupting pesticides on reproductive function in adults, toxicant-induced alterations in mammalian reproductive development, dose response relationships, critical periods of exposure, *in vivo* tissue levels of the active toxicant, *in vivo* and *in vitro* mechanisms of action, androgenic and anti-androgenic activity, the effects of endocrine disruptors on growth factors and receptors that control spermatogenesis and growth and development of the testis, and *in vitro* and *in vivo* assessment methods.

Investigators

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For More Information

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NCER Project Abstract and Reports:
http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/992/report/0