Couple Based Approach for Assessing Environmental Pollutants and Human Reproduction and Development: Recent Findings from the LIFE Study

Germaine M. Buck Louis, Ph.D., M.S.
On behalf of the LIFE Study Team
Division of Epidemiology, Statistics and Prevention Research, Eunice Kennedy Shriver National Institute of Child Health & Human Development
Developmental Origins of Health & Disease

Periconception Life Course Epidemiology

- Blastocyst
- Embryo/Fetus
- Infant
- Child
- Adolescent
- Adult

Life Course Epidemiology

Paternal & Maternal Exposures
Is Human Fecundity Declining?

- Males
  - Declining 2° sex ratios
  - Earlier pubertal onset
  - Declining semen quality
  - Increasing GU malformations
  - Increasing testes cancer

- Females
  - Earlier pubertal onset
  - Earlier onset gynecologic disorders
  - Declining fecundity (>TTP)
  - Declining fertility

TDS (Skakkebaek et al. 2001)  
ODS (Buck Louis et al. 2010)

“Across the developed world, birth rates are plummeting ... social phenomenon, or is our biological fertility also declining? We don’t yet know...”

Nature 2004
Environmental Influences on Reproduction & development Across the Lifespan

- Fecundity (biologic capacity)
  - Couple TTP
  - Pregnancy loss
  - Conception delay & infertility
  - Gestation

- Fertility (live births)
  - Live birth (multiples)
  - Birth size
  - 2° sex ratios
Prospective cohort designs with longitudinal data collection & biospecimens not feasible
  • Hard to recruit & retain women (couples even harder)
  • Too much participant burden

Selection bias
  • Women will have healthier lifestyles
  • Women with fertility problems will be disproportionately represented
  • Women will minimize time already trying

Men will not participate
  • Men will not keep daily diaries
  • Men will not provide necessary semen samples

Home pregnancy kits will miss pregnancies
Conceptual & Methodologic Challenges Underlying Human Reproduction & Development

- Conceptual
  - Series of timed, highly interrelated & conditional outcomes
  - Some outcomes are “hidden”
  - Defining referent & study populations for couples planning pregnancy

- Methodologic – specifying the etiologic model
  - Endogenous & exogenous nature of reproductive factors
  - Hierarchical data structure
  - Correlated outcomes
  - Multiple exposomes
  - Conditioning on intermediates
  - Missingness & censoring

Trans-disciplinary teams needed for discovery, translation & improving population health...
Longitudinal Investigation of Fertility & the Environment – the LIFE Study

Do persistent environmental chemicals affect human reproduction & development in the context of couples’ lifestyles?

Study outcomes

- 1° Time-to-pregnancy; infertility; pregnancy loss, gestation & birth size
- 2° Menses; ovulation; reproductive profiles; semen quality; sex ratios

Chemicals

- Completed - OCPs, PBDEs, PCBs, PFCs, metal, ctinine, phytoestrogens
- Ongoing - BPA, phthalates, UV filters

Lifestyle

- Alcohol, caffeine, exercise, fish consumption, smoking, stress, vitamins
Population Sampling

- Couples
  - Fecund
    - Using Contraception
      - Intending
      - Not intending
  - Impaired Fecundity
    - At risk for pregnancy
    - Conception Delay
    - Pregnancy Loss
  - Infecund
    - Sterile
      - Biological
      - Contraceptive
      - Medical Rx

≈1% population planning pregnancy (Buck et al., 2004)
## Recruitment Strategy – LIFE Study

<table>
<thead>
<tr>
<th>Research Site</th>
<th>Michigan</th>
<th>Texas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Referent population</td>
<td>4 counties</td>
<td>12 counties</td>
</tr>
<tr>
<td>Sampling framework</td>
<td>InfoUSA®</td>
<td>Texas Parks &amp; Wildlife Registry</td>
</tr>
<tr>
<td>Direct contact</td>
<td>Mailing with telephone follow up</td>
<td>Mailing with telephone follow up</td>
</tr>
</tbody>
</table>

Each partner must be contacted separately!
Inclusion Criteria - Couples

- Ages 18-44 years; males aged >18 years
- Able to communicate in English/Spanish
- In committed relationship
- Wishes to conceive in next 6 months
- Planning to stop contraception to become pregnant
Retention Considerations

- Sensitive data collection
- Burden & remuneration
  - Estimating “reproducible” burden
  - $25 blood; $5 urine; $20 saliva; $20 semen
- Data collection options
  - Web based, hardcopy or both
- Supporting web based & hardcopy daily journals
- Iatrogenic harm
  - TTP induced stress
  - Couple differences in journal reporting
Delineating the Exposome

- Male
- Female
- Couple
- Conceptus, embryo, fetus

High dimensional longitudinal mixtures...
(Louis et al., 2011)

...totality of environmental exposures from conception onwards (Wild 2005)

... getting snapshots during critical windows of exposure (Rappaport & Smith 2010)
Time-Based Data Collection

- **Baseline**: Blood, urine, saliva & semen
- **Attempting pregnancy**: Daily
- **hCG pregnancy or 12 months**: Monthly, 8 wks. post-conception
- **Birth**: Urine
Home-Based Data Collection

Daily Journals
Home as Laboratory
Unobservable Outcomes

Ovulation

Fertilization

Cell Division
Home Biospecimen Collection & Testing - FEMALES

**Initial Display**
- Press M button and hold for 5 seconds until "Y" appears

**Display After 'M' Button Is Pressed**
- Fertility Status Bar

**Display Indicates That The Monitor Requires A Test**
- Number 6

**Low Fertility:**
- Very small chance of conception

**High Fertility:**
- Increased chance of conception

**Peak Fertility:**
- Highest chance of conception
Home Biospecimen Collection & Testing - FEMALES cont’d
Home Biospecimen Collection-
MALES
Emerging Results - LIFE Study
## Recruitment

<table>
<thead>
<tr>
<th>Letters Mailed (N)</th>
<th>Recruited* n (%)</th>
<th>Enrolled n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Texas A&amp;M</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(12 counties)</td>
<td>355,087</td>
<td>981 (3%)</td>
</tr>
<tr>
<td><strong>RTI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4 counties)</td>
<td>69,336</td>
<td>203 (1%)</td>
</tr>
</tbody>
</table>

- 42% eligible couples enrolled
- 0.1% recruitment yield
- ≈84% couples not screened
- 36% refused screening

Buck Louis et al., 2011
Sampling Frameworks – LIFE Study

- Few differences by sampling framework or by completion status
  - No difference by partners’ ages, education, health insurance, or women’s gravidity, parity
  - Couples completing study were more likely to be white & have higher household incomes than couples withdrawing
## % Journal Cards Received

<table>
<thead>
<tr>
<th>Card</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Michigan</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Journal</td>
<td>82</td>
<td>84</td>
</tr>
<tr>
<td>• Early pregnancy (daily)</td>
<td>--</td>
<td>80</td>
</tr>
<tr>
<td>• Pregnancy (monthly)</td>
<td>--</td>
<td>76</td>
</tr>
<tr>
<td><strong>Texas</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Journal</td>
<td>85</td>
<td>88</td>
</tr>
<tr>
<td>• Early pregnancy</td>
<td>--</td>
<td>82</td>
</tr>
<tr>
<td>• Pregnancy</td>
<td>--</td>
<td>81</td>
</tr>
</tbody>
</table>
## Biospecimen Collection

<table>
<thead>
<tr>
<th>Biospecimen</th>
<th>First Sample % Obtained</th>
<th>Second Sample % Obtained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>100</td>
<td>--</td>
</tr>
<tr>
<td>Urine (6 mo. &amp; pregnancy)</td>
<td>100</td>
<td>94 (77 &amp; 95)</td>
</tr>
<tr>
<td>Saliva</td>
<td>98</td>
<td>87</td>
</tr>
<tr>
<td>Semen</td>
<td>94</td>
<td>77</td>
</tr>
</tbody>
</table>
Field Lessons to Date

- Challenging, targeting couples planning pregnancy within two months for population based recruitment
  - <1% couples planning pregnancy within two months
  - Some women already pregnant
  - Pregnancy intentions change
- Few language based barriers during telephone contact
- Drop out tend to be early
- Few couples consistently used web for data collection
Emerging environmental results...
# Metals & Fecundability Odds Ratios

<table>
<thead>
<tr>
<th>Adjusted Model</th>
<th>Female FOR (95% CI)</th>
<th>Male FOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium (ug/L)</td>
<td>0.77 (0.62, 0.97)</td>
<td>0.85 (0.71, 1.01)</td>
</tr>
<tr>
<td>Lead (ug/dL)</td>
<td>0.97 (0.85, 1.11)</td>
<td><strong>0.85 (0.73, 0.99)</strong></td>
</tr>
<tr>
<td>Mercury (ug/L)</td>
<td>0.99 (0.87, 1.12)</td>
<td>0.98 (0.86, 1.11)</td>
</tr>
<tr>
<td>Cotinine (ng/ml)</td>
<td>0.98 (0.81, 1.18)</td>
<td>0.96 (0.83, 1.10)</td>
</tr>
<tr>
<td>Serum lipids (ng/g)</td>
<td>0.93 (0.82, 1.06)</td>
<td>0.98 (0.87, 1.10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td><strong>0.80 (0.70, 0.91)</strong></td>
<td>0.85 (0.75, 0.97)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.91 (0.79, 1.04)</td>
<td>0.99 (0.88, 1.12)</td>
</tr>
<tr>
<td>Site (Michigan/Texas)</td>
<td>1.23 (0.91, 1.66)</td>
<td>1.30 (0.96, 1.76)</td>
</tr>
<tr>
<td>Parity (null/parous)</td>
<td><strong>1.72 (1.34, 2.21)</strong></td>
<td><strong>1.66 (1.31, 2.11)</strong></td>
</tr>
</tbody>
</table>
## Couples’ Metal Exposures & Fecundability Odds Ratios

<table>
<thead>
<tr>
<th>Adjusted Model*</th>
<th>FOR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female cadmium (ug/L)</td>
<td>0.81 (0.64, 1.02)</td>
</tr>
<tr>
<td>Female lead (ug/dL)</td>
<td>1.05 (0.91, 1.23)</td>
</tr>
<tr>
<td>Female mercury (ug/L)</td>
<td>1.00 (0.86, 1.16)</td>
</tr>
<tr>
<td>Male cadmium (ug/L)</td>
<td>0.93 (0.78, 1.12)</td>
</tr>
<tr>
<td><strong>Male lead (ug/dL)</strong></td>
<td><strong>0.83 (0.70, 0.98)</strong></td>
</tr>
<tr>
<td>Male mercury (ug/L)</td>
<td>0.98 (0.84, 1.14)</td>
</tr>
<tr>
<td>Female age</td>
<td>0.81 (0.70, 0.94)</td>
</tr>
</tbody>
</table>

*Adjusted for couples’ cotinine, lipids, BMIs, female age (years) & difference between couples' ages
Summary

- Feasibility of population based sampling
  - <1% of targeted samples planning pregnancy
  - 42% of recruited couples enrolled in study
  - 69% of enrolled couples completed study (drops out tend to be early)
  - Men did as well as women with study protocol

- Emerging environmental signals
  - Magnitude comparable to age & other lifestyle factors
  - Various classes of persistent compounds associated with reduced couple fecundity
Conclusions & Future Directions

- Emerging evidence supportive of a relation between environmental factors & couples fecundity
  - Effect comparable in magnitude to age & lifestyle
  - Is effect mediated through anovulation, altered menses or semen quality?
  - What are the implications for other fertility outcomes?
- Concerted efforts to define the exposome for both partners of the couple to delineate underlying mechanisms
- Implications for child health remain to be established

Males matter!
LIFE Study – Research Team

- **NICHD**
  - Drs. Zhen Chen, Sungduk Kim, Enrique Schisterman & Rajeshwari Sundaram

- **Texas A & M University**
  - Drs. Anne Sweeney

- **RTI International**
  - Dr. Tim Wilcosky

- **The EMMES Corporation**
  - Dr. Rob Gore-Langton

- **Ohio State College of Medicine**
  - Dr. Courtney Lynch

- **Emory University**
  - Dr. Dana Boyd Barr

- **CDC**
  - Drs. Antonia Calafat, Steven Schrader, Andreas Sjödin