NIEHS Meeting:
Women’s Reproductive Environmental Health Consortium
January 20, 2012

Directory of Researchers
Women’s Reproductive Environmental Health Consortium  
NIEHS Meeting: January 20, 2012  
Agenda

8:00 a.m. Arrive NIEHS, Room: Rodbell ABC
8:15-8:30 Welcome and Goals: Gwen Collman, Jerry Heindel, Karin Russ
8:30-9:50 Research Summary Presentations I- Epigenetics
   1. Kevin Osteen- Epigenetic biomarkers
   2. Kaylon Bruner Tran- Placental, ovarian and sperm quality, preterm birth
   3. David Crews- Gene expression, timing of puberty, neurobiological changes
   4. Andrea Gore- Sexually dimorphic effects of EDCs on brain and behavior
   5. Alexander Suvorov, Nicholas Lodato/David Waxman’s lab- Genome-wide transcriptional profiling
   6. Nina Holland- Candidate genes and pathways for obesity and pubertal timing
9:50-10:10 Coffee break
10:10-11:00 Scientific Session A: Cheryl Walker-New Advances in Epigenetic Methods
11:00-12:10 Research Summary Presentations II
   7. Lou Guillette- Sexually dimorphic epigenetic signatures
   8. Satomi Kohno- Xenoestrogens and sex determination
   9. Ana Soto- Reproductive system, neuroendocrine, and mammary gland
  10. Shuk Mei Ho- Tumorigenesis in the ovary, endometrium and breast
12:10-1:10 Lunch at NIEHS cafeteria
1:15-2:05 Scientific Session B: John McLachlan- Developmental Estrogenization Syndrome
2:05-3:20 Research Summary Presentations III- Fertility & Fetal outcomes
   11. Shanna Swan- TDS, PCOS, neurodevelopment, growth and obesity
   12. Michael Bloom- Oocyte quality, embryo quality in ART patients
   13. Vasantha Padmanabhan- Neuroendocrine development, birth weight
   14. Hugh Taylor- IVF, DES exposure, PCOS, pregnancy loss
   15. Carmen Williams- NIEHS Reproductive Medicine Group
3:20-3:40 Break
3:40-4:30 Developing Collaborations- Research Opportunities:
   Discussion Leaders: Jerry Heindel, Kim Gray, Caroline Dilworth
4:30-5:00 Goals of the Consortium- Getting the Message Out: Karin Russ
Research Summaries of Presenters

Session I – Epigenetics

Speaker 1

Kevin G. Osteen, PhD
Vanderbilt University Medical Center
Women’s Reproductive Health Research Center

Overview: Progesterone exposure is a negative risk factor for the development of endometriosis in humans due, in part, to its anti-inflammatory nature; however, women with endometriosis often exhibit reduced endometrial responsiveness to progesterone. TCDD (or dioxin), disrupts the “protective” actions of progesterone. Early life TCDD exposure of mice leads to a uterine phenotype which mimics the progesterone resistant endometrial phenotype observed in women with endometriosis-related infertility. Significantly, our toxicant-induced murine phenotype is also associated with an increased sensitivity to inflammatory signals, which disrupts fertility across multiple generations.

Current project: Our primary goal is to identify the TCDD-mediated cellular and molecular modifications in somatic and immune cells which negatively impact progesterone action related to reproductive tract function. Using short-term cultures of human endometrial cells, we have identified a loss of stromal cell progesterone receptor-B (PR-B) mRNA and protein expression following treatment with TCDD; a change that increases the negative impact of pro-inflammatory signals on differentiation.

Models: Primary cultures of human endometrial cells acquired from women with and without endometriosis. Transgenerational model of in utero TCDD exposure in C57bl/6 mice.

Toxicant: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin)

Methods: In vivo and in vitro functional outcomes (fertility, decidualization capacity) following TCDD exposure, including the use of isolated human endometrial cells, chimeric models of experimental endometriosis and transgenerational observations following early life TCDD exposure.

Endpoints and results: Biomarker analysis of cellular and molecular responses to progesterone in the presence or absence of TCDD exposure (uterine/endometrial expression of relevant genes and proteins, including epigenetic marks and with or without epigenetic modification therapy); Histological and immunohistochemical examination of reproductive and non-reproductive tissues generated from our murine model of early life TCDD exposure.
Selected publications:


Nayyar T, Bruner-Tran KL, Piestrzeniewicz-Ulanska D, Osteen KG. Developmental exposure of mice to TCDD elicits a similar uterine phenotype in adult animals as observed in women with endometriosis. Reprod Toxicol. 2007 Apr-May;23(3):326-36. Epub 2006 Sep 30.


Speaker 2

Kaylon L. Bruner-Tran, PhD

Vanderbilt University Medical Center, Women’s Reproductive Health Research Center

Overview: Our laboratory examines the mechanisms by which early life TCDD exposure leads to the development of reproductive disorders, particularly those which impact pregnancy establishment and maintenance. Concomitant with these studies is the examination of nutritional intervention strategies which may reduce the negative consequences of a previous (or ancestral) TCDD exposure on adult reproductive tract function.

Current project: A major, current project within the laboratory is to examine the ability of preconception fish oil supplementation of male or female mice to prevent the transgenerational impact of TCDD exposure. Developmental TCDD exposure of either male or female mice leads to an increased risk of infertility or, in the event of pregnancy, an increased risk of preterm birth in the adult (F1) animal. Significantly, a similar increase in infertility and risk of preterm birth was observed in subsequent generations (F2-F4), even in the absence of an additional TCDD exposure. We recently published data demonstrating the ability of preconception fish oil supplementation of toxicant-exposed male mice to prevent preterm birth in his unexposed female partner. We now have similar data demonstrating that preconception treatment of female mice also prevents the developmental TCDD exposure-associated increase in preterm birth. Current studies are examining reproductive outcomes of the F2-F3 generations of mice with ancestral exposure to TCDD in the presence and absence of fish oil supplementation of only the F1 animals. These studies will determine the ability and potential mechanisms by which nutrition influences the transgenerational impact of developmental TCDD exposure on reproductive tract function.
Model: In utero TCDD exposure of C57bl/6 mice

Toxicant: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD or dioxin)

Methods: Functional outcomes (observe pregnancy/preterm birth); euthanasia during pregnancy for examination of placental/decidual expression of relevant genes and proteins, histologic examination of tissues/protein expression, sperm morphology/number, ovarian histology.

Endpoints and results: Preterm birth in mice able to become pregnant was associated with premature placental inflammation, reduced progesterone response and an increased sensitivity to inflammation regardless of which parent had been exposed to TCDD. Infertility in F1 males was associated with reduced sperm density and altered sperm morphology while infertility in F1 females appears to be multifactorial. Fish oil supplementation of F1 males improved sperm number and morphology which was associated with a significant improvement in fertility and full-term pregnancy in an unexposed female. Preconception fish oil supplementation of F1 females led to a non-significant improvement in fertility and was associated with full-term pregnancy in mice becoming pregnant. Studies examining the F2 and F3 offspring of fish oil supplemented F1 mice are underway.

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Selected publications:


Speaker 3

David Crews, PhD

University of Texas at Austin

Overview: Environmental epigenetics; interaction of transgenerational and stress-induced epigenetic modifications and their effects on neuroendocrine systems controlling physiology, behavior, and neurobiology.

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Current project: Epigenetic Transgenerational Inheritance of Stress Response-Sex Differences
**Model:** Rat – Epigenetically modified rats.

**Toxicant:** Vinclozolin.

We study how ancestral exposure to an endocrine disrupting compound causes epigenetic reprogramming that changes how descendant individuals respond to life challenges. A two ‘hit’ paradigm is used. The first ‘hit’ consists of embryonic exposure to Vinclozolin. This has been to create a permanent epigenetic imprint that is permanently incorporated into the germline and, hence is manifest each generation in the absence of the original causative agent. The second ‘hit’, 3 generations removed from the first, consists of chronic restraint stress (CRS) during adolescence. Stress in adolescence has powerful and permanent effects on brain and behavior, including epigenetic modifications to the nervous system. The behavioral tasks during adulthood of the F3 descendants were selected because they manifest sexually dimorphic responses. We have demonstrated the feasibility and validity of such studies, revealing substantial interactive effects on brain and behavior. For example, studies indicate the epigenetic modifications induced by ancestral exposure to Vinclozolin alter how descendant males respond to CRS at the level of physiology, behavior, brain metabolic activity, target gene expression, and genome networks, thereby changing the stress response fundamentally. Taken together our data demonstrate that environmentally-induced epigenetic transgenerational inheritance alters brain development and genome activity to modify stress-induced behavioral responses in a sex-specific manner.

**Endpoints and results:** Measurements are made at the physiological (i.e. endocrine physiology), behavioral (social behavior, learning and memory, and anxiety), and neurobiological (metabolism and gene regulation in identified brain areas) levels, combined with modern systems biology (brain genomics and gene networks).

**Selected publications:**


Overview: Environmental endocrine disruption of brain and behavior – especially neuroendocrine systems controlling reproductive physiology and behavior. We are also completing a transgenerational study looking at effects of prenatal PCB exposures on F1-F3 generations.

Current project: Sexually Dimorphic Effects of Endocrine Disruptors on Brain and Behavior.

Model: Rat - prenatal exposure to PCBs.

Toxicant: PCBs (Aroclor 1221), estradiol benzoate.

Methods: Fetal rats are exposed to a low dose of PCBs or estrogen during gestation, days 16 and 18 (during the last trimester). After these exposures, offspring (F1 rats) are left untreated. F1 males and females are allowed to develop, are behaviorally characterized, then euthanized for gene expression and protein immunohistochemistry. In the transgenerational study, exposures limited to the F0 dams, and no further exposures to F1-F3 animals. In these studies we are searching for molecular changes to the nervous system, including gene expression and epigenetic modifications (DNA methylation).

Endpoints and results: We have accumulated evidence for effects of prenatal PCB (and other endocrine disruptor) exposures on the developing hypothalamus. One day after birth, hypothalamic gene expression is already altered. Puberty is accelerated in females, and delayed in males. In young adults, gene and protein expression of key neurotransmitters, receptors, and other neuroendocrine molecules is significantly changed. Concomitant with these neurobiological changes, reproductive physiology (estrous cycles) and behavior (mating) is disrupted. Reproductive aging is also accelerated, together with changes in the molecular biology (DNA methylation and gene expression) of the hypothalamus. Ongoing work is looking at potential transgenerational effects of endocrine disruptors, as well as more detailed behavioral analyses of changes caused by prenatal exposures.

Selected publications:


Speaker session 5

**David J. Waxman**
Professor of Cell and Molecular Biology
Professor of Medicine
Boston University
Department of Biology

**Alexander Suvorov**
Postdoctoral Research Associate in molecular toxicology

**Nicholas Lodato**
PhD student in cell and molecular biology

Current project: Gene Expression and Histone Modifications in Mouse Uterus in Response to Prenatal Exposure to BPA.

Model: CD-1 mouse

Toxicant: Bisphenol A (BPA), Diethylstilbestrol (DES)

Methods: Pregnant CD-1 mice were exposed to 5, 50 or 500 µg/kg/day BPA or 5 µg/kg/day DES by oral administration on days E9 through E18, inclusive. Uterine tissue was harvested from adult mice at estrus or proestrus and used for gene expression analysis (microarrays and qPCR) and histone modification analysis (ChIP-PCR and ChIP-seq).

Endpoint: Genome-wide transcriptional profiling, chromatin marks/histone modifications
Selected publications:


Speaker 6

![Image of Nina T. Holland, PhD]

**Nina T. Holland, PhD**  
Director of Children’s Environmental Health Laboratory and SPH Biorepository  
CERCH, School of Public Health  
University of California, Berkeley

**Overview:** Molecular Epidemiology of Children’s Environmental Health, Reproductive Toxicology, Functional Genomics (PON) and Epigenetics

**Current project:** Epigenetic Effects of Prenatal Exposures to Pesticides and Other Pollutants on Puberty.

**Model:** Agricultural cohort Mexican-American mothers and children from CA (CHAMACOS)

**Toxicant:** Organochlorines, OPs, PBDEs and other pollutants, assessed during pregnancy and childhood development

**Methods:** Pyrosequencing (Alu and LINE-1) and Illumina Infinium 450K methylation BeadChip

**Endpoints and results:** Global and site-specific DNA methylation was assessed in 254 newborn- and 9-year-old CHAMACOS children. We found that global DNA methylation increased with age and differ by sex but the measures were not correlated across the three assays. 15.5% of all investigated CpG sites, representing >15,000 genes, were differentially methylated between children at birth and 9 years of age. More than 2% of CpG sites investigated in >1,900 genes showed significant differences in methylation by sex. Candidate genes and pathways involved in age and sex differentiation, and in response to early life exposures, have been identified for future analyses of their effects on obesity and puberty.
Selected publications:


Overview: We are studying the mechanisms by which EDCs engage the cell’s epigenetic machinery to induce developmental reprogramming of the epigenome to increase cancer risk. Our focus is on non-genomic signaling of nuclear hormone receptors (i.e. ER), signaling pathways that regulate epigenetic programming (i.e. PI3K and MAPK) and the “readers, writers and erasers” of the epigenetic code that are the target of these pathways (i.e. histone methyltransferases).

Current project: We are studying how developmental reprogramming by EDCs increase risk of developing uterine and prostate cancer.

Model: Rat and mouse models, human cell lines.

Toxicant: Primarily xenoestrogens including DES, genistein and BPA.

Methods: Animal models (immunohistochemistry), epigenetics (histone and DNA methylation), cell signaling (westerns, IP) and protein-protein interactions (protein domain microarrays).

Endpoints and results: We have developed the following model for how EDCs disrupt the cell’s epigenetic machinery to induce developmental reprogramming.
Selected Publications:


Overview: Environmental influences (e.g., seasonal variation, contaminants, climate change) on the evolution and development of the reproductive system in vertebrates, including sex determination of the ovary, development and evolution of the female reproductive tract and development of external genitalia in males and females. We use multiple sentinel species (e.g., fish, amphibians and reptiles) as well as humans.

Current Projects: 1) Ongoing studies of contaminant (e.g., pesticides, PCBs and metals) effects on the development of the endocrine and reproductive systems of wildlife such as the American alligator, with special focus on altered gene expression. 2) Newly initiated studies examine epigenetic markers. An ongoing study of genital development assessed with ultrasound measurements during human pregnancy, as it relates to urinary and plasma/serum contaminant concentrations (e.g., BPA, phthalates, OCs, metals). In data collection phase, with current pregnancy and babies being born – males and females are receiving well baby checkup and assessment using previously validated procedures (e.g., those used by Swan et al.). 3) Initiated study examining ovarian follicular fluid (contaminant profiles with lipidomics / proteomics) and granulosa/thecal cells (transcriptomics and proteomics, QPCR) obtained during oocyte retrieval in ART patients. Data is then matched with pregnancy establishment and outcomes followed by well baby checkups. 4) IPS cell and primary cell studies using wildlife (e.g., pig, whale and alligator), mouse and human model systems to understand the influence of contaminants on the fate of cell differentiation.

Models: Sentinel species (varied including alligators, dolphins) and human – in ovo or in utero environmental exposures.

Toxicant: varied from agricultural (pesticides & fertilizers), industrial (PCBs, metals), dietary (BPA & pesticides) and personal care product (phthalates).

Methods: Both descriptive sampling (during natural pregnancy in humans and wildlife) or experimental treatments. For human samples, collection at multiple points in pregnancy and post partum period. In wildlife, treatment during various critical windows of development and harvest of samples either during embryonic development or either during neonatal or juvenile phases. Samples examined by genomic and functional genetics (QPCR, NexGen sequencing) as well as advanced analytical chemistry (GC or LC/MS/MS; AA; ICP).

Endpoints and Results: Human studies are ongoing but the wildlife studies have documented long term developmental abnormalities at the physiological, morphological and genetic levels. Current studies with the American alligator have shown that the genetic markers displayed by ovaries exposed to contaminant mixtures from agricultural sites are similar to those reported in women experiencing PCOS.
and premature ovarian failure. Key genes such as follistatin, aromatase and ESR1 expression are altered.

Selected Recent Publications:


Speaker 8

Medical University of South Carolina (MUSC)

**Overview:** To understand the endocrine alterations, the mechanisms need to be investigated. American alligator, Alligator mississippiensis, is one of the top predators in the wild, and environmental contaminants can concentrate in their body by bioaccumulation. Crocodilians including alligator use the unique sex determination system called temperature-dependent sex determination. This sex determination is sensitive to estrogenic compounds, and it can be overwritten by them. Therefore, American alligator is a unique model animal to investigate the influences of environmental contaminants.
Current project: Sexual plasticity and developmental exposure to endocrine active compounds in American alligators.

Model: American alligators.

Toxicant: Estrogenic compounds, actual contaminants in Lake Apopka (e.g., DDTs & PCBs).

Methods: American alligator eggs were exposed to an estrogen receptor-alpha or -beta specific agonist, or the eggs were collected at Lake Apopka and incubated at the laboratory. Gonadal tissues were analyzed by histology and Q-PCR.

Endpoint: Morphological characteristics and mRNA expression pattern of gonads were used to see their sexual plasticity. Estrogen receptor-alpha specific agonist induced sex-reversal under male-producing condition, whereas ERβ agonist-treatment or eggs from Apopka did not. However, Apopka eggs produced more females than males under the intermediate condition.

Selected publications:

M. R. Milnes et al., Increased posthatching mortality and loss of sexually dimorphic gene expression in alligators (Alligator mississippiensis) from a contaminated environment. Biol Reprod 78, 932 (MAY, 2008).


B. C. Moore et al., Influences of sex, incubation temperature, and environmental quality on gonadal estrogen and androgen receptor messenger RNA expression in juvenile American alligators (Alligator mississippiensis). Biol Reprod 82, 194 (Jan, 2010).

Overview: Our research has centered on a) the control of cell proliferation by sex steroids, b) the fetal origins of adult disease, particularly the role of endocrine disruptors on carcinogenesis, reproductive and behavioral disorders, c) the role of stroma/epithelial interactions on organogenesis and carcinogenesis and d) the role of biomechanics on epithelial organization. We are currently using a systems biology approach to investigate and better understand morphogenesis. In the book The Society of Cells (Taylor and Francis, 1999) we posited that the default state of cells in all organisms is proliferation, and proposed the Tissue Organization Field Theory of Carcinogenesis, in which cancer is viewed as development gone awry. We also work on epistemological issues arising from the study of complex biological phenomena and on theoretical biology.

Regarding endocrine disruption, the laboratory developed assays for detecting estrogenicity and androgenicity (E-SCREEN and A-SCREEN assays) and identified novel xenoestrogens. We are currently studying the mechanisms underlying xenoestrogen-induced alterations of the development of the female reproductive system, the neuroendocrine system and the mammary gland. We have shown that in utero exposure to minute, environmentally relevant quantities of xenoestrogens irreversibly alters the development of the female genital tract and the mammary gland. Our findings indicate that environmentally relevant levels of BPA affect the regulation of the estrous cycle probably at the central level (hypothalamic-hypophyseal-gonadal axis), induce early cessation of ovarian cycles, alter the development and histoarchitecture of the mammary gland, and induce preneoplastic and neoplastic lesions in the mammary gland. We are currently exploring the mechanisms underlying these effects.

Current project: Perinatal Effects of BPA on Female Reproduction and Mammary Neoplasia.

Models: Wistar Furth Rat, Sprague Dawley Rat, CD-1 mouse and C57Bl6 mouse

Toxicant: Bisphenol A

Methods: In utero exposure of rodents to Bisphenol A via osmotic pumps, morphometric analysis of tissues and organs, transcriptome and methylome analysis, metabolomic analysis, several microscopic
modes (confocal, second harmonic generation, immunohistochemistry), 2 and 3 D- tissue culture, organ culture, biomechanics.

**Endpoints:** Body weight, mammary gland development (morphometrics, gene expression, protein expression, collagen fiber organization), fertility, fecundity, mammary cancer, alterations of sexually dimorphic brain structures, epigenetic markers, metabolomic markers of exposure.

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**Selected publications:**


Overview:
Dr. Ho’s research focus on (1) hormonal carcinogenesis of the prostate, breast, ovaries and endometrium; (2) the developmental bases of disease susceptibility by applying epigenetics to endocrine-related cancers and other complex diseases such as asthma, and (3) the development and validation of molecular biomarkers for the measurement of toxic exposure and various disease states in clinical and epidemiological studies. She uses a host of “omics” discovery platforms to address two of the important challenges in environmental exposure and human health – interactions among multiple exposures with various developmental stages, and the trans-generational effects of exposure.

Current project: The developmental effects of in utero exposure to bisphenol A with high fat diet in mammary cancer risk in later-life.

Model: Rat
Toxicant: Bisphenol A, high fat (life style modifier).
Methods: Prenatal exposure, DMBA-induced mammary carcinogenesis, disruption of female reproductive function, identification of epigenetic and transcriptional disruption.
Endpoints and results: Dams reproductive capacity, pre-pubertal mammary gland development, susceptibility to DMBA-induced mammary carcinogenesis.

Selected publications:


Scientific Session B: Developmental Estrogenization Syndrome

**Overview:** McLachlan studies the estrogenic chemical induction of differentiation defects in the reproductive system. Cell signaling and transcriptional regulation with an emphasis on epigenetic change underlie the work. McLachlan worked at NIEHS/NIH for twenty years, before moving to Tulane University in 1995 as the Weatherhead Distinguished Chair of Environmental Studies.

**Current project:** Understanding induction of differentiation defects through non-coding RNAs.

**Model:** Cell culture with human reproductive tract and breast cells.

**Toxicant:** EDCs with emphasis on BPA, DDT, genestein, DES.

**Methods:** Cellular, molecular and functional.

**Endpoint:** Understand the initial steps in EDC induced misprogramming.

**Selected publications:**


Overview: Early life exposures and sexually dimorphic reproductive tract development. Exposures of particular interest: phthalates, BPA, stress. Outcomes of particular interest: testicular dysgenesis (including anogenital distance), play behavior and other neurodevelopmental outcomes, growth (growth rate and obesity); risk factors for impaired fertility and semen quality, PCOS.

Current projects: The Study for Future Families (SFF) (children now 5-10 years); The Infant Development and the Environment Study (TIDES) currently enrolling; children now being born; Rochester Young Men’s Study, Spanish Young Men and Women Studies.

Model: Human prenatal, infant, early childhood (multicenter pregnancy cohort, prospective); population-based samples of adult men and women.

Toxicant: Phthalates, BPA, pesticides, stress.

Methods: For pregnancy cohort studies; Urine and blood in collected in pregnancy, assayed for chemicals and hormones, multiple questionnaires, birth exams, follow-up for later developmental endpoints; Recruitment of fertile and healthy men and women for genital measures, semen quality and PCOS; recruitment of testicular cancer cases and controls.

Endpoints: Anogenital distance, testes volume and other genital measures, anthropometry, play behavior, gender identity, games and activities and food preference, asthma and allergy-related symptoms, CBCL, BRIEFS; semen quality, PCOS, testicular cancer.

Selected publications:


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Speaker 12

Michael S. Bloom, PhD

Department of Environmental Health Sciences
Department of Epidemiology and Biostatistics
School of Public Health, University at Albany
State University of New York

Overview: Our group is interested in the effects of long-term, ‘background’ exposures to widespread environmental pollutants on in vitro fertilization (IVF); including organic compounds and elements.

Current project: The Study of Metals and Assisted Reproductive Technologies (SMART).

Model: A cohort of IVF patients and their male partners receiving infertility treatment at the University of California at San Francisco Center for Reproductive Health.

Toxicants: Bisphenol A (BPA), mercury (Hg0), cadmium (Cd), lead (Pb) and other elements.
Methods: Prospective epidemiologic study of 58 couples completing a 1st cycle of IVF at UCSF with collection of bio-specimens from women and men on the day of oocyte retrieval (blood, urine, follicular fluid, seminal fluid).

Endpoints and results: Inverse association between female serum unconjugated BPA and estrogen synthesis in response to controlled ovarian stimulation; inverse association between female serum unconjugated BPA and oocyte maturity following hCG ‘trigger’ in Asian women; inverse association between female and Asian male serum unconjugated BPA and oocyte fertilization; association between male BPA and embryo quality indicators; inverse association between female blood Pb and oocyte maturity; inverse association between male urine Cd and oocyte fertilization; association between female blood Pb and embryo quality indicators; association between male blood Hg and Pb and embryo quality indicators; inverse association between female blood Hg and embryo implantation. Associations for female blood Hg and Pb, and serum unconjugated BPA with DNA methylation at GSTM1/5, COL1A2 and TSP50, respectively.

Selected publications:


Overview: Our research focus is on the effects of BPA on developmental programming.

Current projects:

NIH ES016541 Bisphenol A and reproductive dysfunction
Hypothesis: prenatal exposure to BPA at levels similar to what human fetuses are exposed to, will disrupt adult reproductive function by disrupting the mechanisms controlling postnatal neuroendocrine feedback controls of LH secretion and ovarian sensitivity to gonadotropins. Further, postnatal adiposity would exacerbate severity of reproductive disruptions in prenatal T-treated sheep. In parallel, insulin sensitivity is being monitored.

NIH ES016541 Bisphenol A and reproductive dysfunction (ARRA supplement)
Hypothesis: prenatal exposure to BPA at levels found in humans disrupts free fatty acid (FFA) balance and induces oxidative stress at the systemic and adipose tissue level. Four species (mouse, rat, sheep and human) are used to assess the impact of prenatal BPA exposure on oxidative-stress marker and FFA profiles. Mouse samples are provided by Dana Dolinoy and rat samples by Heather Patisaul.

NIH ES17005 Endocrine disruptors and fetal development
The goal of this is to determine if poor pregnancy outcomes and low birth weight offspring in humans are correlated with increased exposure to BPA and consequent epigenetic modifications. Maternal and cord blood samples are being collected during first trimester at the time of birth.

Model: NIH ES016541: Sheep
NIH ES016541 (ARRA supplement): Sheep, rat, mouse and human
NIH ES17005: human
Toxicant: Bisphenol A

Methods:
NIH ES016541: Exposure of pregnant sheep to bisphenol A from days 30-90 of gestation.
NIH ES016541 (ARRA supplement): Measures of FFA and oxidative markers in blood samples from prenatal BPA-treated female fetuses of mouse, rat and sheep, plus cord samples from subjects with high and low maternal BPA levels.
NIH ES17005: Collect first trimester maternal and term maternal and cord samples for measures of BPA and epigenetic modifications (cord only)

Endpoints:

NIH ES016541: Cycle disruption, neuroendocrine feedback, ovarian disruptions and insulin resistance

NIH ES016541 (ARRA supplement): Measures of FFA and oxidative stress.

NIH ES17005: BPA measures, epigenetics and birth weight.

Selected publications:


Speaker 14

Hugh Taylor, MD
Section Chief, Reproductive Endocrinology and Infertility; Director, Yale Center for Endometrium and Endometriosis; Director, Yale Center for Reproductive Biology

Overview: Homeobox genes, embryo implantation, endometriosis, stem cells, menopause, uterine development, endocrine disruption, developmental programming.

Current project: Effect of DES/BPA on HOX Gene Expression

Model: Mouse.
Toxicant: DES, BPA.
Methods: ChIP analysis.

**Endpoint:** Alteration of the HOX code, *In utero* alterations in uterine HOXA10 Methylation induced by BPA, Reporter activity driven by the methylated and unmethylated ERE.

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**Selected publications:**


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Speaker 15

![Carmen J. Williams, PhD, MD](image)

**Overview:** We use a mouse model to study the effects on female reproductive health of neonatal exposure to the phytoestrogen genistein or to DES. Female mice treated neonatally with genistein are completely infertile even after superovulation. Defects in both the oviduct and uterus contribute to this phenotype. We are currently examining how genistein or DES exposure leads to these defects in the female reproductive tract environment. Overall, this project has direct relevance to understanding how endocrine disrupting chemicals or other environmental factors can impact early reproductive events and potentially lead to reproductive failure in women.

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**Current project:** Effects of environmental chemical exposures on early reproduction.

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**Model:** Mouse.

**Toxicant:** Genistein and DES.

**Methods:** Immunohistochemistry, microarrays, qPCR, ChIP.

**Endpoint:** Alterations in gene expression or chromatin modifications.
Selected publications:


Overview: Research on female fecundability, early pregnancy, reproductive hormones, menopause, and uterine fibroids.

Current project: Examining menstrual-cycle-specific BPA levels and time to pregnancy, early pregnancy loss, and events of early pregnancy such as time to implantation and corpus luteum rescue.

Model: Human study.

Toxicant: BPA.

Methods: Epidemiology.

Endpoints: Multiple.

Selected publications:


Overview: I am a postdoctoral fellow working with Dr. Ken Korach in the Receptor Biology Group on the Stimulation of Estrogenic Responses. Our research focus has been to study the development of endometriosis-like lesions in an estrogen receptor mediated manner. We use a mouse model of endometriosis to examine both host genotype and lesion genotype on the initiation and progression of disease.

Current project: Project focus will move to the treatment of mice with BPA and access the progression of endometriosis-like disease in this mouse model.

Model: Mouse model of endometriosis.

Toxicant: BPA.

Methods: Mouse model of endometriosis, ELISA, real time-PCR, histology, immunohistochemistry.

Endpoint: The focus will be on the development and growth of endometriosis-like lesions after treatment of environmental toxicants to determine the potential relevance of these toxicants on human disease.

Selected publications:


Barbara Cohn, PhD, MPH, MCP
Director Child Health and Development Studies
Public Health Institute

Overview: Early life predictors of health over the life-span and across generations.

Current project: Multiple endpoints: 50 year follow-up of 20,000 pregnancies, continuing active follow-up of 2nd and 3rd generation females, with male follow-up and 4th generation follow-up planned.

Model: Human, epidemiological studies.

Toxicant: Multiple-environmental chemicals including pops, pharmaceuticals, tobacco, caffeine, alcohol.

Methods: Prospective epidemiological studies.

Endpoints and results: Pregnancy complications and outcome, placenta characteristics, infant and child health, growth and development, neurodevelopment, social development, reproduction (e.g. PCO, pre-eclampsia), semen quality, time to pregnancy, obesity, aging (menopause) cancer, cardiovascular disease and risk factors for these, mental health and mental health symptoms.

Selected publications:


Overview: Dr. Davis’s longstanding research interests are in the discovery of gene-environment interactions that contribute to cancer over the lifespan of an individual. She is internationally recognized for her expertise in female reproductive pathology and oncology having spent a significant portion of her career at the National Institutes of Environmental Health Sciences, NIH, as Head of the Female Reproductive Pathology group and Acting Chief of the Laboratory of Women’s Health. Current research initiatives focus on multigenerational effects on female endocrine systems and the reproductive tract, including toxicity and cancer.

Current project: Effects of Multigenerational Exposure to BPA on Female Endocrine Systems and Reproductive Tract, in collaboration with Ana Soto.

Model: Rat
Toxicant: BPA
Methods: Reproductive assessment, histopathology
Endpoint: Toxicity, cancer

Selected publications:


Overview: Our research focuses on understanding molecular mechanisms of tumor cell proliferation/inhibition, and delineating the role of growth factor receptor signaling and signaling pathway interactions in uterine fibroids. We evaluate the effects of environmental chemicals on tumor cell growth and disease progression. Our lab also studies the molecular mechanisms of toxicity and carcinogenicity of chemicals evaluated by the NTP testing program.

Current project: Molecular pathogenesis of female reproductive tract diseases and the role of environmental chemicals.

Model: Cell Cultures, Tissue samples (human, rats, mice).

Toxicant: Genistein, Fenvalerate

Methods: RNA and protein analyses, FACS, gene transfection, immunoprecipitation, immunohistochemistry/fluorescence, and light/confocal microscopy, histopathology

Endpoint: Identifying and understanding signaling pathways/novel signaling proteins that regulate disease processes and how environmental chemicals interact with these pathways/proteins.

Selected publications:


Overview: My research focuses on the impact of environmental chemicals on development and reproductive function, specifically chemicals that disrupt endocrine signaling. These chemicals include persistent chlorinated compounds, pesticides, phthalates and bisphenol A. My study population consists of couples using assisted reproductive technologies (ART) for infertility. This provides an innovative model for studying embryo-fetal development as it relates to both paternal and maternal exposure to environmental chemicals. ART allows for the identification of the biological mechanisms underlying infertility and early pregnancy loss, including disruption of gametogenesis, oocyte fertilization, and pre- and post-implantation embryonic development.

Current project: BPA, phthalates, fertility and pregnancy outcomes.

Model: Humans (ART).

Toxicant: BPA, phthalates.

Methods: Prospective Pre-conception Cohort Study of couples undergoing ART.

Endpoints and results: Ovarian response to hyperstimulation, ovarian reserve, IVF outcomes, implantation, pregnancy loss. Specific endpoints of interest:

1. Male endpoints: conventional semen parameters, reproductive hormones, and sperm DNA damage.

2. Pregnancy endpoints: failure of pre-implantation development, implantation failure, pre-clinical pregnancy loss, spontaneous miscarriage, stillbirth, and altered fetal growth.
Selected publications:


Sylvia C. Hewitt
Biologist, Receptor Biology Section
Laboratory of Reproductive and Developmental Toxicology
NIEHS Intramural

Current project: Linking Mouse Uterine Estrogenic Transcriptional, Cistromic and Biological Endpoints.

Model: Ovariectomized mouse uterus.
Toxicant: BPA, HPTE.
Methods: In Vivo Microarray, RT-PCR, ChiP seq, ChIP PCR following acute dosing (0-72 hours).
Endpoint: Changes is transcription, ER and Pol2 interaction sequences, cell proliferation, apoptosis.
Selected publications:


Patricia Hunt, PhD
Meyer Distinguished Professor
School of Molecular Biosciences
Washington State University

Overview: Fertility studies in male and female mice, meiotic analyses of human fetal oocytes.

Current project: Meiotic Studies of Chemicals with Estrogenic Activity.

Model: Mouse and human.
Toxicant: Bisphenol A and ethinyl estradiol.
Methods: a) Mouse: Oral or time release pellet implants during fetal and perinatal development.
b) Human: collection of maternal serum, urine and amniotic fluid, fetal ovaries, liver and placenta from intentional terminations of pregnancy.
Endpoints: a) Mouse: fertility studies in males and females, meiotic analyses, and assessment of aneuploidy levels b) Human: meiotic analyses of fetal oocytes

Selected publications:


Matthew P. Longnecker, PhD, MD
Epidemiology Branch, NIEHS Intramural

Overview: Our research program focuses on the health effects of early-life exposure to environmental contaminants. He has ongoing projects to examine the effects of DDT, bisphenol A, and organophosphate pesticides. These projects are being conducted in Africa, Mexico, the Netherlands, and Norway.

Current project: Perfluorinated compounds and preeclampsia

Model: Norwegian Mother and Child Cohort Study
Toxicant: Perfluorooctane sulfate, perfluorooctanoate.
Methods: Observational, biomarker-based measures of exposure, prospective design.
Endpoint: Preeclampsia.

Selected publications:


Hazel Nichols, PhD
Epidemiology Branch, NIEHS Intramural

Brief bio: Hazel Nichols joined the Epidemiology Branch as a Research Fellow in 2011. She completed her PhD in Cancer Epidemiology at Johns Hopkins University in 2011 and a MS in Reproductive Epidemiology at the Harvard School of Public Health in 2003.

Current project: Hormonal factors associated with breast cancer risk & survival in the Sister Study.

Model: Humans
Toxicant: N/A
Methods: Epidemiology
Endpoint: Breast cancer incidence, chronic disease among breast cancer survivors.

Selected publications:


Overview: Our research focuses on elucidating the cellular and genetic mechanisms by which contaminants in the environment affect the reproduction of wildlife and humans. Our lab has previously shown that alligators living in environments contaminated with EDCs are characterized by the alteration of sexually dimorphic gene expression. This perturbation of sexually dimorphism appears to have origins in early development, as eggs removed from contaminated environments and placed in the lab yield juveniles in which these abnormalities still persist. Currently, we are investigating sexually dimorphic epigenetic signatures in alligators to identify pathways affected by early exposure to endocrine disrupting contaminants. The aim of these studies is to provide insights into the mechanisms by which EDCs negatively affect reproduction in a long-lived sentinel species.

Selected publications:


Overview: Brain sexual differentiation, hormone-dependent organization of GnRH signaling pathways, pubertal timing, sexually dimorphic behavior (including reproductive behavior) and estrous cycle quality.

Current project: Impact of BPA on neuroendocrine development and behavior

Model: Rats (Wistar or Long Evans).
Toxicant: BPA and soy phytoestrogens.
Methods: Neuroanatomy, behavioral analysis, ovarian histology, in situ hybridization and immunohistochemistry.

Selected publications:


Overview: I am a research fellow in Dr. Ken Korach Lab at laboratory of reproductive and developmental toxicology. Our research interests are the effects of xenoestrogenic compounds on reproductive function as well as the roles of tissue specific estrogen receptor (ER) α in reproduction.

Current project: The roles of tissue specific ERα (and its ligands) in the female reproductive tract on fertility and preimplantation embryo development.

Model: Tissue specific ERα knockout mouse model.

Toxicant: Estrogen, phytoestrogens.

Methods: IVF, oviduct/uterine embryonic transfer, preimplantation embryo collection.

Endpoint: Fertilization rate and number of preimplantation embryos compared between control and conditional knockout mice.

Selected publications:


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