Environmental Toxicants and Reproductive Health

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Disclosures

• Quest Diagnostics, Academic Associate
• American Society for Reproductive Medicine, President 2012-1013
• World Endometriosis Society 2014, President
• World Endometriosis Research Foundation Board of Directors, 2011-2014.
• March of Dimes Scientific Advisory Board, Member, 2013-2016.
Today

• Review trends in reproductive health disorders.
• Review industrial/pharmaceutical chemical trends.
• Understand the evidence linking select environmental contaminants with reproductive outcomes.
• Learn what health care professionals can do to minimize risk for patients globally.
Human Disease Trends

Over recent decades there has been:

- significant increase in reproductive disorders in some regions of the world, suggesting a strong role for unidentified environmental factors in disease etiology
- increase in endocrine cancers
- increase in chronic diseases
- decrease in human fertility rates
- increasing number of chemicals to which all humans in industrialized areas are exposed

- Obesity
- CVD
- Diabetes
- Metabolic syndrome

Top: Richiardi et al., Cancer Epidem. Biomark. (2004);
Bottom: based on data from http://data.euro.who.int/hfadb/
Courtesy: J. Heindel, NIEHS 2013
Change in Percent of Impaired Fecundity in the U.S. over 20 Years

2002 - 12%
1995 - 10%
1988 - 8%
1982 - 8%

National Survey for Family Growth
National Center for Health Statistics
2002

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>15-24</td>
<td>4.3</td>
<td>4.8</td>
<td>6.1</td>
<td>8.3</td>
<td>+90%</td>
</tr>
<tr>
<td>25-34</td>
<td>10.0</td>
<td>9.6</td>
<td>11.2</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td>35-44</td>
<td>12.1</td>
<td>10.6</td>
<td>12.8</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>8.4</td>
<td>8.4</td>
<td>10.2</td>
<td>11.8</td>
<td>+40%</td>
</tr>
</tbody>
</table>

Swann, Hertz-Picciotto. Family Planning Persp 1999;31:156-157
Schettler. Infertility and related reproductive disorders, 2003 online:
Age of menarche in Europe and the US from 1790 to 1980

Caucasian  10.14 years
Hispanics  10.40 years
African Americans  9.14 years

What has changed over the past 50 years?

Nutrition

Surveillance/Detection bias

Diagnostic criteria

Diagnostic acumen

Genetics?

*Other?
U.S. and Global Chemical Production

Chemical production has increased 23.5-fold between 1947 and 2007

Federal Reserve G.17

- 80,000 chemical substances registered for use in U.S. commerce
- 3,000 chemicals manufactured or imported >1 million lbs/yr
- 700 new industrial chemicals introduced into commerce/yr
Nearly everyone has environmental tobacco smoke, lead, mercury, phthalates, bisphenol A, perfluorinated compounds, perchlorates.
Pathways of Exposure

Chlorinated byproducts, pesticides, microorganisms, inorganic & organic chemicals, radionuclides

pesticides, heavy metals (Hg), persistent organic pollutants (DDT, PCBs)

ozone, particulate matter, lead. hazardous pollutants

household dust, furniture PBDEs, phthalates, formaldehyde

phthalates, Bisphenol A
# Contaminants in Pregnant Women!

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Percent of US population with measurable levels*</th>
<th>Some evidence can disrupt endocrine system?</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phthalates (4 kinds)</td>
<td>80 – 100%</td>
<td>Yes</td>
<td>Flooring, wall covering, medical devices, food wrap, personal care products, lacquers</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>92%</td>
<td>Yes</td>
<td>Polycarbonate plastic, food can lining dental sealant</td>
</tr>
<tr>
<td>Polyfluoroalkyl Chemicals (PFOS) (4 kinds)</td>
<td>91-99%</td>
<td>Yes</td>
<td>Nonstick cookware, stain resistant fabrics, food packaging, dental products</td>
</tr>
<tr>
<td>Parabens (4 kinds)</td>
<td>36-99%</td>
<td>Yes</td>
<td>Personal care products, food</td>
</tr>
<tr>
<td>Benzophenone-3</td>
<td>100%</td>
<td>Yes</td>
<td>Sunscreen, food packaging</td>
</tr>
<tr>
<td>PCBs (many)</td>
<td>100% (with at least one congener)</td>
<td>Yes</td>
<td>Banned in 1977 – persistent through food</td>
</tr>
</tbody>
</table>

Woodruff et al, EHP 2011)
Critical Windows of Susceptibility

Programm ("Barker Hypothesis"): developmental origins of adult health and disease. Process in which a stimulus or insult at a critical/sensitive period in development or perinatal life has permanent effects on structure, physiology, and metabolism and is transgenerational.

Godfrey and Barker 2001

- chemicals
- dose
- duration
- mixtures
Windows of susceptibility in oogenesis

with permission, Pat Hunt
Germ cells migrate to the **fetal ovary** and initiate meiosis.

- **Migration:** ~6 weeks of gestation
- **Mitotic proliferation:** ~6-10 weeks of gestation
- **Meiotic entry:** 8-10 weeks of gestation

**Interfering with these events can reduce the pool of oocytes**

*with permission, Pat Hunt*
Germ cells migrate to the fetal ovary and initiate meiosis

Can chemicals affect early meiotic events?

- The only data comes from bisphenol A (BPA) exposure, a weak estrogen

- BPA affects synopsis and recombination
  - increased oocyte loss
  - problems in creating chromosomally normal eggs

- Susiarjo et al., 2007
Maternal bisphenol A (BPA) exposure disrupts early meiotic events in the fetal ovary

- Susiarjo et al., 2007

Fetal BPA exposure increases the likelihood of chromosomally abnormal eggs and embryos in adult females

A grandmaternal effect

with permission, Pat Hunt
During the second trimester, oocytes become enclosed in follicles.

Exposure to estrogenic substances disrupts the process, leading to the formation of multioocyte follicles

- Chen et al., 2007

- Follicle formation is correlated with oocyte loss due to atresia
- Loss has been estimated to be as high as 80% of the oocyte pool

 Primordial follicles = reproductive reserve

with permission, Pat Hunt
Primordial to Primary Follicles

Reduced capacity to detoxify chemicals

Dioxins
4-vinylcyclohexene diepoxide (VCD)
1,3-butadiene
Polycyclic aromatic hydrocarbons

POI?

- Flaws et al., 1994
- Hoyer et al., 2004, 2009
Late stage follicles are sensitive to estrogenic substances

BPA exposure disrupts chromosome behavior… increasing the likelihood of an aneuploid egg or of an oocyte that cannot complete maturation

BPA decreases *human* granulosa FSH-stimulated production of E$_2$ through PPAR$_\gamma$ signaling pathway. *Kwinkiewicz & Giudice 2009*
Meiotic progression and recombination are affected by Bisphenol-A during in vitro human oocyte development

Fetal ovarian follicles were incubated with BPA (1μM, 3-30μM) or E2 (1nM, 30nM)

Main findings BPA:
• decreased oocyte survival
• increased MLH1 foci (cross-over marker)

Conclusion:
BPA can affect key events in meiotic prophase and oocyte survival in fetal ovary.

MA Brien-Enriquez, P Robles, N Camats-Tarruella, R Garcia-Cruz, I Roig, L Cabero, F Martinez, M Garcia Caldes.
Human Reprod 2011;26:2807-2818
Bisphenol-A and *human* oocyte maturation *in vitro*

As BPA dose increased:
- decrease in % oocytes progressed to MII (P< 0.002)
- increase in % of degenerated oocytes (P< 0.01)
- Increase % oocytes that had undergone spontaneous activation (P< 0.007).

Among MII oocytes, as the BPA dose increased, there were:
- decreased incidence of bipolar spindles (P< 0.0001)
- Decreased incidence of aligned chromosomes (P< 0.02)
Maternal Serum BPA Positively Associated with Miscarriage Risk in Women

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Serum conjugated BPA (ng/ml)</th>
<th>All miscarriage RR(95% CI)</th>
<th>Aneuploid Miscarriage</th>
<th>Euploid Miscarriage RR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=114</td>
<td>n=68</td>
<td>n=46</td>
<td>n=22</td>
</tr>
<tr>
<td>1st</td>
<td>&lt;0.0636</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>2nd</td>
<td>0.0636-0.0873</td>
<td>1.30(0.74-2.25)</td>
<td>1.18(0.57-2.45)</td>
<td>2.11(0.61-7.24)</td>
</tr>
<tr>
<td>3rd</td>
<td>0.0874-0.1650</td>
<td>1.58(0.85-2.63)</td>
<td>16.3(0.86-3.09)</td>
<td>2.50(0.74-8.47)</td>
</tr>
<tr>
<td>4th</td>
<td>&gt;0.1651</td>
<td>1.83*(1.14-2.96)</td>
<td>1.97*(1.08-3.59)</td>
<td>3.33*(1.04-10.71)</td>
</tr>
</tbody>
</table>

*p<0.05.

Women with highest BPA levels had significantly increased risk of miscarriage (euploid and aneuploid), compared to women with the lowest levels.

Environmental tobacco smoke (ETS) affects reproductive health

- reduced fecundity
- decreased ovarian reserve
- decreased success rates in IVF
- earlier menopause (by 1 - 4 years)
- ARH receptor-mediated apoptosis of oocytes (cotinine-induced)
- increased miscarriage rate
- transgenerational

Sharara et al, Fertil Steril 1988
Genuis, Human Repro 2006
Could Epigenetics Be Involved?

- Recently, BPA exposure has been linked to DNA methylation changes, suggesting epigenetic mechanisms relevant to effects of BPA reproductive outcomes.
- Imprinted genes (e.g., IGF2/H19)
  - ~80-100 of them
  - Subjected to differential DNA methylation (DMRs) (Maternal vs. Paternal imprinted/expressed)
  - Regulate fetal, placental and post-natal development
  - Perturbed imprinting → BWS, PW, AS
- DNA methylation across the whole genome (and other modifications)
- Transgenerational
Bisphenol A Exposure Disrupts Genomic Imprinting

Susiarjo M, Sasson I, Mesaros C, Bartolomei MS.

• Investigated effects of BPA exposure on
  – genomic imprinting
  – whole genome DNA methylation.

• Maternal BPA exposure during late stages of oocyte development and early stages of embryo development significantly disrupted imprinted gene expression in embryonic day (E) 9.5 and 12.5 embryos and placentas.

• Affected genes: Snrpn, Ube3a, Igf2, Kcnq1ot1, Cdkn1c, and Ascl2 (mutations and aberrant regulation of these genes are associated with imprinting disorders in humans).

• Exposure outside of the epigenetic reprogramming window did not cause significant imprinting perturbations.

BPA Exposure Reduced Genome-Wide DNA Methylation in the Placenta, Placentomegaly and Small Labyrinth

- Majority of affected genes were expressed abnormally in the placenta with DMRs including the Snrpn imprinting control region (ICR) and Igf2 DMR1.
- Exposure significantly reduced genome-wide methylation levels in the placenta, but not the embryo.
TCDD and JP-8 (jet fuel mixture) exposure of F₀ resulted in abnormalities in the F₃ generation:

- Primordial follicles decreased 30-40%.
- 2 day earlier puberty (=2 years in humans).
- 50-60% lower T levels in males.

Skinner & Colleagues EHP 2012
The Developing Female Reproductive Tract

Interactions between the mesenchyme and epithelium are important for FRT formation and differentiation.

Glatstein and Yeh, 1995
Vulnerability of Developing Uterus to EDCs

- DES causes changes in expression of Wnt 7A, Hoxa10, Hoxa11-genes involved in tissue patterning → altered uterine morphogenesis (Ma et al, 1998; Miller et al 1998; Block et al, 2000).

- *In utero* exposure of mice to DES (Bromer et al 2009) results in:
  - Hypermethylation Hoxa10 promoter
  - Over-expression of DNMT1 and DNMT3B

- DES daughters have abnormal vaginal adenosis (Jeffries et al, 1984)

- Vaginal adenosis was also found in 80% of stillborns and neonates exposed *in utero* to DES in the first trimester (Johnson et al, 1979).
In utero DES exposure alters Hox gene expression in the developing Müllerian system.

Resulting in infertility, ectopic pregnancy, increased miscarriage, vaginal adenosis/cancer.

Endometriosis?

In Utero Exposure to DES Increases a Woman’s Risk of Endometriosis as an Adult

Prospective cohort study
- 116,678 female nurses
- Baseline questionnaire in 1989
- Age range in 1989 = 25 – 42 yo
- Follow-up in 2-year intervals

Prevalence at baseline = 6,203 (5%)

Incidence: 2,941 laparoscopically confirmed cases
- Pain symptoms prompted diagnosis = 77%
- Infertility work-up prompted diagnosis = 23%
- Exposure to DES: 80 % increased risk of endometriosis
- Low birth weight
- Earlier menarche

Missmer et al., Fertil Steril  2004
Might endometriosis be an epigenetic disorder?

Persistent Changes in Endometrium in Adult Women with Endometriosis

- P and cAMP-resistance is transgenerational in hESF cultured 1-4 passages (Giudice et al 2012).
- Altered promoter methylation status and gene expression in eutopic and ectopic lesions:
  - hypomethylation of SF1 (increased aromatase and E_2 biosynthesis in lesions and eutopic endometrium)
  - hypomethylation of ERβ
  - hypermethylation of HoxA10
  - hypermethylation of PRB
  - hypermethylation of E-cadherin
- DNA methylation, HDAC1, HDAC2, miRNAs.
- Up-regulation of mini-chromosome maintenance genes (Burney, Talbi, Giudice, 2007 Endocrinology)
Global DNA Methylation in Endometrium Differs Across the Menstrual Cycle and between Women With and Without Endometriosis

Figure 2. Heat map of statistically significant probes from the Infinium assay with differential DNA methylation in endometrium from normal women (-) in different cycle phases (Panel A) and from women with (+) versus without (-) endometriosis in different cycle phases (Panel B).

Houshdaran et al 2013
Human Studies of Endocrine Disruptors and Endometriosis in Adult Women

14 human studies published to date
- 12 case-control
- 2 retrospective cohort studies
- Dozens of congeners evaluated
- Largely inconsistent findings

Crain et al, 2008

Women with highest quartile of banned and persistent organochlorine pesticides
b-HCH (lindane byproduct) = 70% increased risk of endometriosis; highest levels
of Mirex = 50% increased risk.
2.5 times greater risk of endometriomas with highest blood levels of b-HCH vs
lowest levels.

Upson et al EHP, Nov 2013
Promotion of Endometriosis by Organochlorines (OCs)

Dioxin (TCDD)
pesticides -methoxychlor and DDT
polychlorinated biphenyls

- Evidence is overwhelming in adult laboratory animals that endometriosis can be promoted by many OCs.
- Data linking OC exposure and other EDCs and endometriosis in adult women are equivocal.

Weaknesses of observational epidemiology studies
  - Limited sample sizes
  - Confounding variables.
  - Epidemiologic study designs/cohorts

- Data linking in utero exposure to DES and endometriosis in adult women are consistent with biological plausability of other estrogen-like compounds being a FRT disrupter, given developmental programming.
Well-defined developmental periods of sensitivity when EDC exposure greatly increases the risk for reproductive disorders.

A. Polycystic ovarian syndrome
B. Meiotic disruption during oogenesis
C. Multiocytic follicles
D. Endometriosis
E. Uterine fibroids
F. Duration of lactation
G. Early breast development
F. Premature menarche

Pregnancy Outcomes and Environmental Tobacco Smoke

- A known risk factor for
  - low birth weight
  - preterm birth

California Environmental Protection Agency. 2005. Identification of Environmental Tobacco Smoke as a Toxic Air Contaminant
Particulate Matter and Preterm Birth During Steel Mill Closure, Utah Valley

Geneva Steel, Utah Valley, 1989 ($PM_{10} = 150 \mu g/m^3$)

Parker, Mendola, Woodruff
Epidemiology 2008
Environmental Phthalate Exposure and Preterm Birth

**Design:** prospective observational nested case-control cohort study between 2006-2008.

130 cases of PTB and 352 controls.

3 time points in pregnancy, urine samples analyzed for levels of phthalate metabolites.

**Results:**
- Geometric means of DEHP, MEHP and MECPP, and MBP, were **significantly higher in cases vs. controls**
- In adjusted models, MEHP, MECPP, and $\Sigma$ DEHP metabolites were associated with **significantly increased odds of PTB**.
- When **spontaneous PTB** was examined alone, MEHP, MECPP, $\Sigma$ DEHP, MBP, and mono-(3-carboxypropyl) phthalate metabolite levels were **all associated with significantly elevated odds of PTB**.

Ferguson et al JAMA Pediatr 2013
BPA exposure in Mexico City and risk of PTB: a pilot nested case control study

N=60 Last trimester of pregnancy in Mexico City and the relationship of concentrations of urinary BPA levels to risk of PTB.

Maternal Exposure to Particulate Air Pollution and Term Birth Weight: A Multi-Country Evaluation of Effect and Heterogeneity

Payam Dadvand, Jennifer Parker, Michelle L. Bell, Matteo Bonzini, Michael Brauer, Lyndsey A. Darrow, Ulrike Gehring, Svetlana V. Glinianaia, Nelson Gouveia, Eun-hee Ha, Jong Han Leem, Edith H. van den Hooven, Bin Jalaludin, Bill M. Jesdale, Johanna Lepeule, Rachel Morello-Frosch, Geoffrey G. Morgan, Angela Cecilia Pesatori, Frank H. Pierik, Tanja Pless-Mulloli, David Q. Rich, Sheela Sathyanarayana, Juhee Seo, Remy Slama, Matthew Strickland, Lillian Tamburic, Daniel Wartenberg, Mark J. Nieuwenhuijsen, and Tracey J. Woodruff

> 3 million births in this meta-analysis.

PM$_{2.5}$ and OR BW for 10 $\mu$g/m$^3$ change

<table>
<thead>
<tr>
<th>Center</th>
<th>OR (95% CI)</th>
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<tbody>
<tr>
<td>Atlanta</td>
<td>1.05 (0.92, 1.21)</td>
</tr>
<tr>
<td>California</td>
<td>1.06 (1.03, 1.08)</td>
</tr>
<tr>
<td>Connecticut and Massachusetts</td>
<td>1.45 (1.23, 1.71)</td>
</tr>
<tr>
<td>New Jersey</td>
<td>1.40 (1.08, 1.82)</td>
</tr>
<tr>
<td>PIAMA</td>
<td>0.51 (0.16, 1.56)</td>
</tr>
<tr>
<td>Seattle</td>
<td>0.99 (0.98, 1.01)</td>
</tr>
<tr>
<td>Vancouver</td>
<td>1.63 (1.13, 2.36)</td>
</tr>
<tr>
<td>Overall</td>
<td>1.10 (1.03, 1.18)</td>
</tr>
</tbody>
</table>
Advocacy

What health professionals can do to:

– strengthen professional education in reproductive environmental health

– share what we know with our patients, colleagues
  • disparities in exposures
  • protect our communities locally, globally

– advocate for chemical policy reform

– advocate for scientific research to reverse population risk

– work on a global scale to minimize exposures to ALL populations
Actions Can Reduce Exposure

• 23 children monitored for metabolites before/after organic diet
• Levels of urinary metabolites for chlorpyrifos and malathion reduced to non-detectable
• Again elevated on re-introduction of conventional diet

American Congress of Obstetricians and Gynecologists Survey (ACOG) (N=2514)

78% of obstetricians surveyed feel that they can reduce patient exposure

Yet…..
<25% report they take an environmental health history
What Do Obstetricians Ask About?

100%

- Image 1: A burger and a pregnant woman.

- Why?
  - “Bigger fish to fry”

< 20%

- Image 2: Various ingredients.

- Why?
  - “Won’t know what to say”
  - “Pandora’s Box”
Our Professional Organizations at Work

Chemical Exposures During Pregnancy: Dealing with Potential, but Unproven, Risks to Child Health

Lead Screening During Pregnancy and Lactation

Reproductive Health and the Environment

Joint Committee Opinion ACOG/ASRM
Strength of the Evidence

2008
Woodruff T, Jansen S, Guillette, L, Giudice LC 2008

2009
Diamanti-Kandarakis E et al. 2009

2012
Aäke Bergman, Jerrold J. Heindel, Susan Jobling, Karen A. Kidd and R. Thomas Zoeller
WHO/UNEP 2012

Program on Reproductive Health and the Environment
Thank You!

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