Environmental Exposures: Do they Affect Child and Reproductive Outcomes?

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Environmental Exposures: EDCs

- One type of environmental exposures is a group of chemicals or contaminants known as “Endocrine Disrupting Compounds”, or EDCs
- EDCs are very common and widespread, and they may interfere with normal hormonal regulation
- Many EDCs are “lipophilic”, accumulate over time, and are persistent
- Types of EDCs include:
  - Dioxins and PCBs
  - Pesticides (eg., metabolites of DDT, like p,p,’-DDE)
  - Heavy metals (lead, mercury)
  - Plasticizers (phthalates, bisphenol A (BPA))
  - Parabens (used in personal care products)
  - Flame retardants (used in baby products, furniture, pajamas)

Possible outcomes of EDC exposure

- Impaired growth
- Delayed or accelerated pubertal maturation
- Metabolic problems (insulin resistance, diabetes)
- Fertility or reproductive outcomes
  - Embryo development
  - Time to pregnancy
  - Low birth weight, prematurity
  - Semen quality

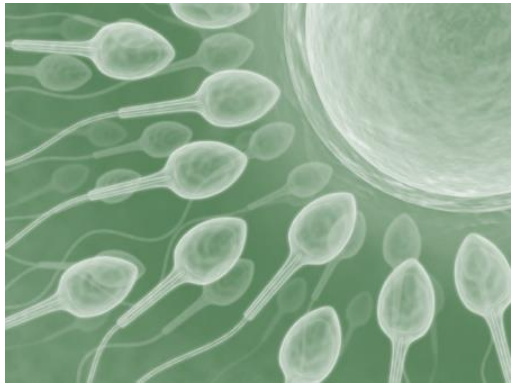
Although almost everyone is exposed to EDCs, children and pregnant women may be most vulnerable.

Early life exposures may impact later adult health outcomes.

Studies Evaluating EDCs

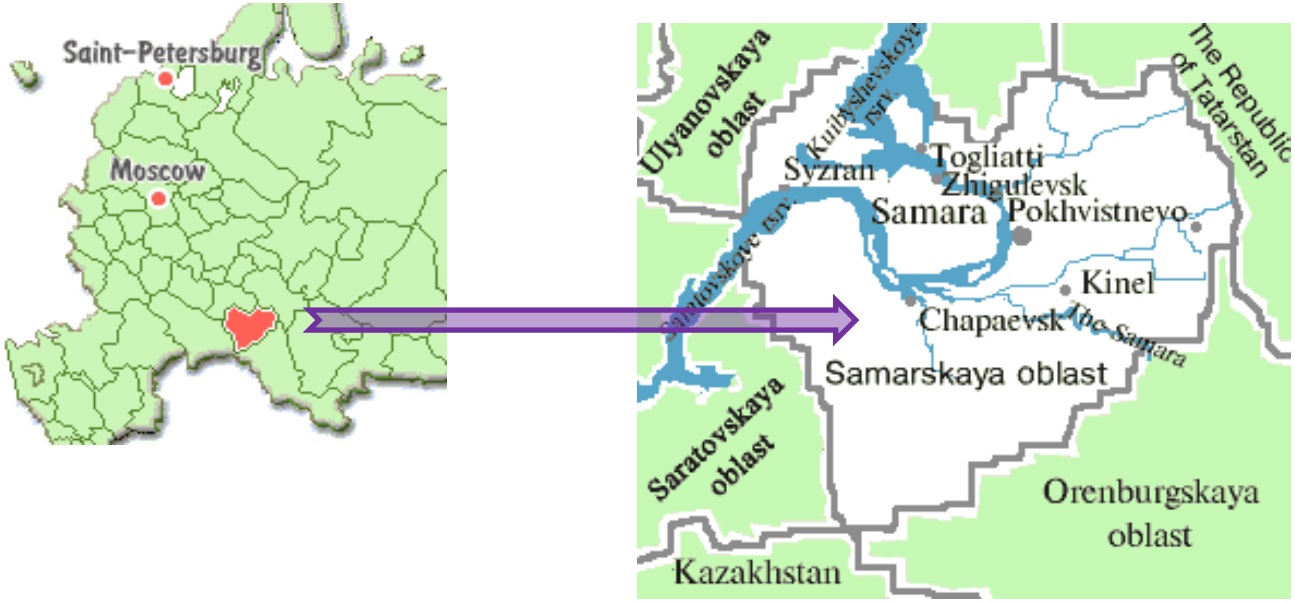
- **Russian Children's Study** – cohort study of 499 boys enrolled at age 8-9 and followed for ~10 years with annual visits, to evaluate association of EDCs (specifically lead, dioxins, PCBs and pesticides) with growth, pubertal development and maturation, and reproductive capacity (eg., semen quality)

- **EaRTH Study** – Environmental and Reproductive Health Study: an ongoing study of environmental factors and fertility among couples attending the Massachusetts General Hospital (MGH) fertility center.

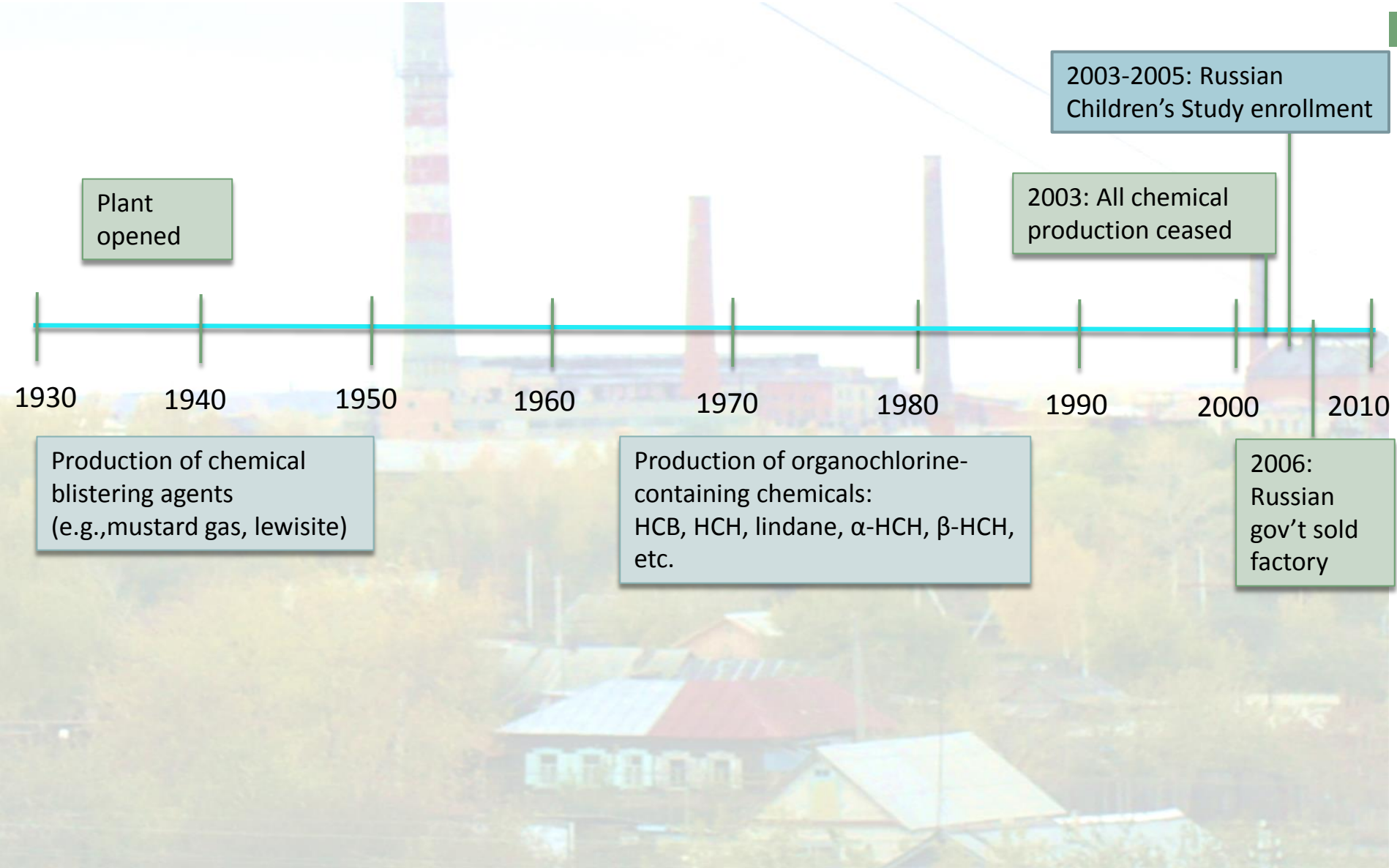


Russian Children's Study

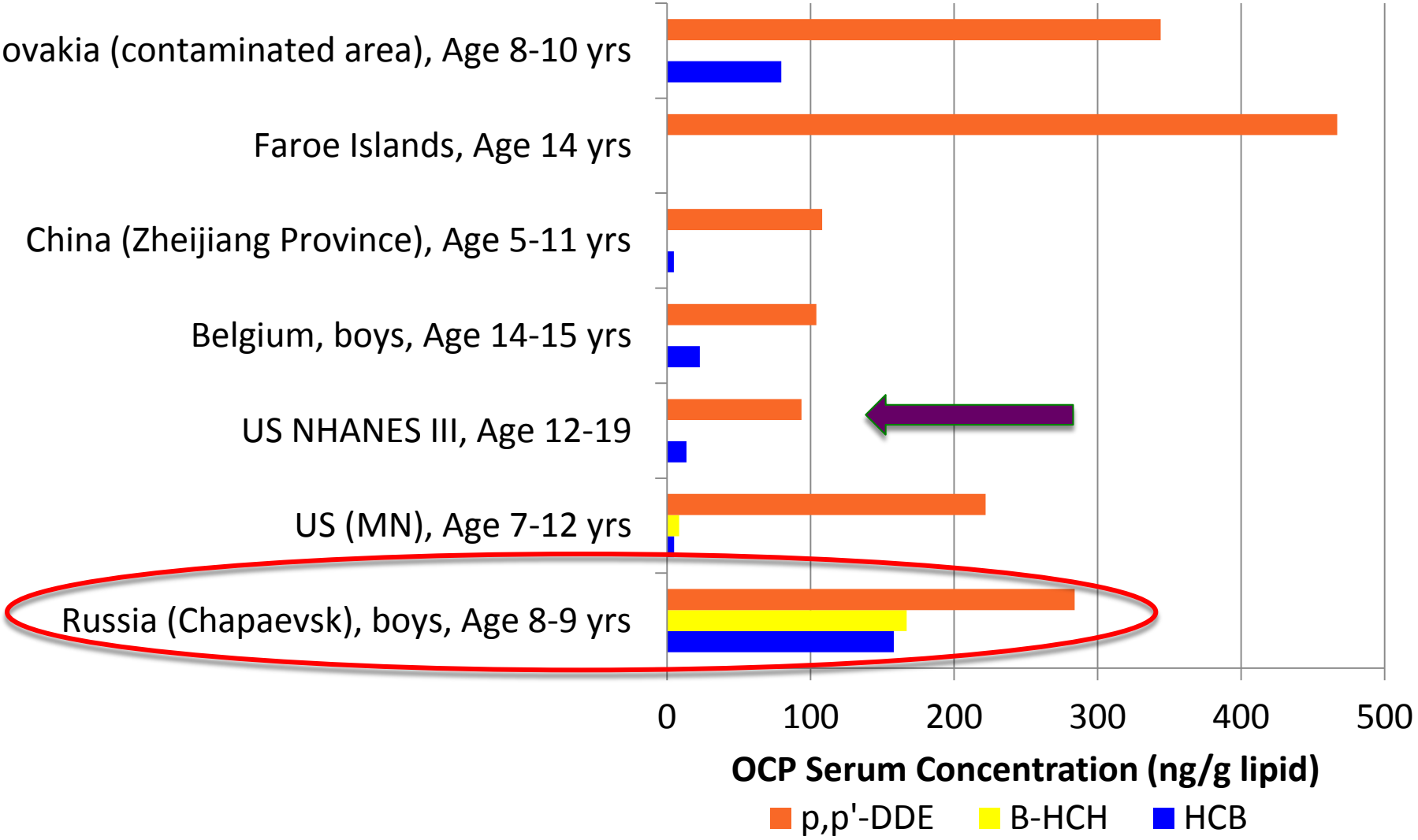
- Conducted in Chapaevsk, Russia, a small industrial city in Russia (Samara region)
- Former home to a large complex of chemical factories that produced agricultural and industrial organochlorine compounds



Chemical Plant Timeline

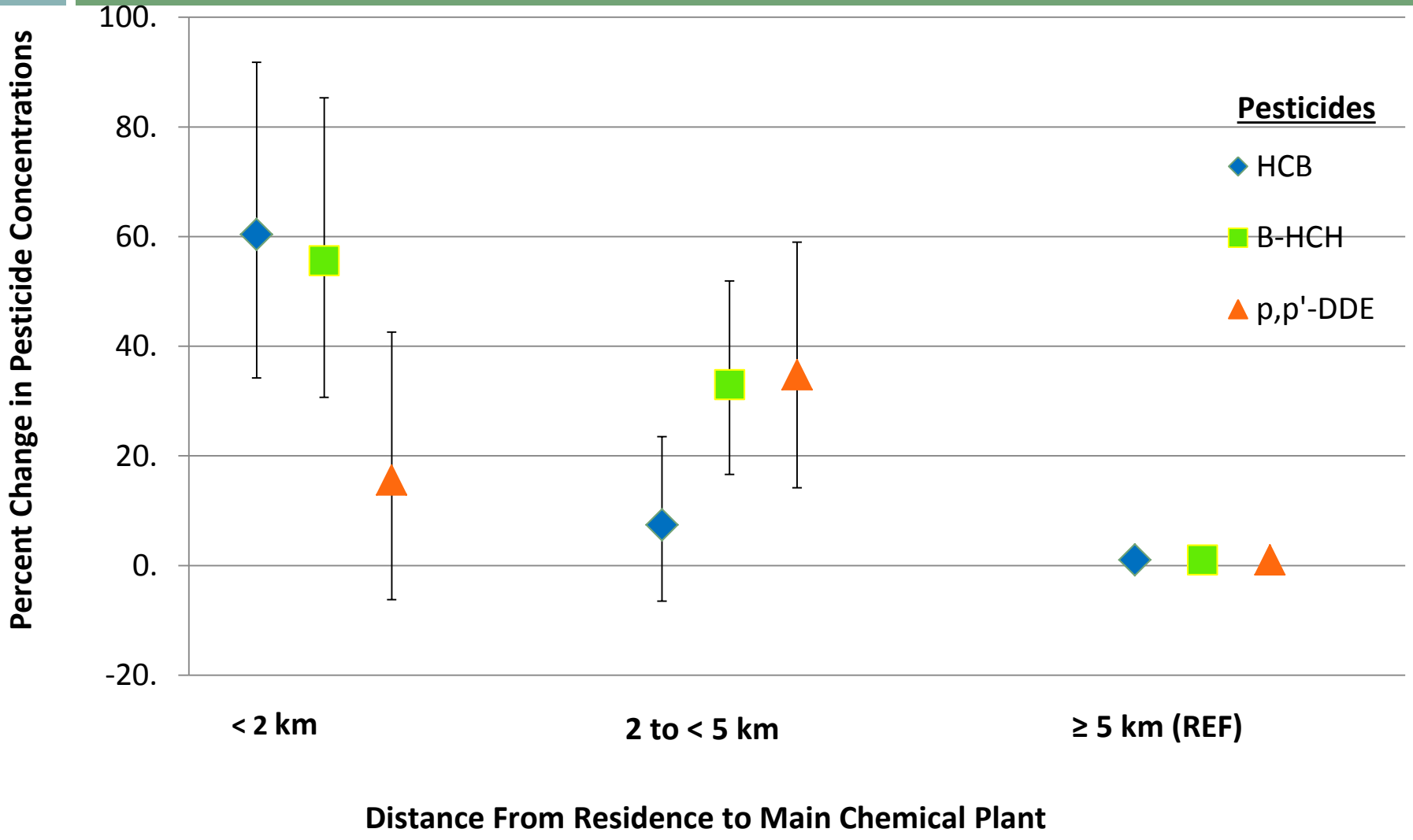


Median Pesticide Levels for Russian Childrens Study Compared to Other Child Populations



Note: Studies include both boys and girls unless otherwise specified

Percent Change in Boys' Serum Concentrations By Residential Distance to Chemical Plant

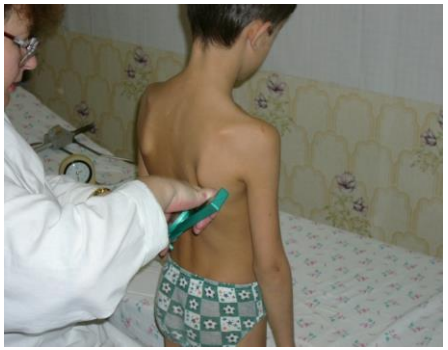


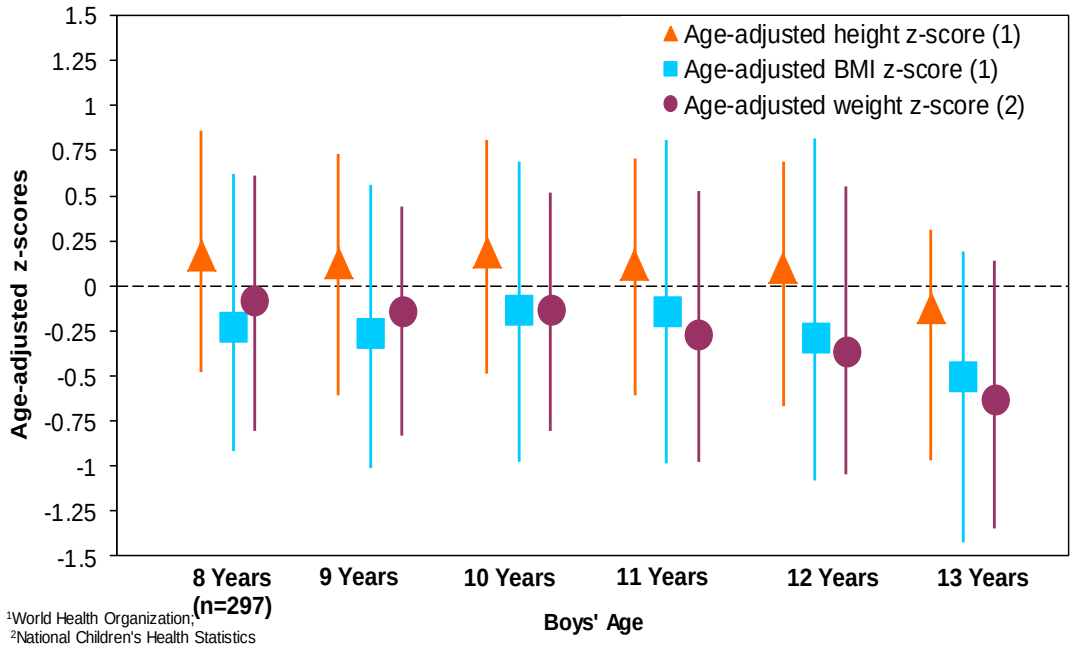
Scientific questions related to Russian Children's Study

- What are the sources of and risk factors for exposures to various types of EDCs? (diet, proximity to plant, socioeconomic factors, birth characteristics, home environment)
- Do higher levels of EDCs affect **growth** of boys?
- Do higher levels of EDCs affect **timing of pubertal onset** and sexual maturity?
- If so, **how do we explain the mechanisms** behind these effects?
  - Are there effects of EDCs on hormone levels?
  - Are there effects of EDCs on cholesterol and triglycerides?
  - Since growth of boys affects timing of puberty, is this the main way that EDCs might affect pubertal onset, or are there other pathways?

Interesting statistical issues in addressing these scientific questions

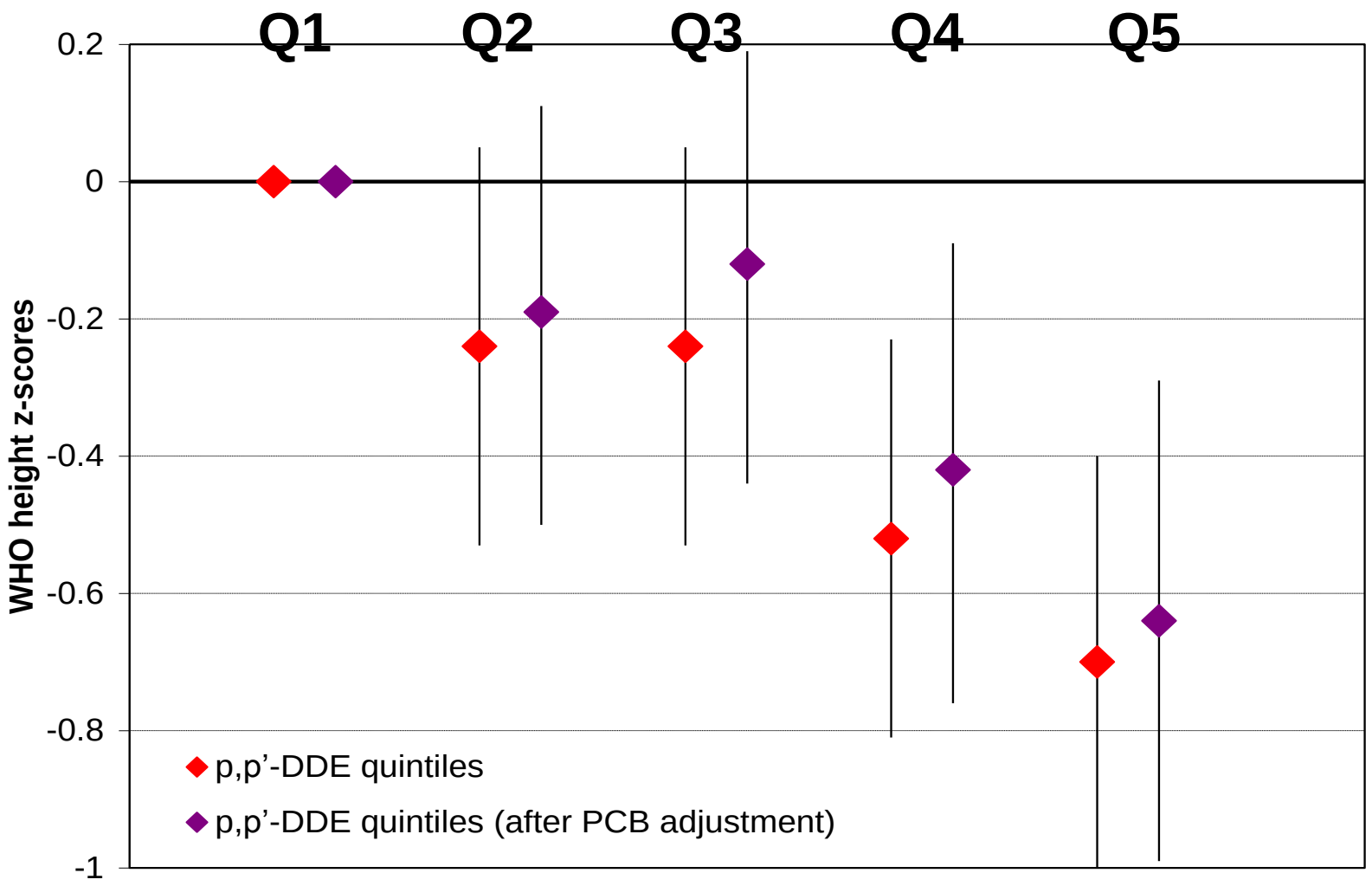
- Growth is measured every year (height, weight, BMI, other measures), so we need to account for correlation in repeated measures over time.
- We are essentially looking at whether exposures to EDCs change the “trajectories” of growth as boys go through adolescence.





[1]World Health Organization;
[2]National Children's Health Statistics

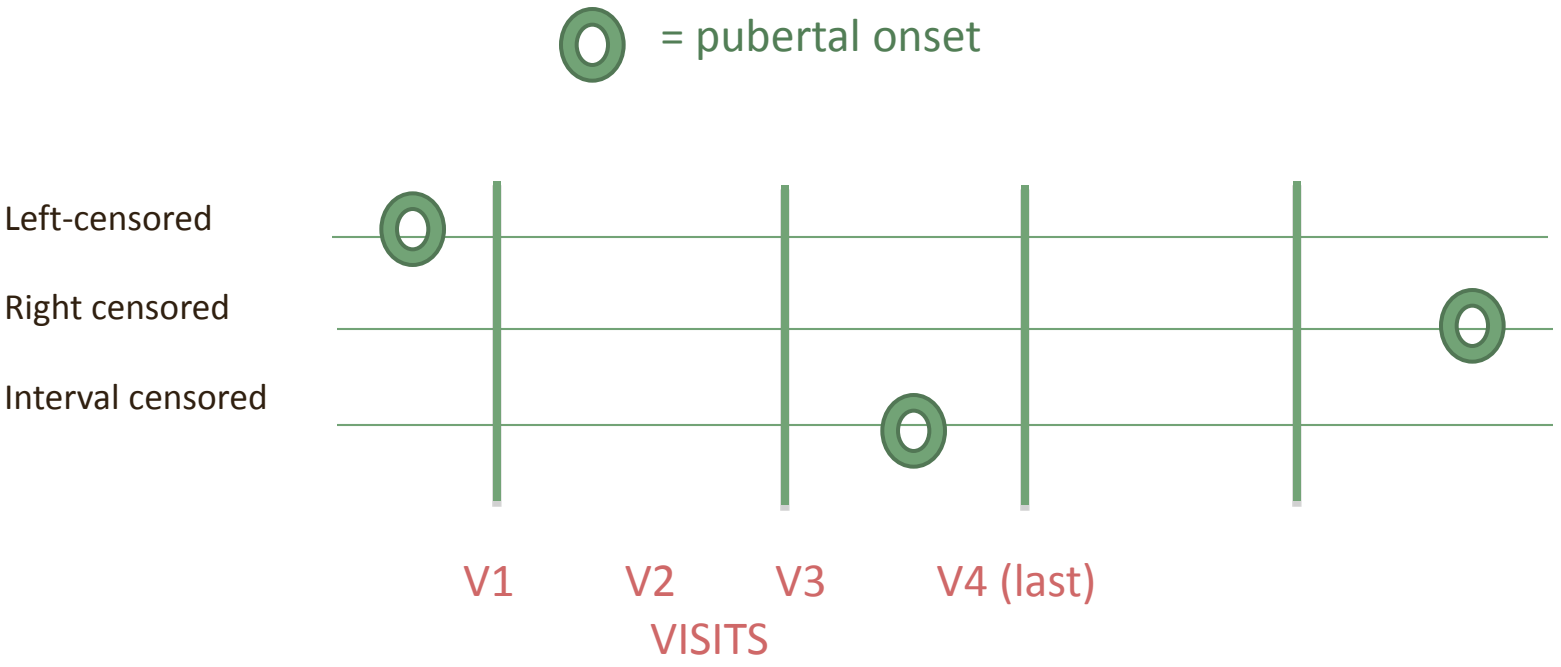
Serum p,p'-DDE Quintile Associations with WHO Height Z-scores



More interesting statistical issues

- Pubertal onset is not an event that occurs on a single day, but is more of a continuous process.
- We typically only know that puberty has occurred before boys were enrolled (at age 8-9), between two study visits, or was never attained.
- Statistical methods for “interval censored data” need to be used.

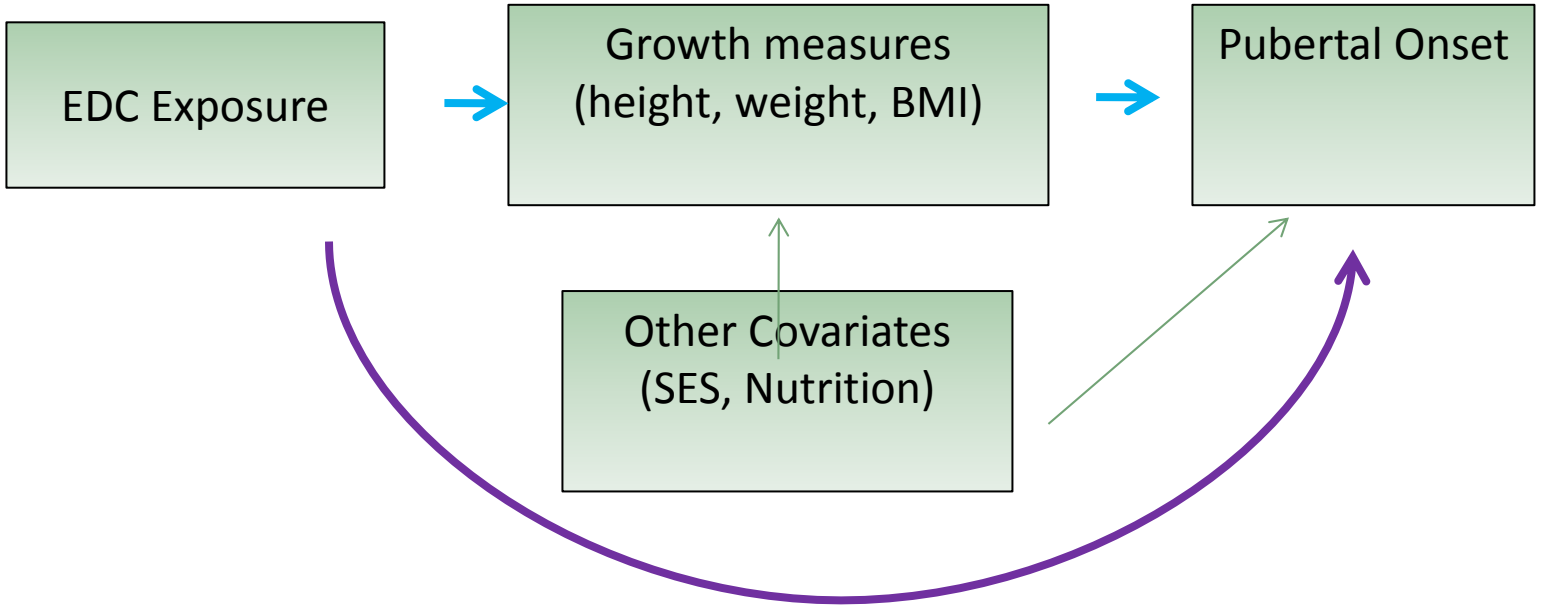
Illustration of interval censored approach



Left censored – child already had pubertal onset before first visit
Right censored – as of last visit on study, child still had not started puberty
Interval censored – onset occurred since the last study visit
Assumed event times for Cox models: midpoint between visits for interval censored, midpoint between age 7 and first visit for left-censored

Even more interesting statistical issues

- We have demonstrated that exposures to many EDCs (including lead, pesticides, dioxins and PCBs) are associated with reduced growth.
- Growth (height, BMI) is one of the main predictors of pubertal onset
- If we adjust for growth, we may be adjusting away true EDC effects



Mediation Analysis for Survival Outcomes

- **Accelerated failure time model** for age at onset T, as a function of exposure E (HIV status), mediator M (BMI Z-score), and other covariates Z:

$$\log(T) = \theta_0 + \theta_1 E + \theta_2 M + \theta_3 Z + \gamma\varepsilon$$

- **Linear regression model:** for mediator M (BMI Z-score) as a function of exposure E (HIV) and other covariates Z (with normal errors):
  - $E[M|E,Z] = \beta_0 + \beta_1 E + \beta_2 Z$
- **Then effects of interest are:**
  - natural direct effect: $\theta_1 (E - E^*)$
  - natural indirect effect: $\beta_1\theta_2 (E - E^*)$
- Tein and MacKinnon showed that the difference in the θ_1 coefficient for the AFT model with and without the mediator is equal to the "product" method of coefficients $\beta_1\theta_2$ for a Weibull model, but VanderWeele showed that it held regardless of the distribution of ε

Direct and Indirect Effects of EDCs on Pubertal Onset

Using this framework, we have the following direct and indirect effects of blood lead on age at pubertal onset (controlling for other covariates):

Mediator	Tanner Stage	TOTAL Lead effect* (without growth)	Direct Lead effect (with growth)	Δ	Indirect Effect of Lead	% Direct Effect	% Indirect Effect
BMI Z-score	B2	8.28	5.88	2.39	2.29	72%	28%
	G2	10.71	9.39	1.32	1.32	88%	12%
Height Z-score	B2	8.28	3.97	4.31	4.21	48%	52%
	G2	10.71	5.59	5.13	5.20	52%	48%

* Comparing high lead (>5 ug/dl) to lower lead levels

Mediation Analysis for Survival Outcomes

- Usually, the difference between the total effect and the direct effect (eg., adjusting for the mediator) is equivalent to the product of coefficients $\beta_1\theta_2$. However, this relationship does not seem to hold for interval censored data.
- Mediation techniques to allow calculation of indirect and direct effects have not been addressed in the context of interval censored data.
- Impact of specific distribution on discrepancy between indirect effect estimates needs to be evaluated, along with other open questions in this area.

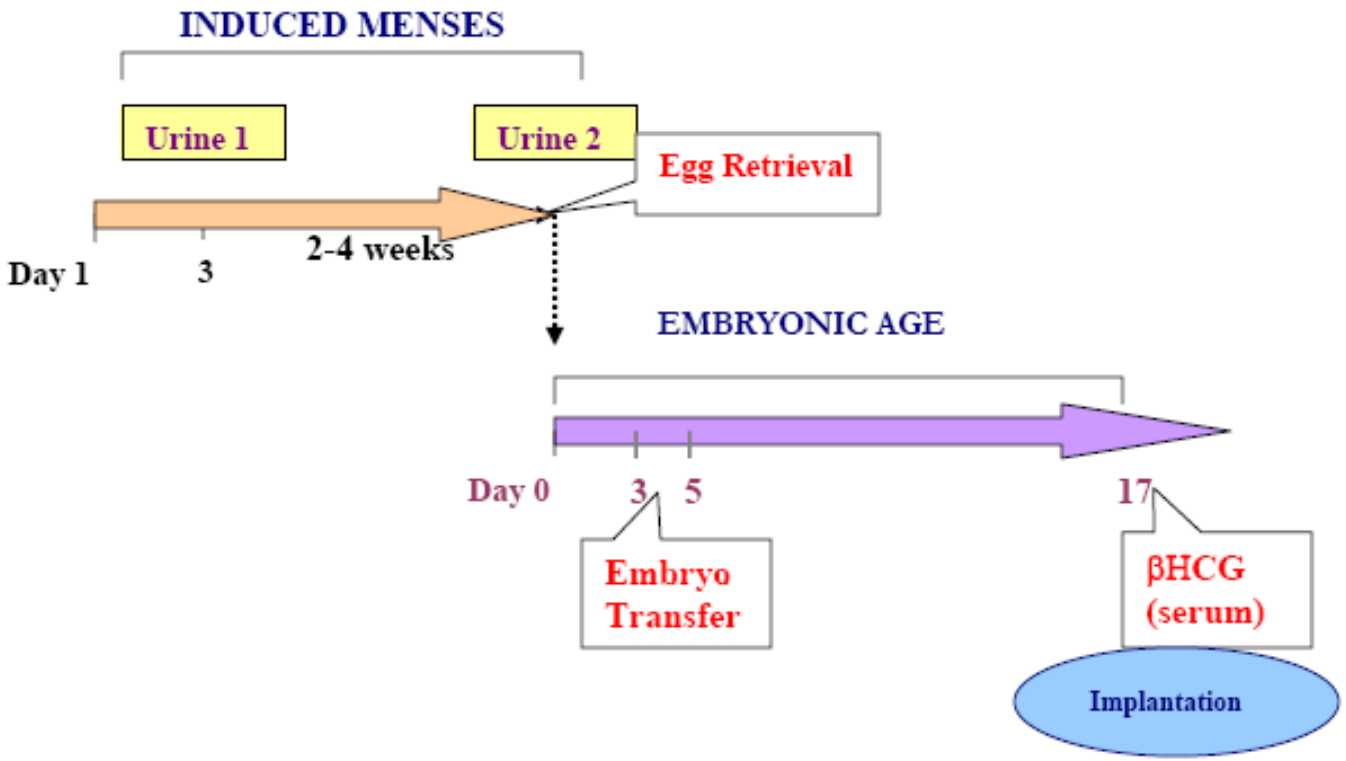
EaRTH Study

- Couples presenting at the MGH Fertility Center
  - Females: 18 to 45 yrs
  - Males: 18 to 55 yrs
  - Using own gametes for ART
  - Completed at least 1 ART cycle
- The couples were followed for 1-4 cycles of ART

ART=assisted reproductive technology

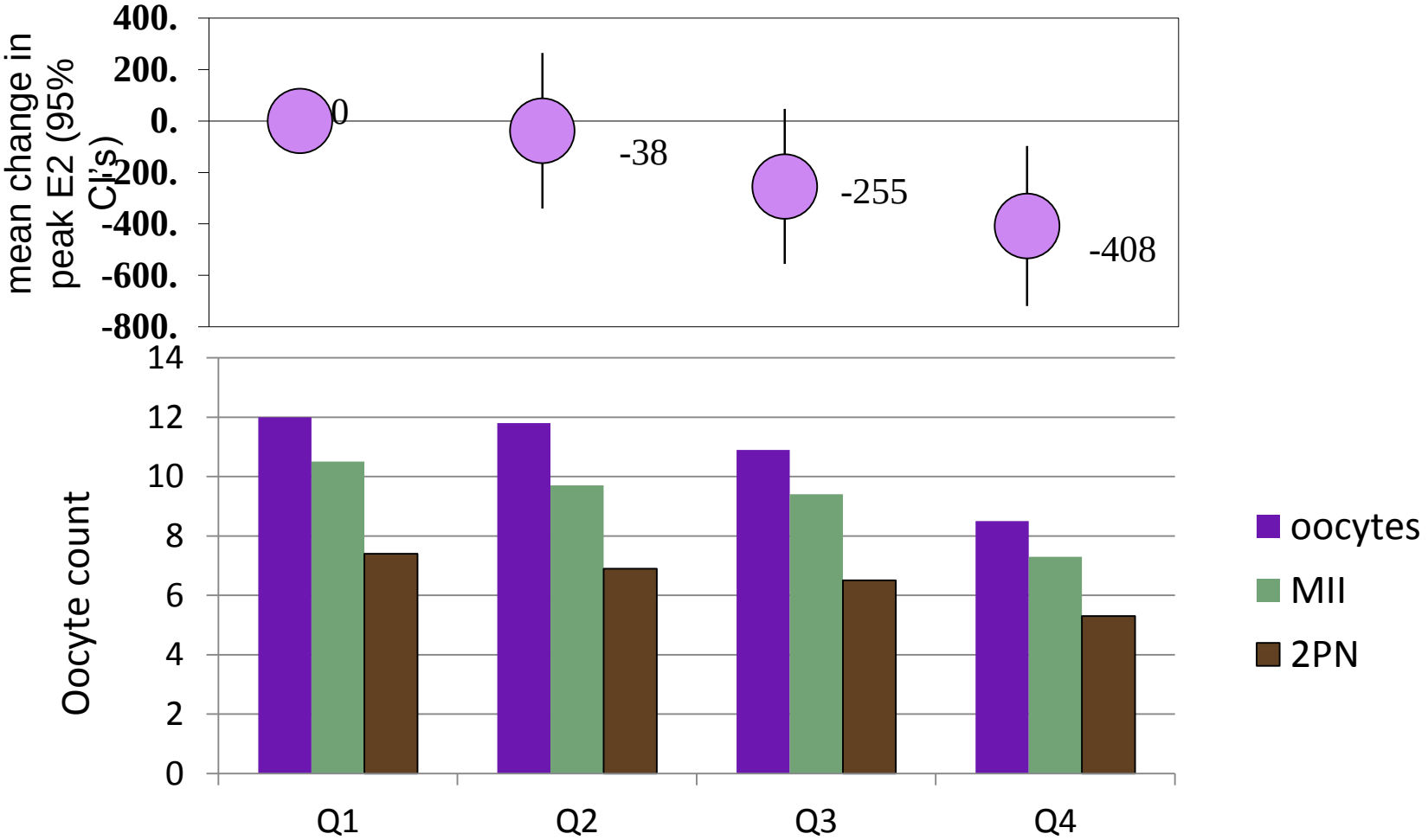
EaRTH Study

- Using an in vitro fertility (IVF) allows us as researchers to observe parts of the reproductive process we wouldn't ordinarily be able to examine
- Male and female partners provide regular urine and blood specimens, which can be evaluated in terms of environmental exposures



EaRTH Study: some findings for BPA

- Higher Female BPA levels were associated with lower levels of estradiol and lower numbers of oocytes (overall, and mature (MII)).



EaRTH Study: Statistical issues

- There are multiple outcomes for each IVF cycle (embryo fertilization, implantation, live birth), with multiple embryos evaluated for quality and maturation.
- For a successful outcome, each successive stage must be passed successfully.
- Many couples have more than one IVF cycle before attaining a live born baby, and the multiple cycles are not independent.
- Although there are statistical approaches for accounting for correlation among multiple cycles, they typically assume that imbalance in # cycles is random.

EaRTH Study: more statistical issues

- The exposure levels of both male and female partners may affect these outcomes; our recent studies of both parabens and phthalates in male partners suggests that men matter!
- However, evaluating joint effects and possible interactions requires a much larger study design.
- In addition, there are mixtures of chemicals which may act synergistically. Efficient strategies for screening mixtures of chemicals need to be applied.
- There may also be interactions with overall diet, especially for critical dietary factors such as folate and vitamin D.

Summary and Discussion

- Findings from our Russian Children's Study have shown that exposures to environmental contaminants, particularly EDCs, is associated with reduced growth, increased odds of metabolic problems, delay in pubertal onset, and similar delays in sexual maturity
- Findings from our EaRTH IVF study have shown that other EDCs such as phthalates, parabens, and bisphenol A can be associated with reduced levels of estradiol (a reproductive hormone) and reduced oocytes, implantation failure, and lower odds of live birth
- There are lots of open statistical research problems that are motivated by these type of collaborative studies!