Endocrine Disrupting Chemicals and Women's Health Symposium

A Virtual Symposium

July 18–19, 2023

9am–4pm ET

OASH | Office on Women's Health

Welcome

Dorothy Fink, M.D.
Deputy Assistant Secretary for Women's Health
Director, Office on Women's Health
Department of Health and Human Services

Introduction To EDCs and Their Impacts On Women

Andrea Gore, Ph.D.

Moderated by Keiva Nelson

Introduction To EDCs and Their Impacts On Women

Andrea Gore, Ph.D.

Professor and Vacek Chair in Pharmacology

University of Texas at Austin

Introduction to EDCs and Their Impacts on Women

Andrea C. Gore, Ph.D.

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Of the hundreds of thousands of manufactured chemicals, about 1000 have been shown to be endocrine-disrupting chemicals (EDCs)

Endocrinology 153: 4097 (2012)

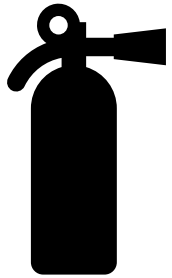
POSITION STATEMENT

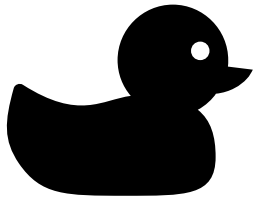
Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society

R. Thomas Zoeller, T. R. Brown, L. L. Doan, A. C. Gore, N. E. Skakkebaek, A. M. Soto, T. J. Woodruff, and F. S. Vom Saal

"An exogenous chemical, or mixture of chemicals, that interferes with any aspect of hormone action"

Categories of EDCs

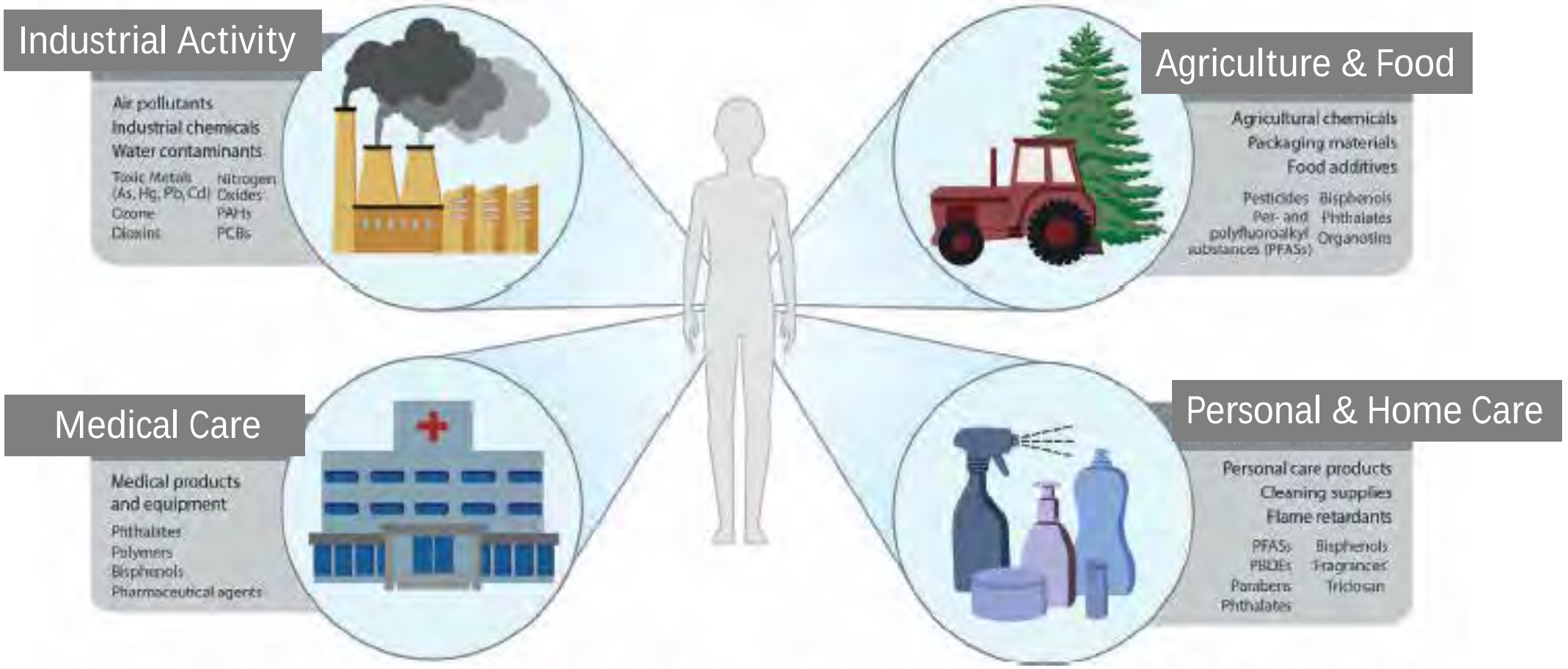




Category/Use	Example EDCs
Pesticides	DDT, chlorpyrifos, atrazine, 2,4-D, glyphosate
Children's products	Lead, phthalates, cadmium
Food contact materials	BPA, phthalates, phenol
Electronics and Building materials	Brominated flame retardants, PCBs
Personal care products, medical tubing	Phthalates
Antibacterials	Triclosan
Textiles, clothing	Perfluorochemicals

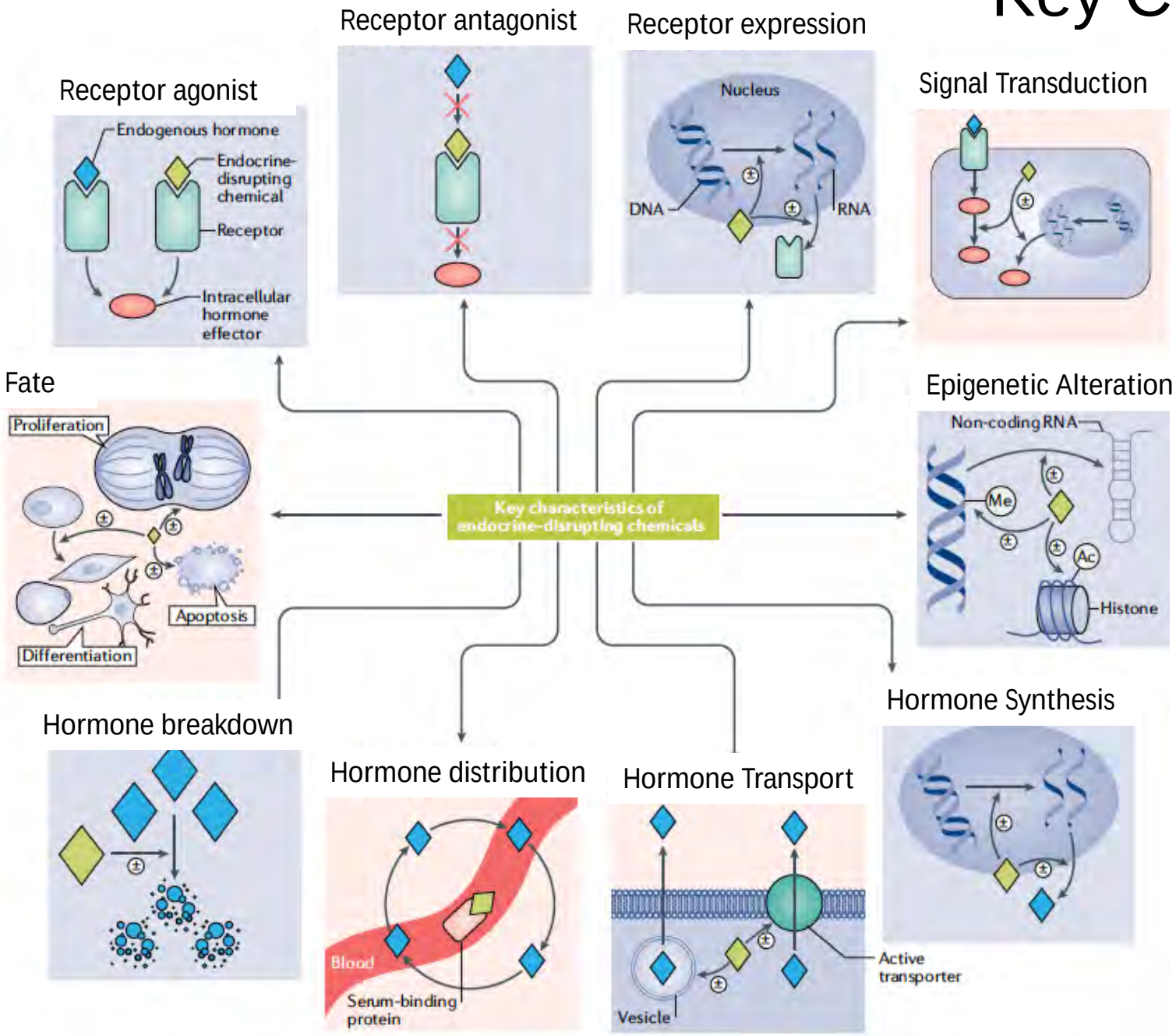
Gore et al. 2014, Introduction to EDCs (IPEN)

Human exposures to EDCs



Schulz & Sargis (2021) Advances in Pharmacology 92: 419.

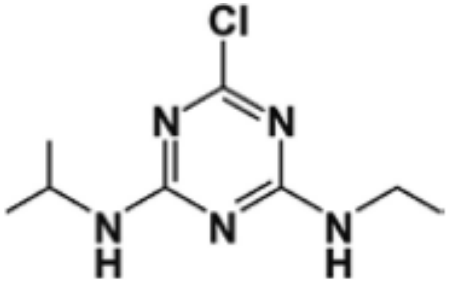
Key Characteristics of EDCs



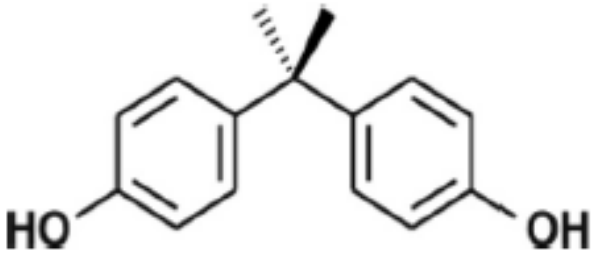
La Merrill et al. 2020, Nat. Rev. Endocrinol. 16: 45

EDC Examples & Structures

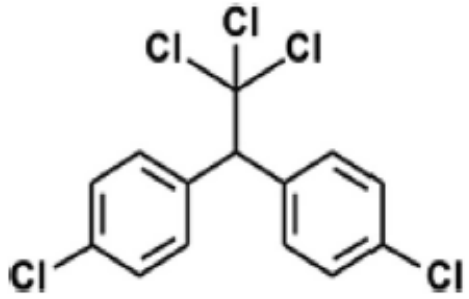
Atrazine (herbicide)



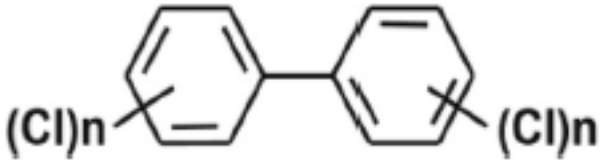
Bisphenol A (plastics)



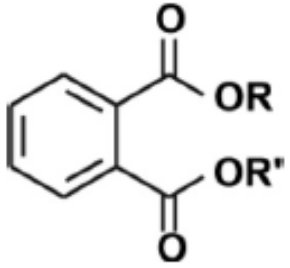
DDT (pesticide)

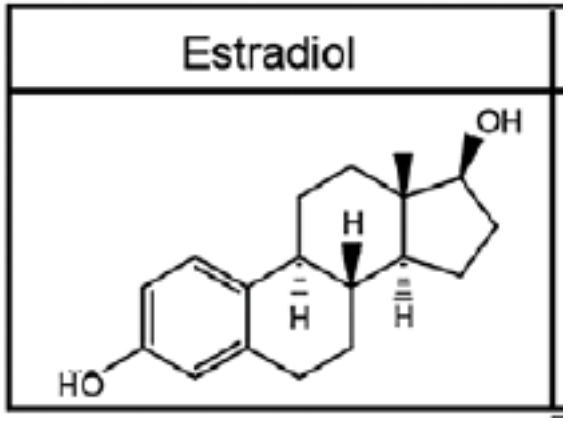


PCBs (industry)

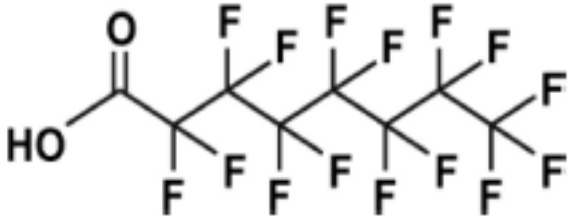


Phthalates (plastics, cosmetics)

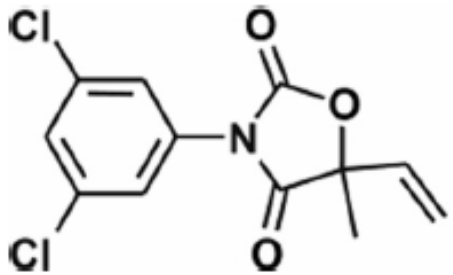




PFOA (surfactant)



Vinclozolin (fungicide)



Evidence that EDCs Affect Human Health

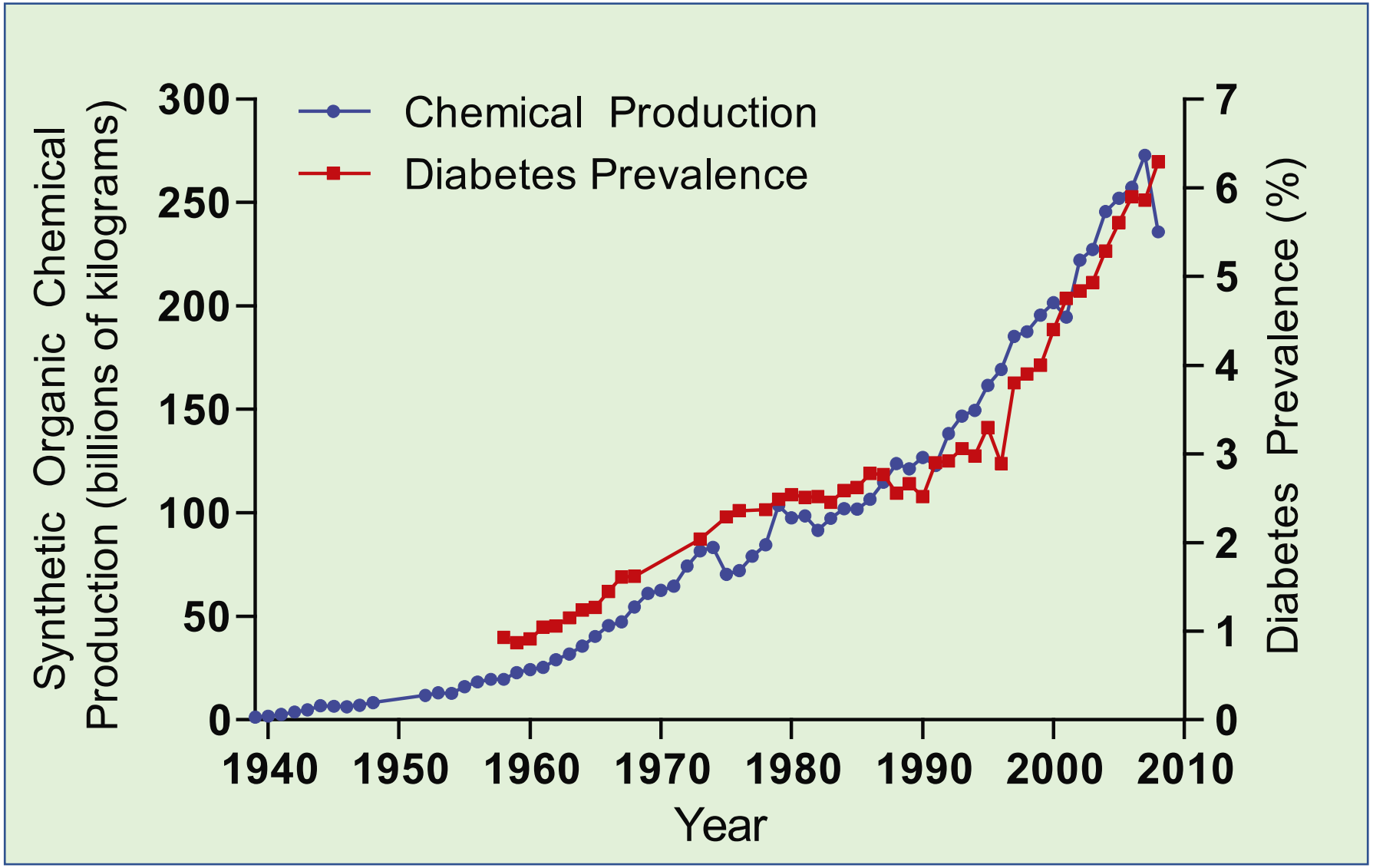
Disasters and Industrial Accidents:

- Seveso, Italy – dioxins
- Japan, Taiwan – industrial chemicals (PCBs)

Epidemiology:

- Agent Orange exposure and prostate cancer
- Twin studies showing that environmental factors play the principle role in hormone-sensitive cancers
- Correlations between increased chemical synthesis and chronic diseases

Correlations between chemical production and diabetes



Neel & Sargis, Diabetes 60: 1838 (2011)

EDCs and Human Health

Biomonitoring:

- NHANES database (US CDC) shows EDCs detectable in human fluids (urine, blood, breast milk, umbilical cord blood)
- Individual studies overwhelmingly detect chemicals in all humans

EDCs and Human Health

It is not possible to prove cause and effect in humans in the case of EDC exposures and chronic, complex diseases.

This is where experimental animal studies have been invaluable and irreplaceable.

The convergence of human observational and epidemiological data, together with lab animal studies, have led to the strong conclusion that EDCs are environmental factors increasing risk of endocrine and neurological disorders.

Moving EDCs into the Mainstream: Endocrine Society Scientific Statements (2009, 2015)

Endocrine-Disrupting Chemicals

An Endocrine Society Scientific Statement

Evanthia Diamanti-Kandarakis, Jean-Pierre Bourguignon, Linda C. Giudice, Russ Hauser, Gail S. Prins, Ana M. Soto, R. Thomas Zoeller, and Andrea C. Gore

2009

EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals

A. C. Gore, V. A. Chappell, S. E. Fenton, J. A. Flaws, A. Nadal, G. S. Prins, J. Toppari, and R. T. Zoeller

2015

THE ENDOCRINE SOCIETY®

Endocrine Society Scientific Statements (2009, 2015)

- Obesity, diabetes, and cardiovascular disease
- Female reproductive health
- Male reproductive health
- Hormone sensitive cancers in females
- Prostate gland
- Thyroid gland
- Neurodevelopment and neuroendocrine systems

Endocrine Society Scientific Statements (2009, 2015)

- Obesity, diabetes, and cardiovascular disease
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Female Reproductive Health

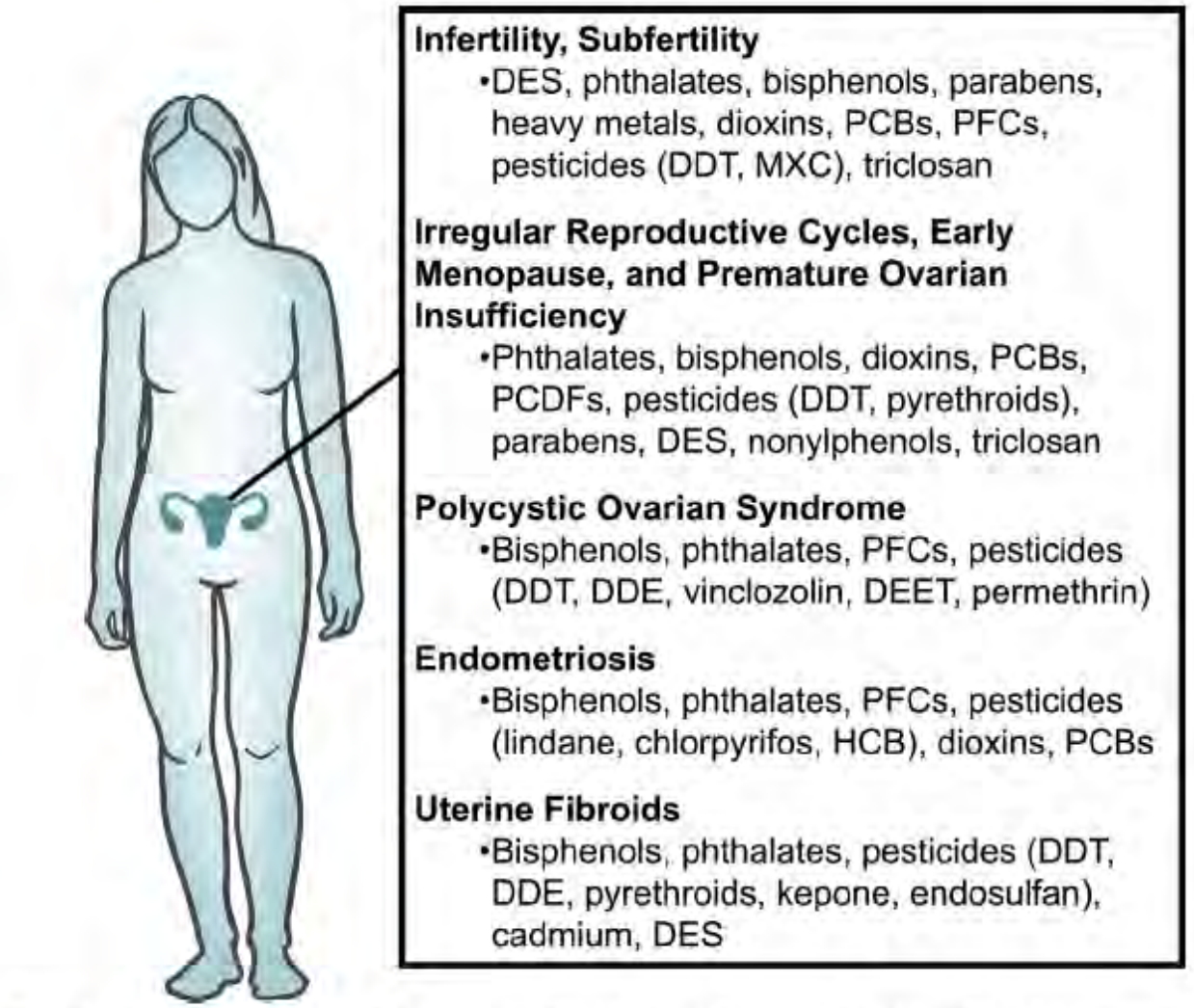


Fig. 1 A summary of EDCs associated with female reproductive disorders.

Laws…Flaws (2021) Advances in Pharmacology 92: 151.

EDCs: Special Considerations for Women

- EDCs have a disparate impact dependent on race and ethnicity, socioeconomic status, and other factors.
- Women have more body fat than men and can accumulate lipophilic substances such as EDCs.
- Women tend to use more personal care products (e.g. cosmetics, hair products).
- Women have unique physiological demands during pregnancy.
- Environmental exposure of a pregnant women can potentially affect the developing fetus.

Ø Effects of EDCs can differ profoundly between females and males.

Personal Care Product Exposure Tied to Girls' Early Puberty

— Associations weren't seen among boys using parabens, phthalates

by Kristen Monaco, Staff Writer, MedPage Today December 28, 2019

THESE CHEMICALS DISRUPT THE SEXUAL DEVELOPMENT OF CHILDREN — AND THEY'RE EVERYWHERE

Chemicals in cosmetics, soaps tied to early puberty in girls

By Lisa Rapaport, Reuters Health

5 MIN READ

Use Patterns of Leave-on Personal Care Products among Swiss-German Children, Adolescents, and Adults

Eva Manová [1], Natalie von Goetz [1,*], Carmen Keller [2], Michael Siegrist [2] and Konrad Hungerbühler [1]

Int J Environ Res & Publ Health 2013

Maternal Concentrations of Polyfluoroalkyl Compounds during Pregnancy and Fetal and Postnatal Growth in British Girls

Mildred Maisonet,[1,2] Metrecia L. Terrell,[1] Michael A. McGeehin,[2] Krista Yorita Christensen,[1] Adrianne Holmes,[2] Antonia M. Calafat,[2] and Michele Marcus[1,2,3]

The Exposure of Fetuses and Children to Endocrine Disrupting Chemicals: A European Society for Paediatric Endocrinology (ESPE) and Pediatric Endocrine Society (PES) Call to Action Statement

Niels E. Skakkebaek, Jorma Toppari, Olle Söder, Catherine M. Gordon, Sara Divall, and Martin Draznin

JCEM 2011

Environmental Exposures During Pregnancy and the Developing Fetus

DOHaD: Developmental Origins of Health and Disease

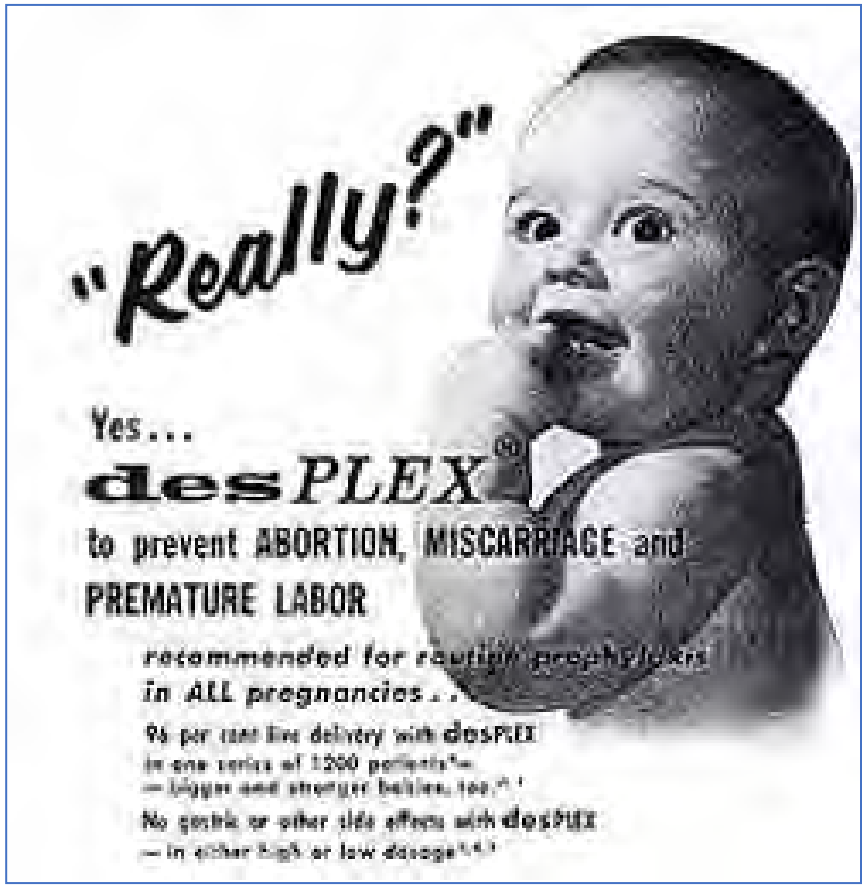
- The timing of exposure to EDCs is everything – fetus, infant, child, puberty
- The manifestation of disease may not occur for years or decades

Critical periods

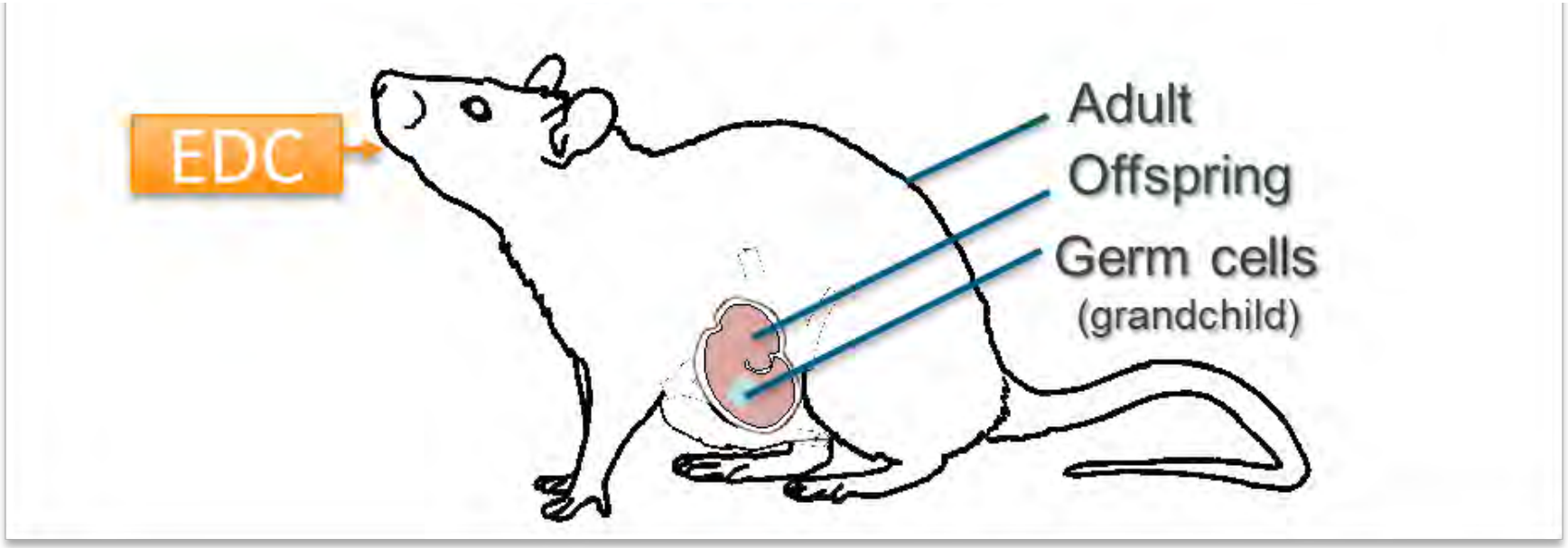
- Particular vulnerability during life stages when there is rapid developmental change and hormone sensitivity.

DES (Diethylstilbestrol) as the poster child for DOHaD in humans

- Pharmaceutical estrogens can cross the placenta
- The fetus is sensitive to these substances
- Developmental ‘programming’ happens in a sex- and timing-specific manner

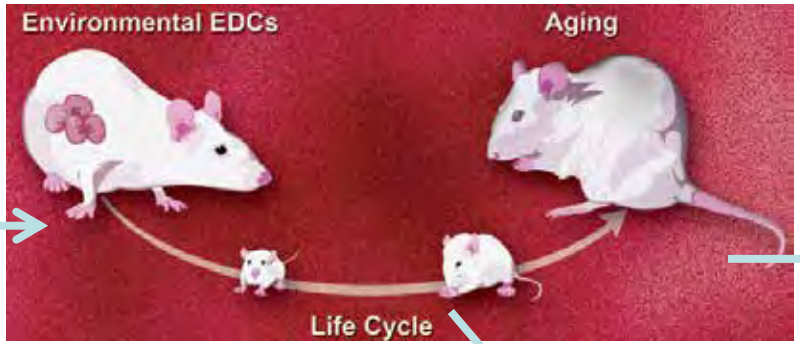


The Rodent DOHaD Model: Proving cause and effect



Modeling EDC effects using real-world scenarios

Developmental exposure *in utero*



Future Generations

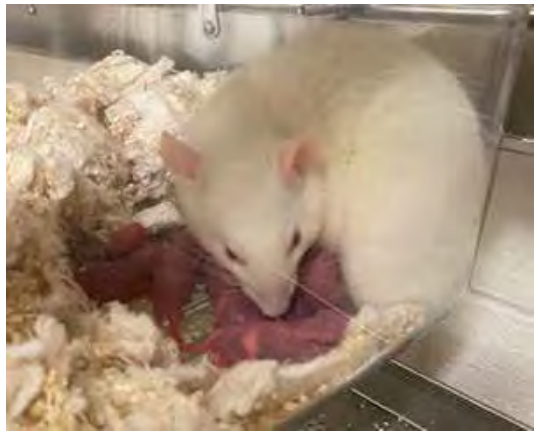
- Legacy vs. contemporary EDCs
- Combination of direct and heritable effects
- Lineages (maternal, paternal)
- Sex differences
- Epigenetic programming mechanisms

Direct Developmental Exposure (DOHaD)

- Multiple chemicals across the lifespan
- EDCs in combination with other life stressors
- Sex differences

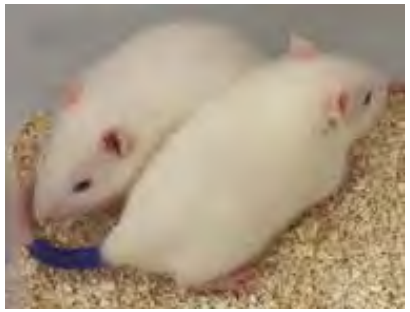
Overview of EDC effects in our DOHaD model

Neonates



- Body weight
- Anogenital distance
- Hypothalamic gene expression
- Hypothalamic protein expression
- Sex differences

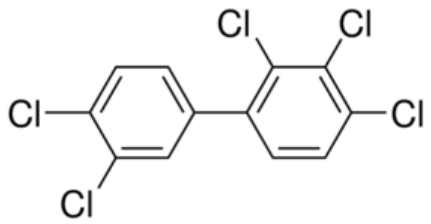
Adolescents



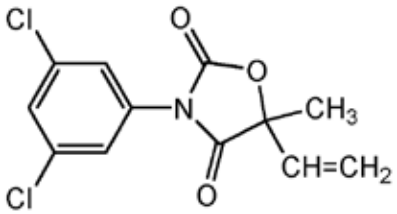
- Body weight
- Timing of puberty
- Hypothalamic gene expression
- Hypothalamic protein expression
- Social behaviors
- Sex differences

Adults & Aging

- Body weight
- Hypothalamic gene expression
- Hypothalamic protein expression
- Social behaviors
- Sexual behaviors
- Mate choice
- Reproductive aging
- Epigenetic marks (DNA methylation, microRNAs)
- Sex differences



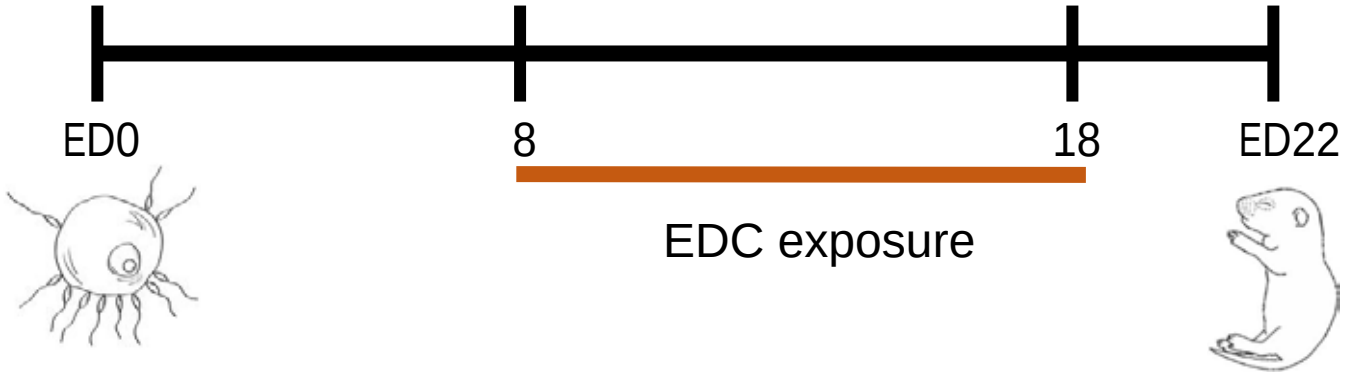
Aroclor 1221



Vinclozolin

-polychlorinated biphenyl (PCB)
-weakly estrogenic
-used as industrial lubricant
-dielectric fluid in transformers, capacitors
-banned 1979
-contaminated soil/water

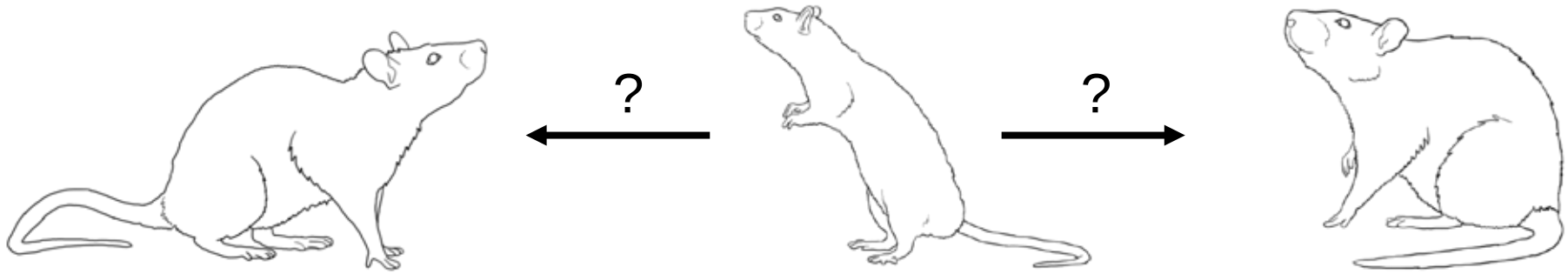
-dicarboxamide fungicide
-anti-androgenic
-used on vineyards, sod, fruits and vegetables
-banned EU, AUS (but not U.S.)
-contaminated food/drink



Prenatal EDCs: Effects on mate preference

A fundamental question is whether EDCs impair reproductive success.

Female rat prenatally
exposed to EDC or vehicle

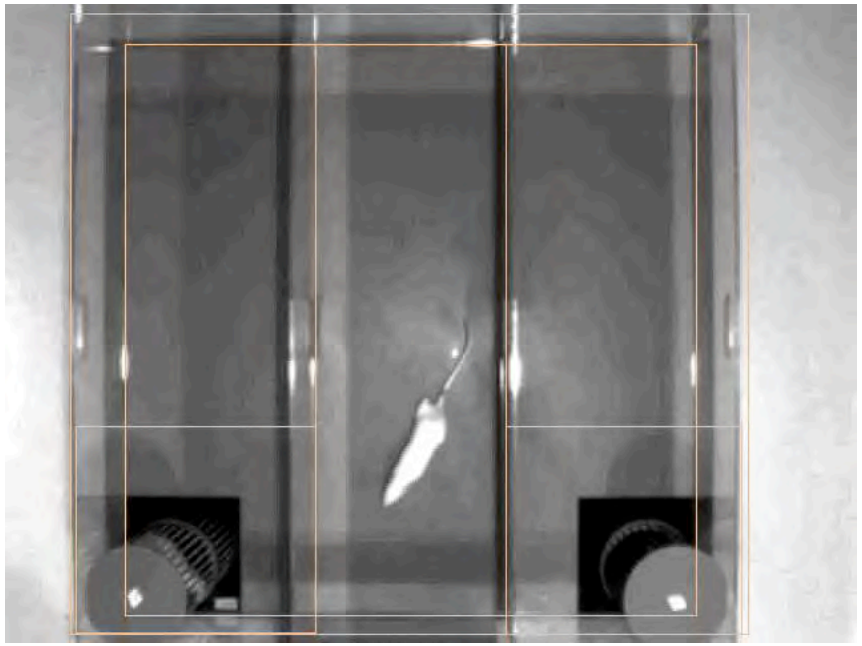


Male rat (no T)

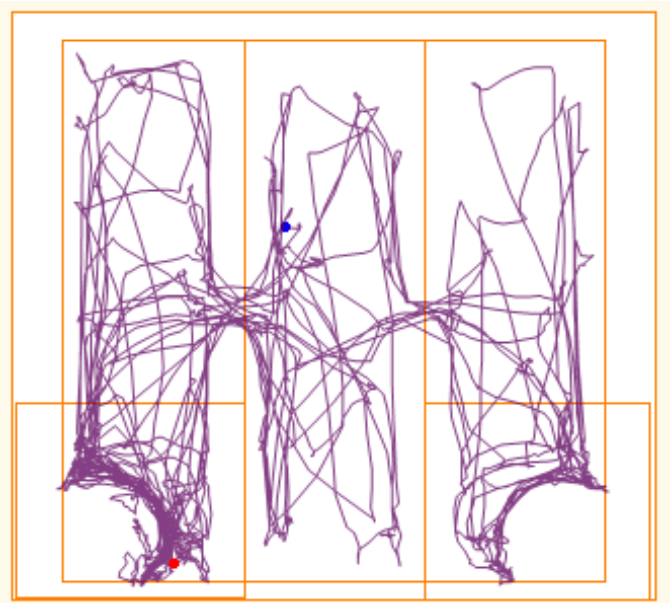
Male rat (with T)

Mate preference test

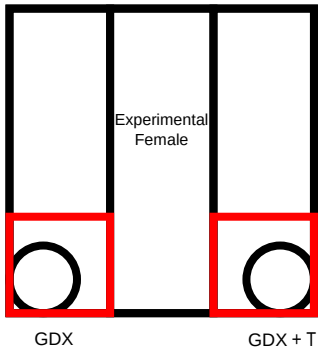
Male 1
(T)

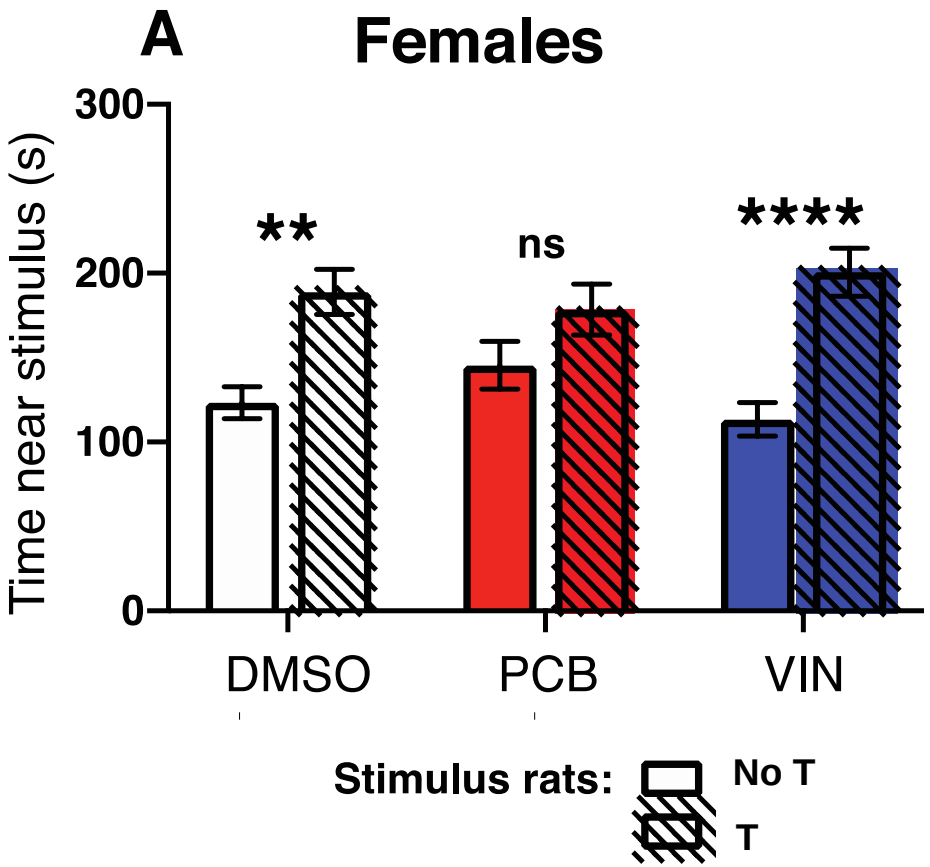


Male 2
(no T)



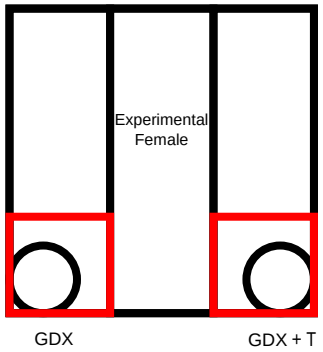
EDC treatment disrupts female mate preference



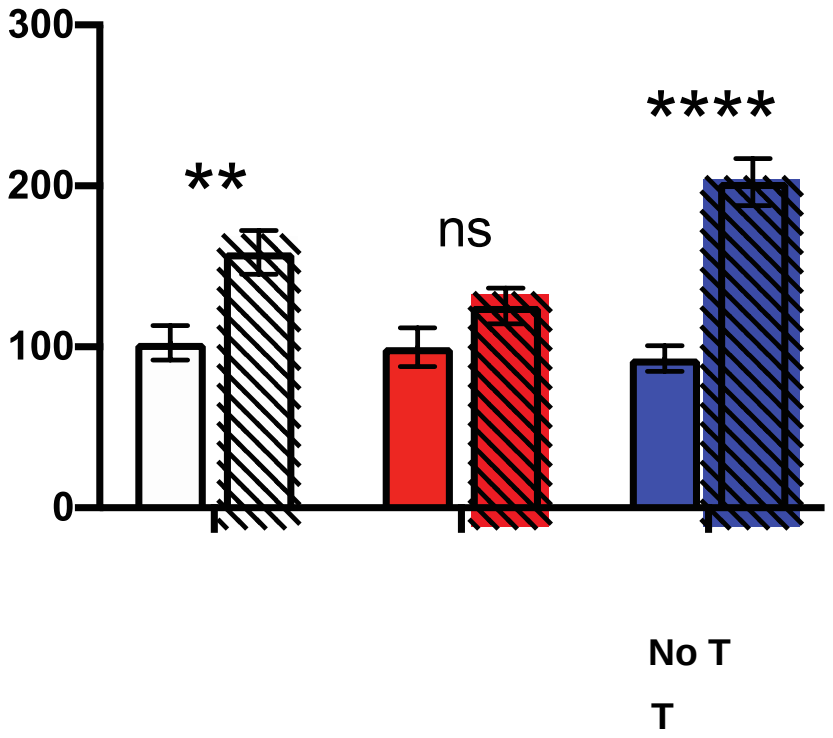


Hernandez Scudder... Gore, Endocrinology 161: bqaa124 (2020), doi: 10.1210/endocr/bqaa124

Deficits in odor preference explain disrupted mate preference

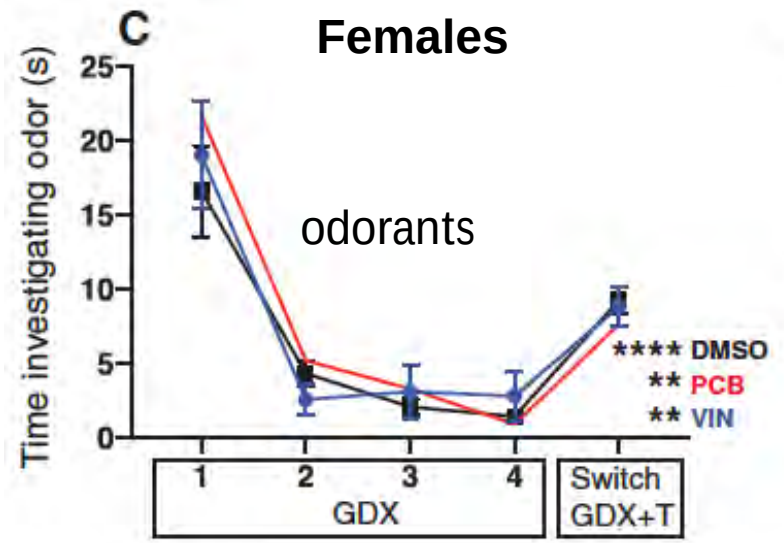


Urine-soaked filter paper



Does prenatal exposure to EDCs impair olfactory discrimination in adulthood?

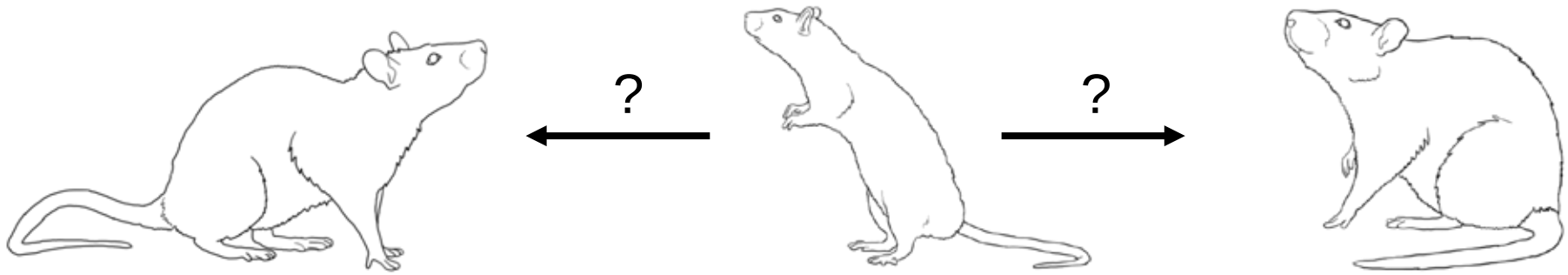
Habituation-Dishabituation



Hernandez Scudder *et al.*, Endocrinology 161: bqaa124 (2020), doi: 10.1210/endocr/bqaa124

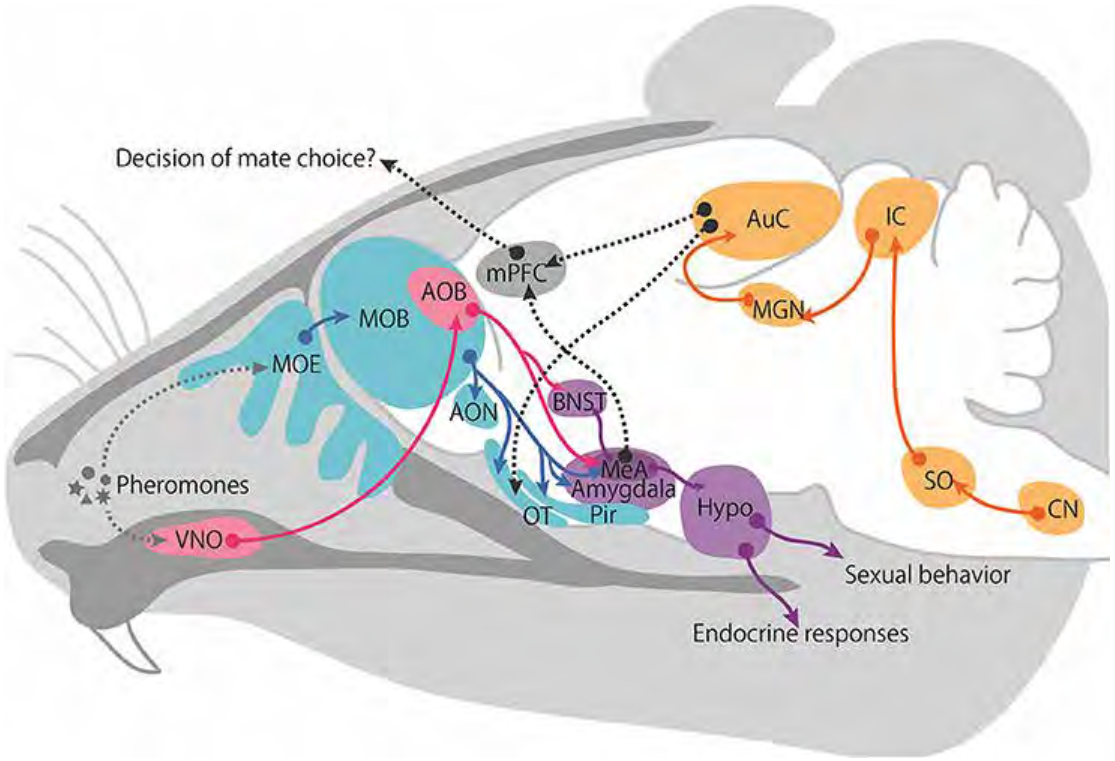
Summary (1)

Prenatal exposure to EDCs disrupts mate preference and odor preference behavior in adulthood, but not by means of impaired olfactory discrimination.



Sensory cues → Signal processing and decision making → Behavioral output

The effects of prenatal EDCs on olfactory processing



Hypothalamus: Ventromedial nucleus (VMNvl) and Preoptic area (POA)

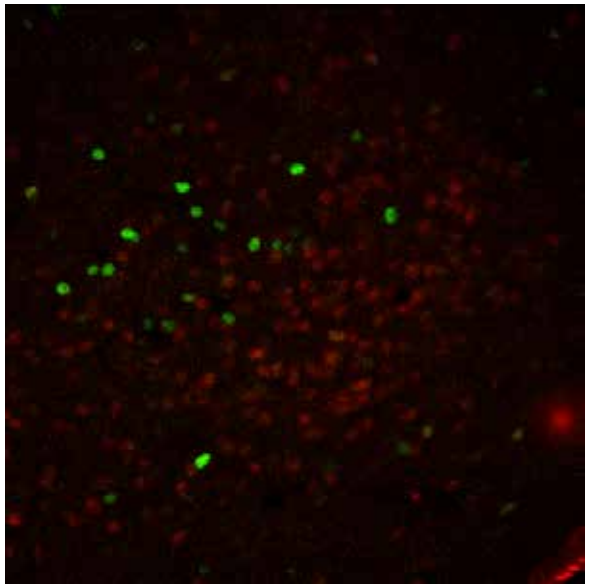
Piriform cortex (anterior, posterior)

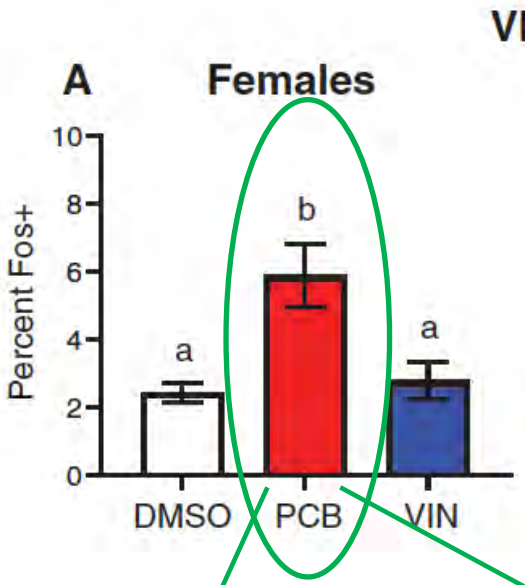
Medial amygdala (posterodorsal, posteroventral)

Fos immunoreactivity as a marker of neuronal activation 1 hr after the odor preference test

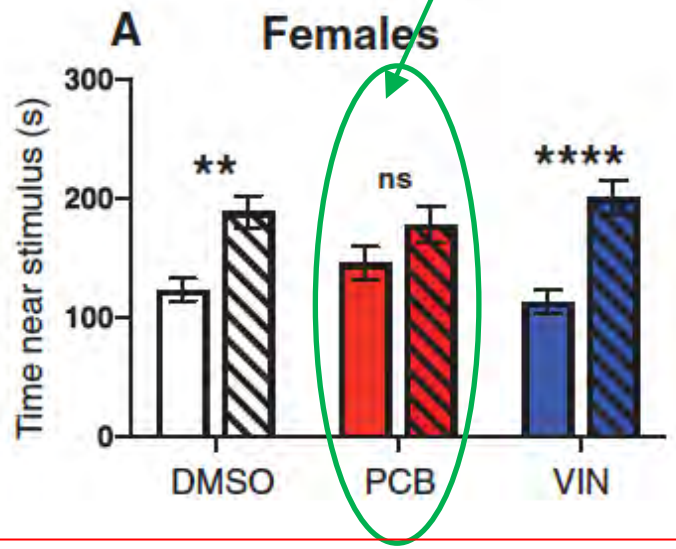
from Akari *et al.* Frontiers in Neuroscience, 2014.

The VMNvl is the only region affected by EDCs, and only in females

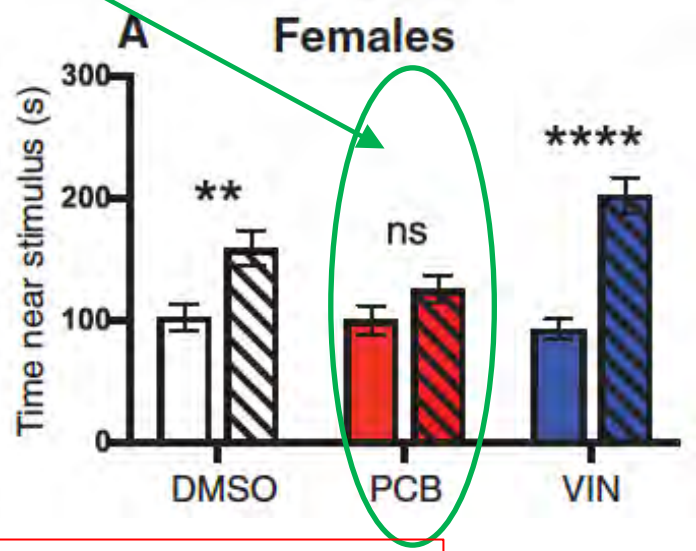




Mate Preference



Odor Preference



No treatment effect in POA, piriform cortex, amygdala.

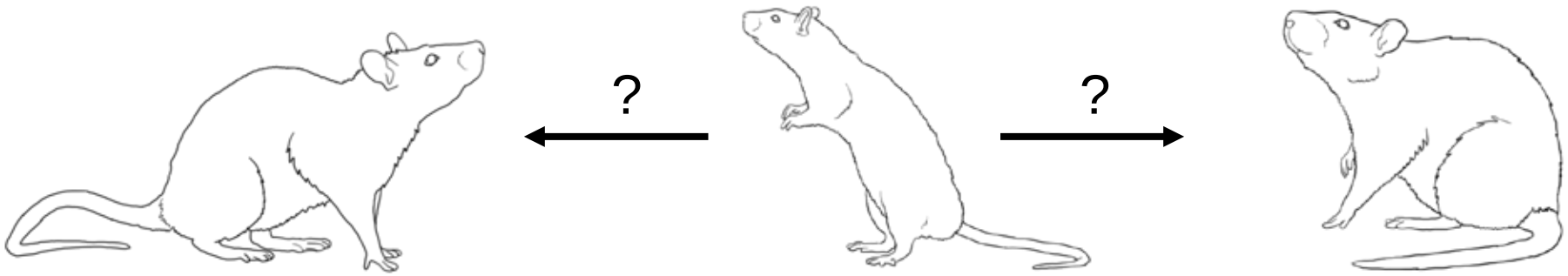
Sex difference in the cortex (F>M).

Hernandez Scudder *et al.*, Endocrinology 161: bqaa124 (2020), doi: 10.1210/endocr/bqaa124

Summary (2)

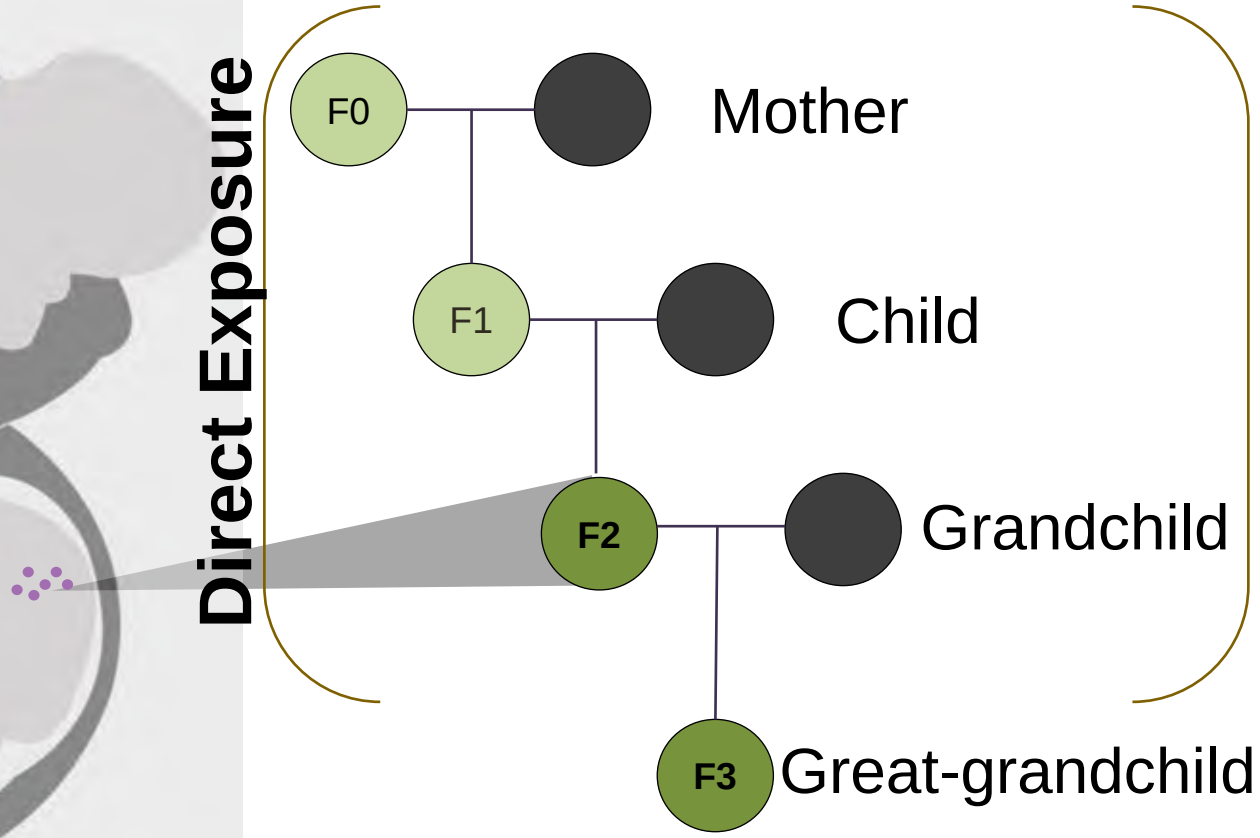
Prenatal exposure to EDCs disrupts mate preference and odor preference behavior in adulthood, but not by means of impaired olfactory discrimination.

Deficits in mate and odor preference behavior are associated with increased Fos activation in the VMNvl in females, but not males.



Multigenerational EDC effects

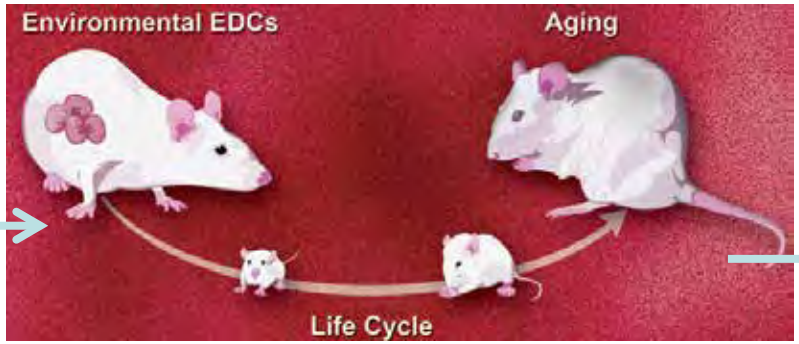
When a pregnant woman is exposed, so is her fetus (F1) and its germ cells (F2)



Gore & Patisaul, 2010

Multigenerational EDC Effects

Developmental exposure *in utero*



Future Generations

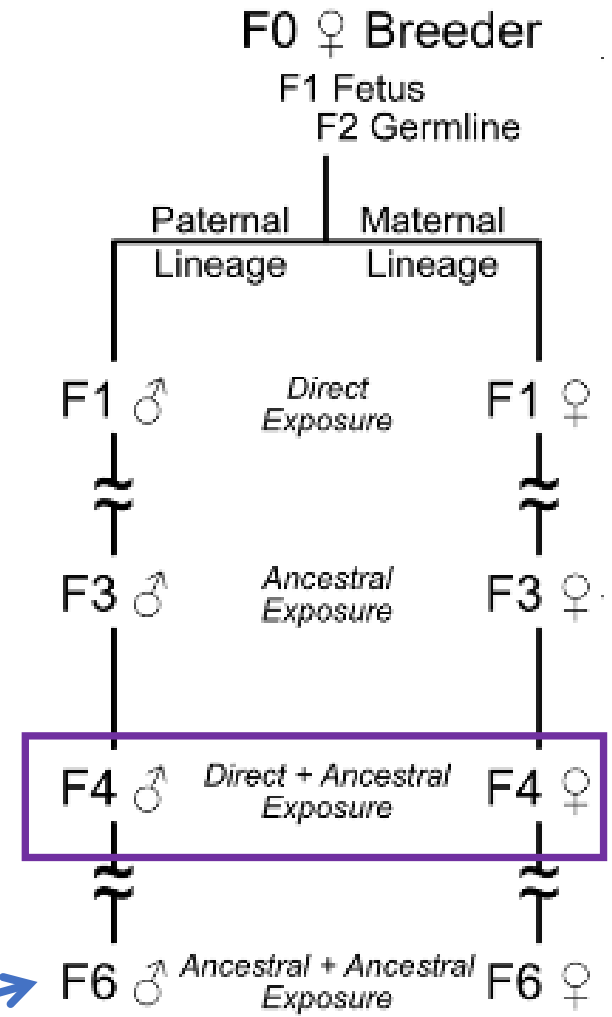
- Legacy vs. contemporary EDCs with different mechanisms
- Combination of direct and heritable effects
- Importance of lineages (maternal, paternal)
- Sex differences
- Epigenetic programming mechanisms

"Two hits of EDCs, 3 generations apart"

Two hits of EDCs 3 generations apart

Expose Prenatally
- DMSO vehicle
- PCBs (A1221)
- Vinclozolin

Expose Prenatally
- DMSO vehicle
- PCBs (A1221)
- Vinclozolin



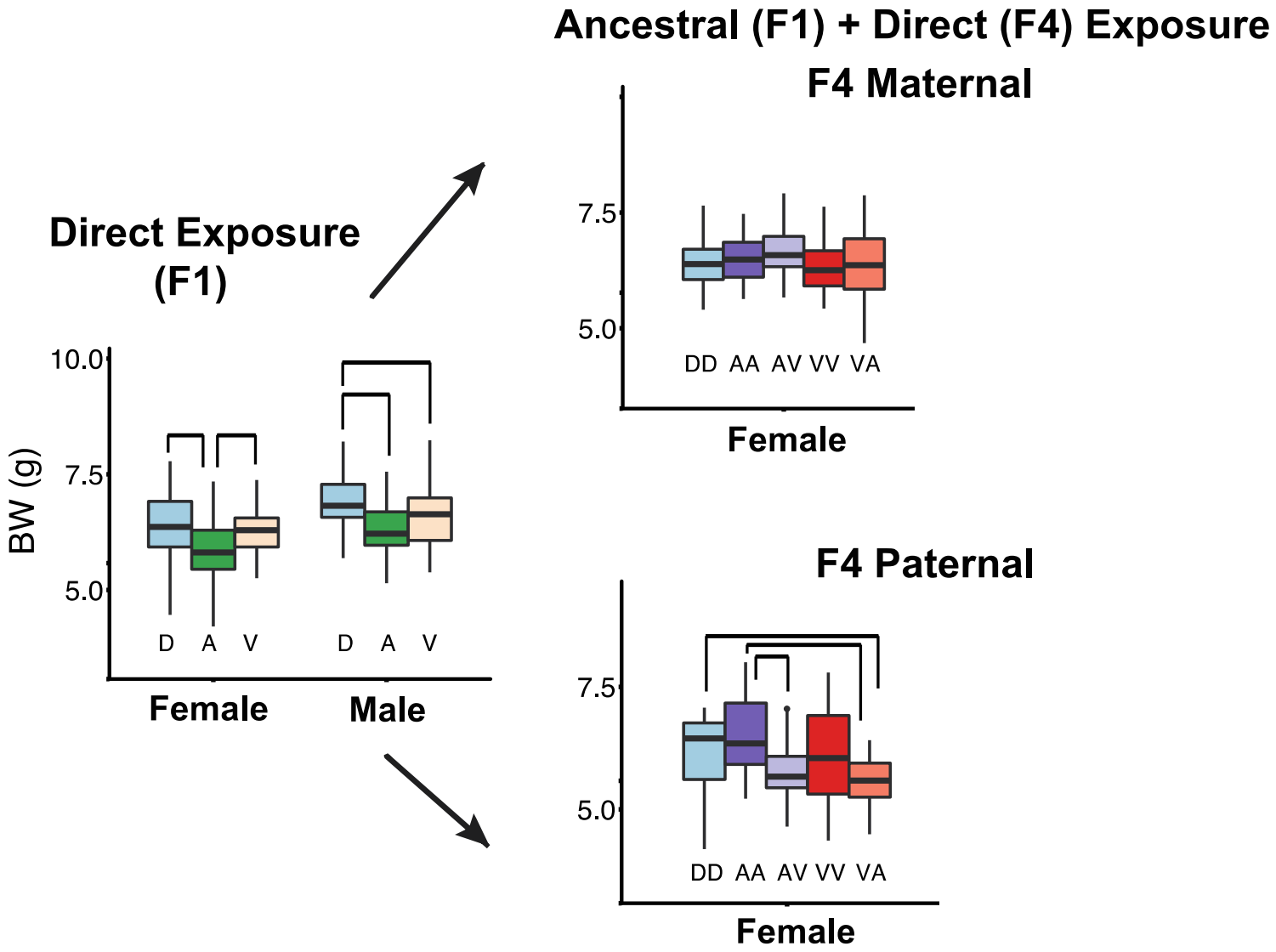
Direct Prenatal Exposures

Ancestral Exposures

Ancestral + Direct Prenatal Exposures

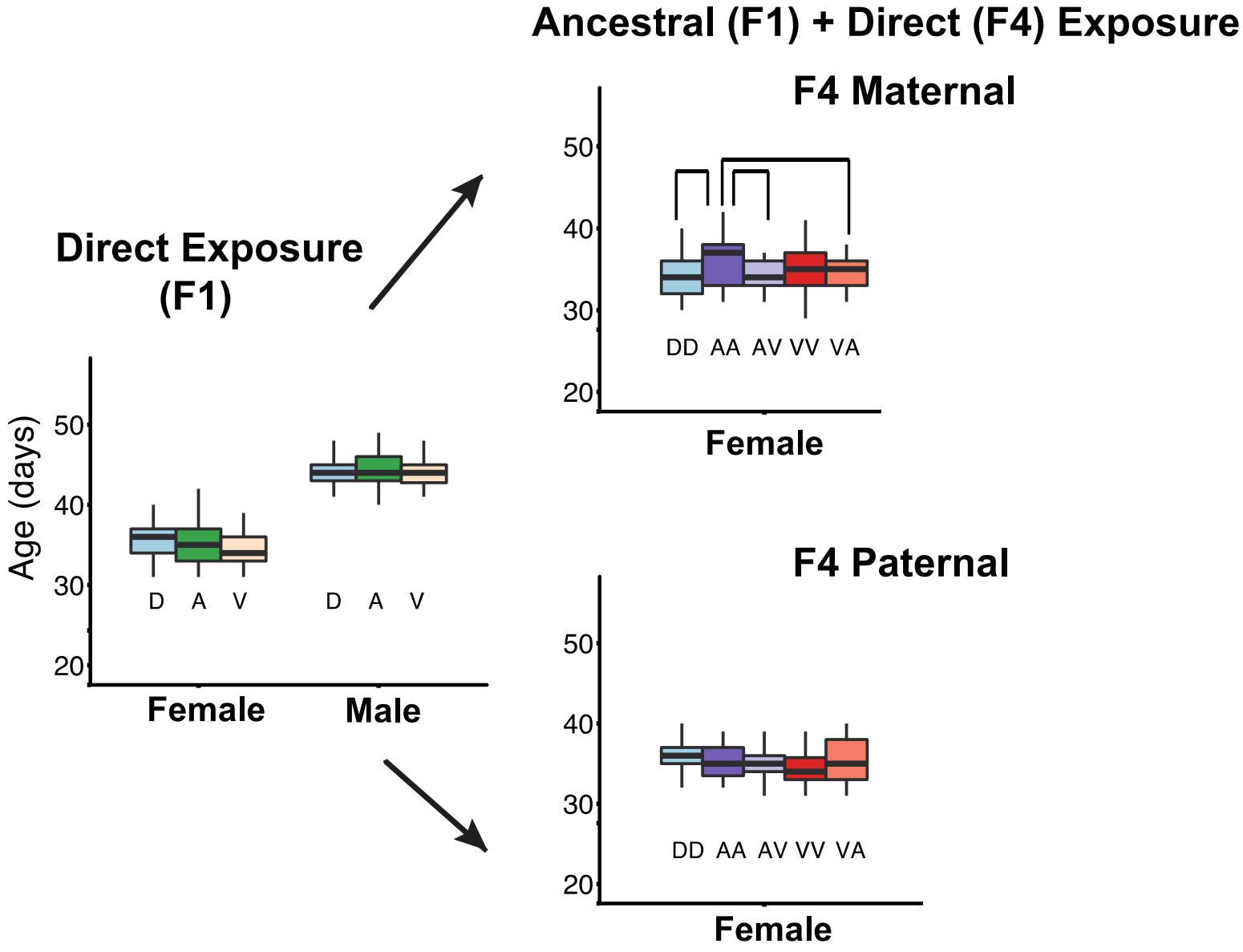
Double Ancestral Exposures

Body Weight on P1



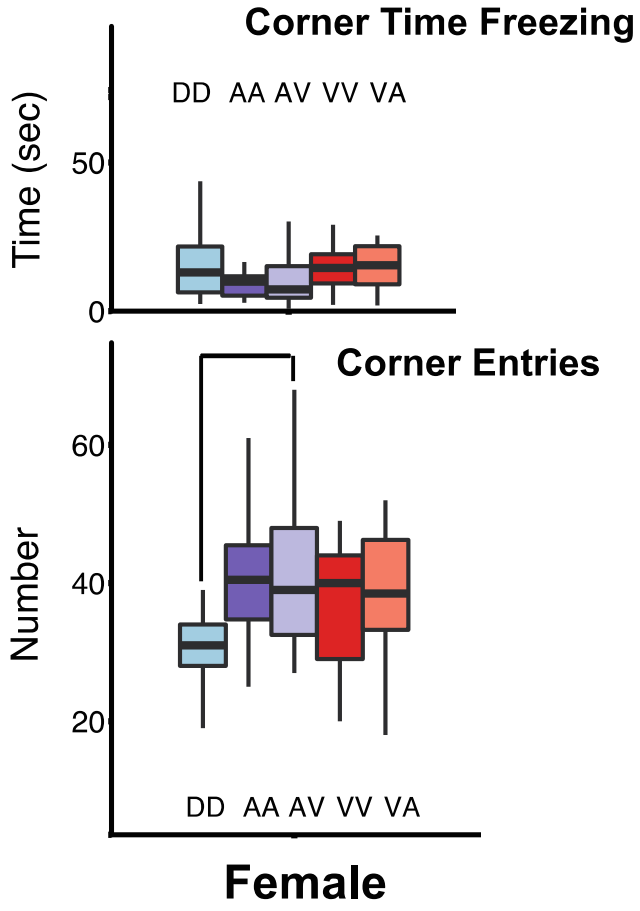
Ross Gillette, Lindsay Thompson, David Crews

Age at Puberty



Open Field (adult)

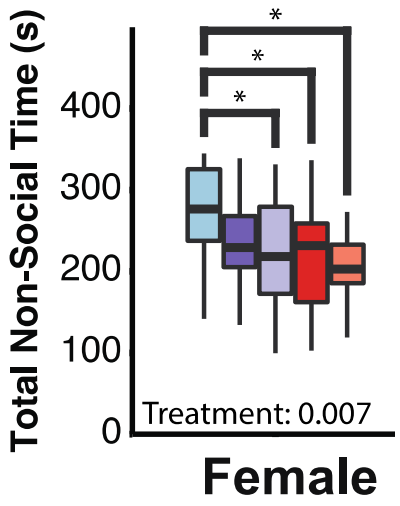
Ancestral (F1) + Direct (F4) Exposure
F4 Maternal



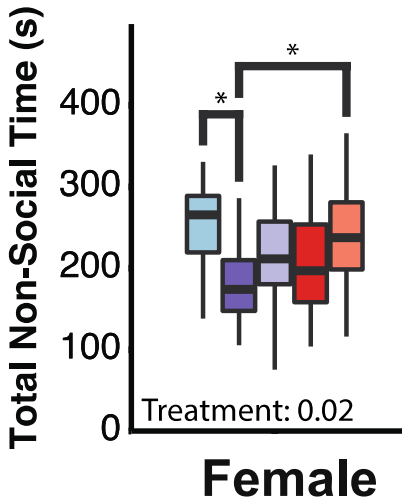
Social Interaction Dynamics (adult)

Ancestral (F1) + Direct (F4) Exposure

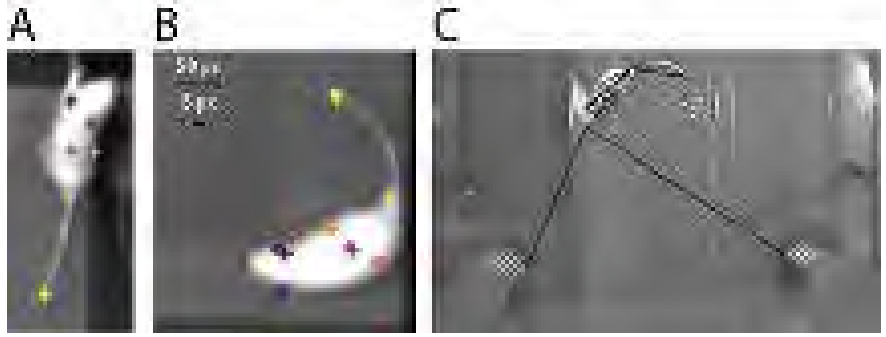
F4 Maternal



F4 Paternal



Dr. Ross Gillette



Gillette, Dias… Gore, Toxics 10: 30 (2022)

Summary (3) – “2 Hits of EDCs 3 Generations Apart”

- Each generation has a unique phenotype in response to EDC exposures.
  - *“Emergent phenotypes” caused by EDCs: there may be no F1 phenotype, but a phenotype emerges, especially after a 2nd hit in the F4 generation.*
- The nature of the second hit (same vs. different EDC) is a key determinant of the outcome.
  - *Ecological relevance: as new EDCs come onto the market, this is overlaid on our historical exposures.*
- Virtually all outcomes, in all generations, were sexually dimorphic.
- Lineage was a key determinant in outcomes.

Take-home messages and Future directions

EDCs affect women's health: this includes reproductive health, as well as other endocrine and neurological domains.

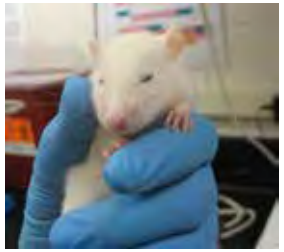
Sex differences are common for EDC effects, underscoring the importance of including females in all studies.

Individuals experience multiple environmental perturbations throughout their life histories such as EDCs and other stressors. The combination of these experiences shapes the individual's phenotypes.

Ancestral epigenetic processes add to the complexity by shaping the response to current and future environmental challenges. This is modeled in the "2 hit, 3 generations apart" paradigm.

Our future direction is to pursue how the transfer of epigenetic information from germline to somatic tissues such as the brain can happen, and result in neurobehavioral changes.

Thank you!



Our happy rats

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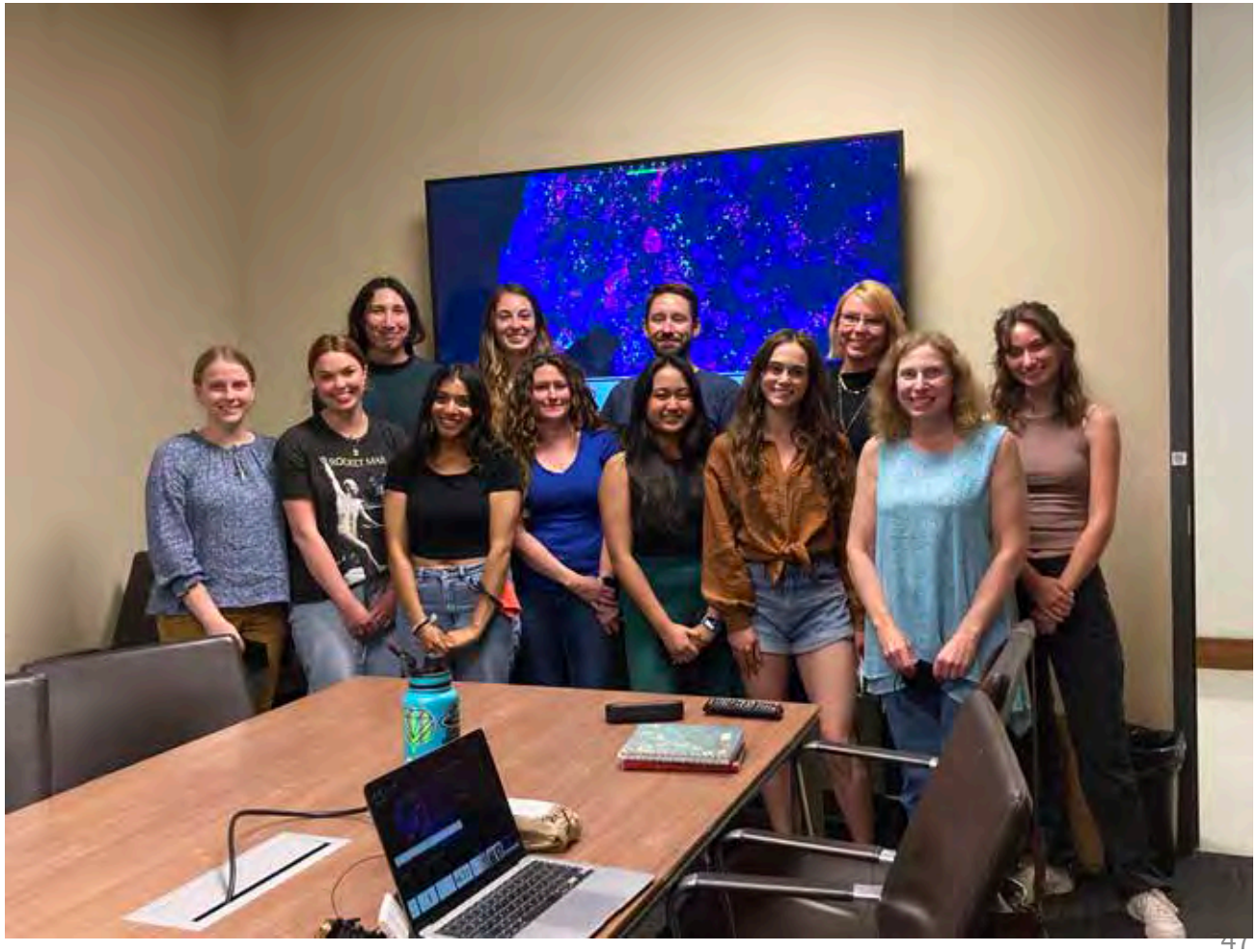
Dr. Ross Gillette
Dr. Emily Hilz
Lindsay Thompson
Madeline Streifer
Dana Sheinhaus

Collaborators:
Dr. David Crews

Everyone else in the Gore Lab, but especially our wonderful undergraduates



NIH Research Panel

Anne Marie Jukic, Ph.D., Francesco DeMayo, Ph.D.,
Carmen Williams, M.D., Ph.D., Janet Hall, M.D., M.S.

Moderated by Keiva Nelson

NIH Research Panel

Anne Marie Jukic, Ph.D.

Investigator, Fertility and Reproductive Health Group

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SCAN ME

We're hiring! Postdoctoral fellowships are available:
https://www.training.nih.gov/postdoc_jobs_nih/view/_31/9294/NIEHS_FRH_PD

NIH Research Panel

Francesco DeMayo, Ph.D.

Chief, Reproductive and Developmental Biology Laboratory

National Institute for Environmental Health Sciences

Molecular Analysis of Uterine Function

Francesco DeMayo Ph.D.

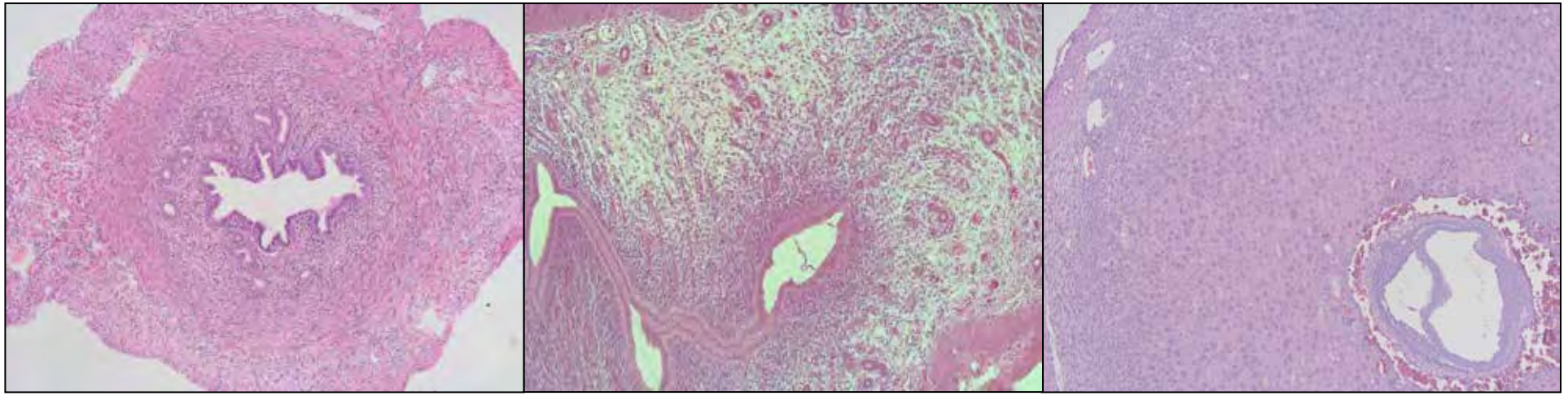
Endocrine Disrupting Chemicals and Women's Health Symposium
Virtual
July 18-19, 2023

Environment and the Female Reproduction

- The female reproductive tract is the most sensitive organ to endocrine signaling.
- Dysfunctions of the uterus such as Infertility, Endometriosis, Endometrial Cancer, Fibroids and Preterm Birth may be the result of hormone deregulation.
- Endocrine disruptors, stress, inflammation and diet can result in infertility, preterm birth and impairment of women's health.

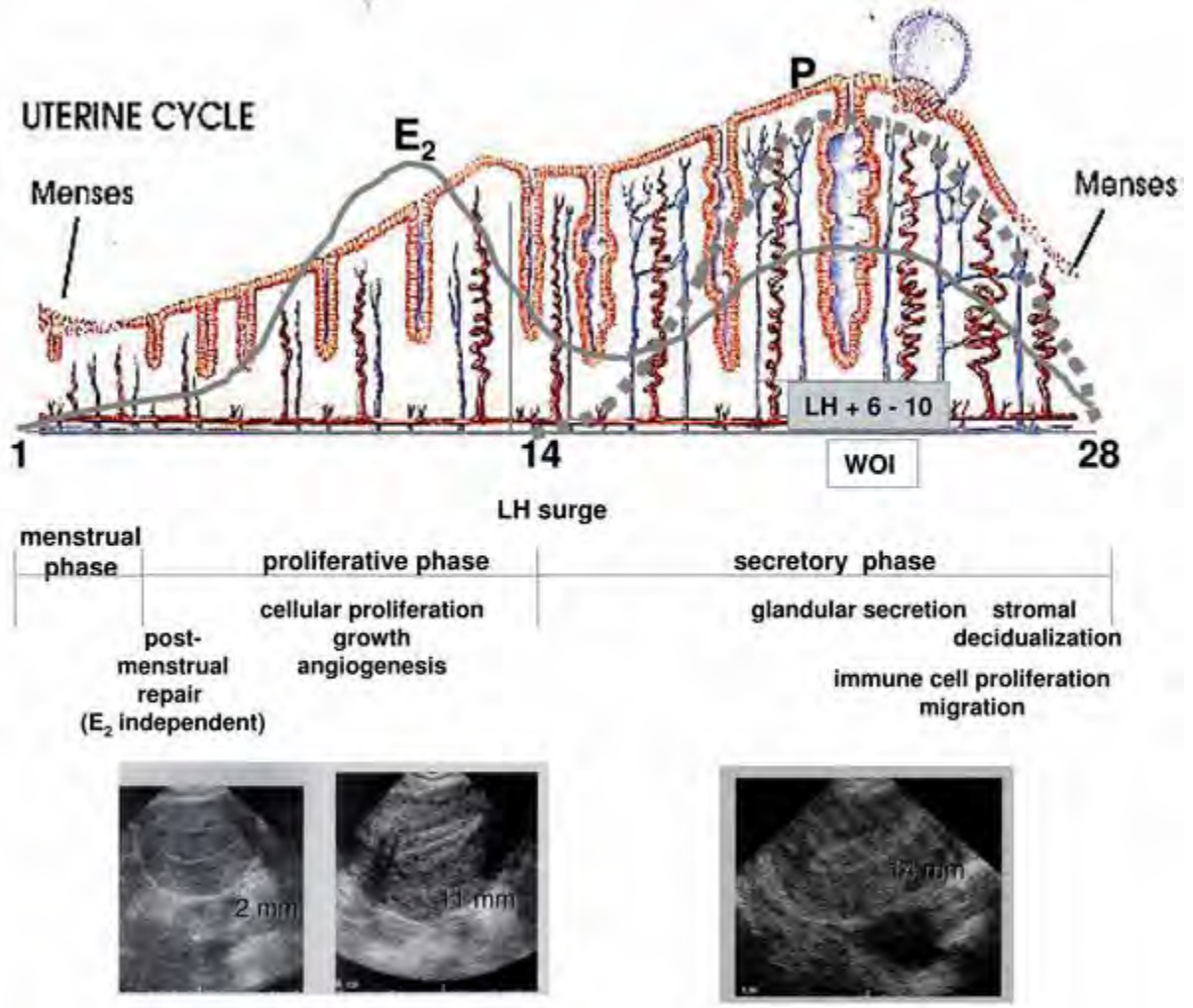
Rashtian et al., 2019 Reprod. Biol and Endo., Bala et al., 2020 Reprod. Sci

The Uterus Undergoes Dynamic Changes During Pregnancy



Nonpregnant Uterus | Peri-implantation Uterus | Decidualized Uterus

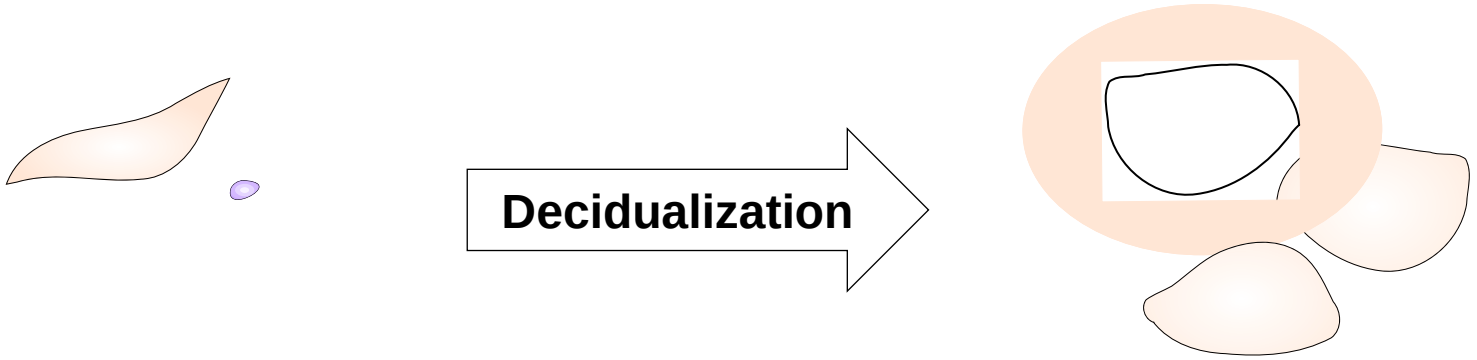
Changes in the human uterus during the Menstrual cycle.



Giudice, Reproductive Biology and Endocrinology (2006) 4(Supp 1):84

Decidualization

- **Endometrial stromal cells differentiate into decidual cells, which serve several functions during early pregnancy:**
  - **Support embryo**
  - **Regulate trophoblast invasion**
  - **Enhance vascularization**
  - **Modulate maternal immunity**



Human endometrial stromal cells (HESCs)

Decidual cells

Regulation of Human Endometrial Stromal (HES) Cell Decidualization

Isolate primary HES cell from 3 different patients

↓

Induce Decidualization
(50 mM db-cAMP, 10nM E2, 1mM MPA)

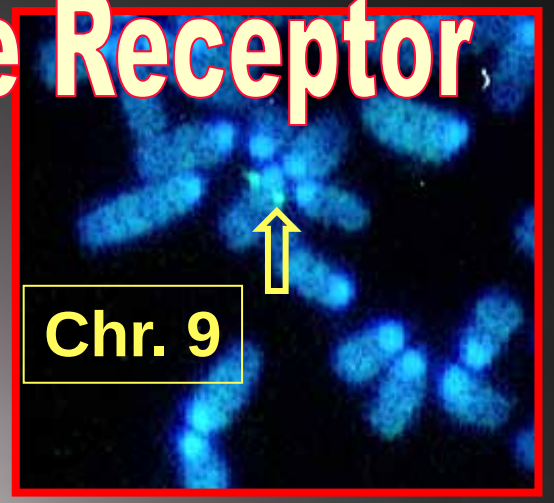
↓

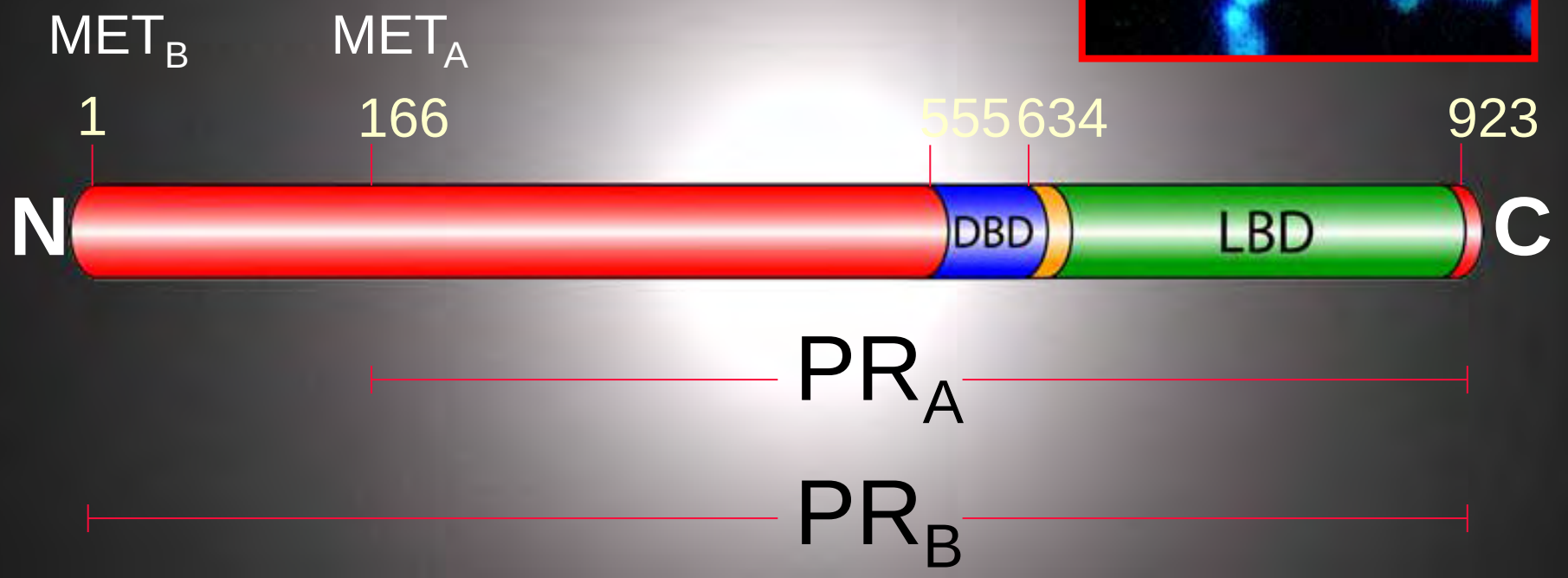
Change in morphology and increased expression of marker proteins IGFBP1 and PRL

Progesterone: the Hormone of Pregnancy

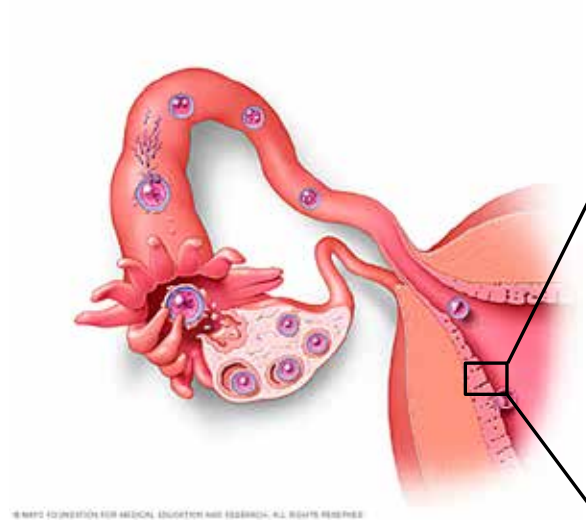
- Progesterone acts through its cognate receptors PGRA and PGRB.
- Regulates the timing of the embryo transport to the site of implantation.
- Negates the mitogenic actions of Estrogen on uterine epithelium.
- Integral part of the paracrine cross talk between epithelium and stroma that regulates uterine function.
- Regulates the ability of the uterus to support embryo invasion and growth.
- Regulates myometrial quiescence and parturition.
- **Regulates the “Window of Receptivity”.**

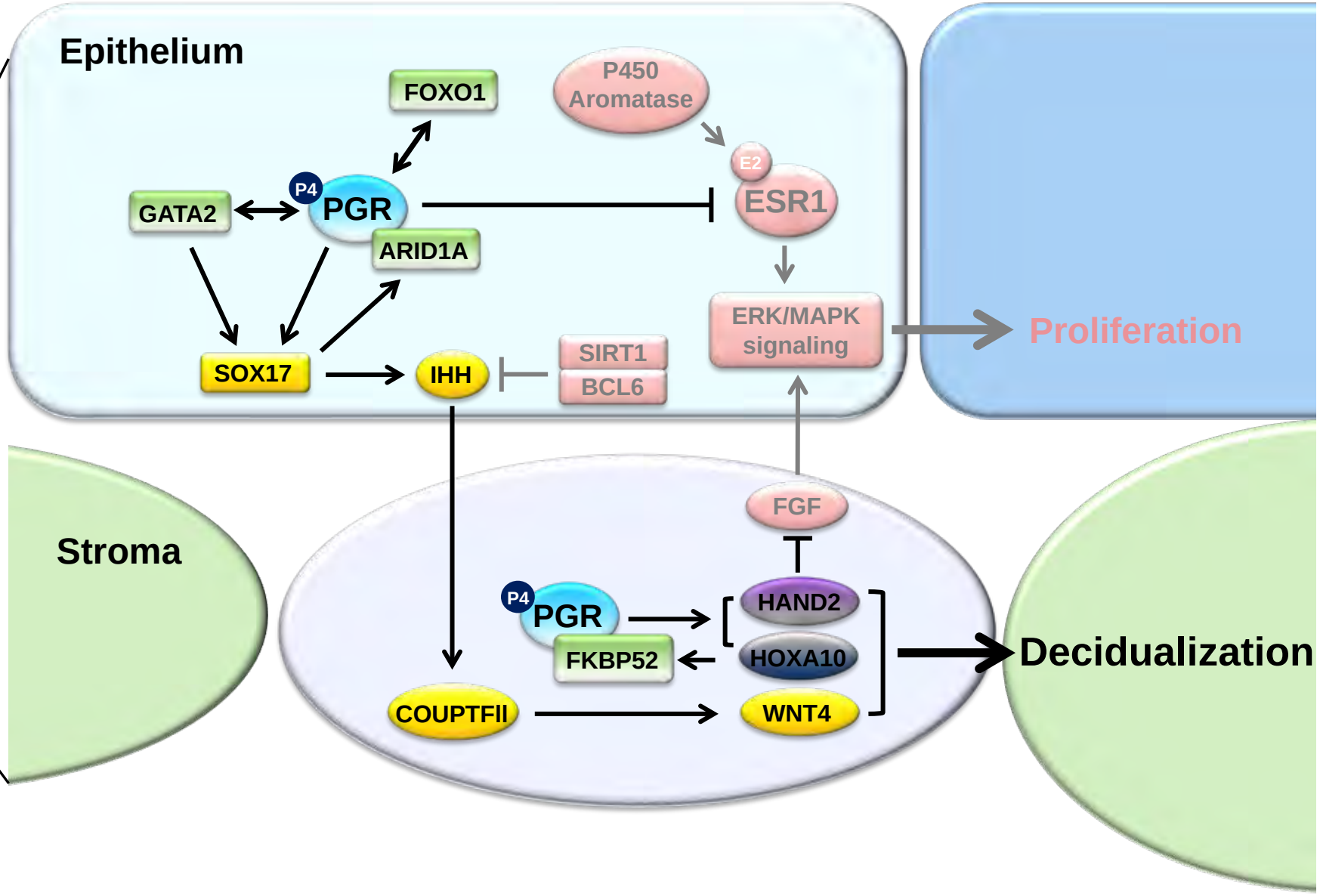
The Murine Progesterone Receptor





Progesterone Receptor (PGR) Function in the Receptive Endometrium





Marquardt et al., *IJMS*. 2019

Serum Response Factor (SRF) as a Potential PGR Co-regulator in the Uterus

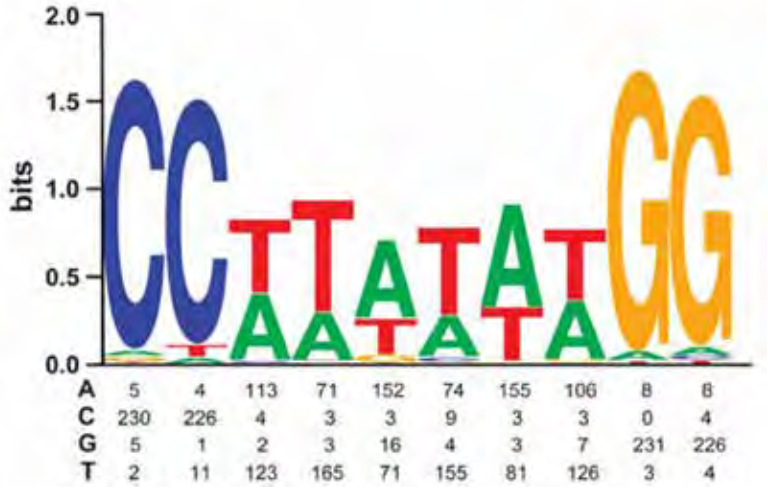
The **SRF binding motif (CArG) is enriched in PGR binding intervals** in term pregnant and non-pregnant human myometrial samples based on PGR ChIP-seq

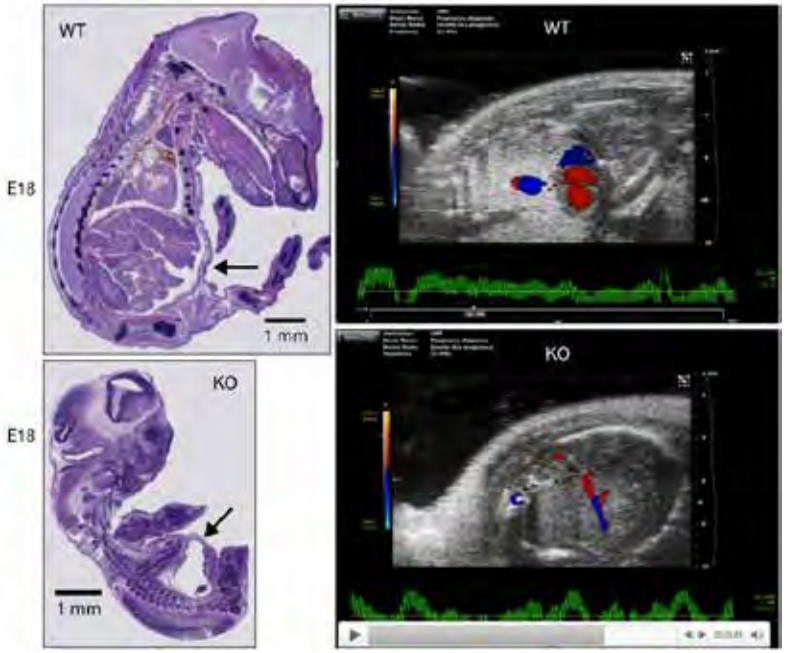
Motif		Log *p*-value of enrichment	
		NP	TP
	PGR	-225.8	-1445
	CArG	-127.1	-66.32

NP: nonpregnant myometrial tissue
TP: term pregnant myometrial tissue

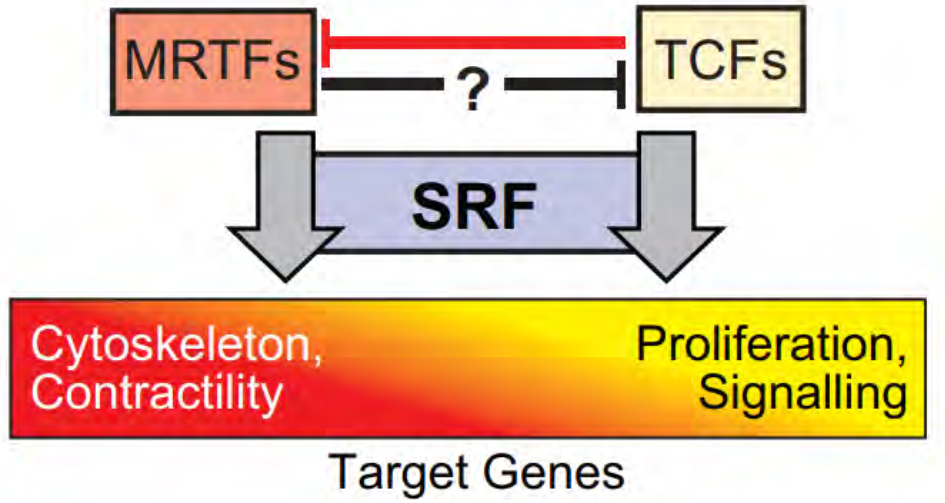
Wu et al., *FASEB J*. 2020

SRF Molecular Function





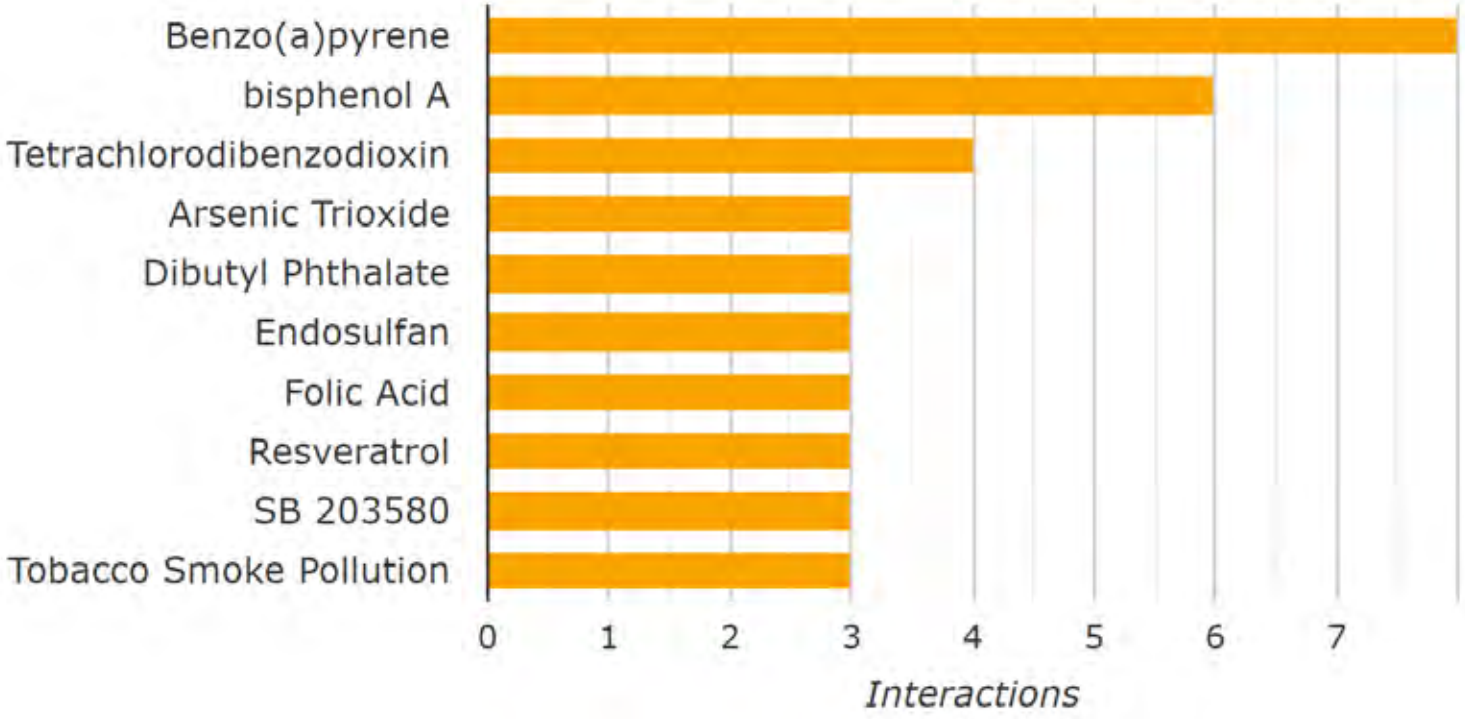
- Widely-expressed transcription factor from the “MADS-box” protein family (Miano, 2010).
- Binds a consensus DNA element CC(A/T-rich)$_6$GG, referred to as the “CArG box” (Miano, 2010).
- Essential for prenatal development of the GI tract and heart (Park et al., 2015).
- Transcriptional output is determined by its cofactors (Gualdrini et al., 2016).



Miano, *Lab. Invest.* 2010; Park et al., *J. Neurogastroenterol. Motil.* 2015; Gualdrini et al., *Mol. Cell* 2016

SRF and the Environment

Top Interacting Chemicals (10/81)



40% (32/81) of known SRF-interacting chemicals also interact with PGR.

ctd *Illuminating how chemicals affect human health.*

Comparative Toxicogenomics Database

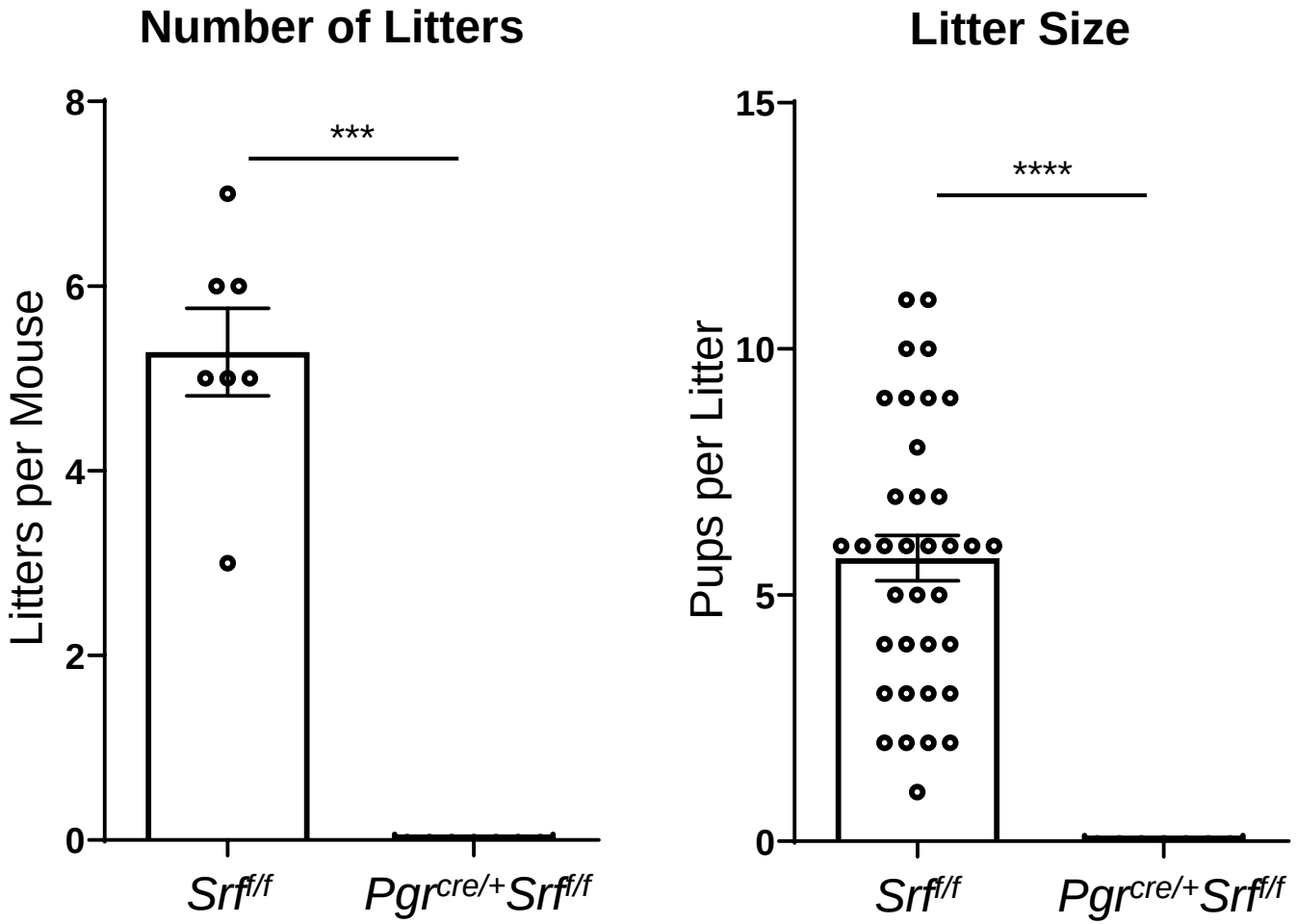
ctdbase.org; wordclouds.com

Hypothesis:

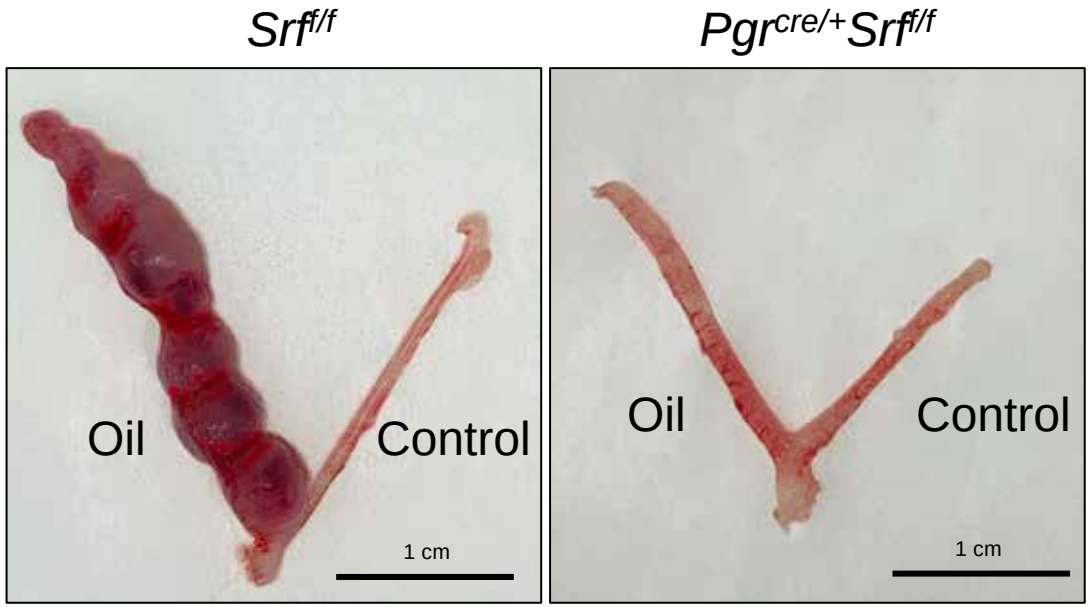
SRF collaborates with PGR to regulate the transcriptional environment needed for uterine function.

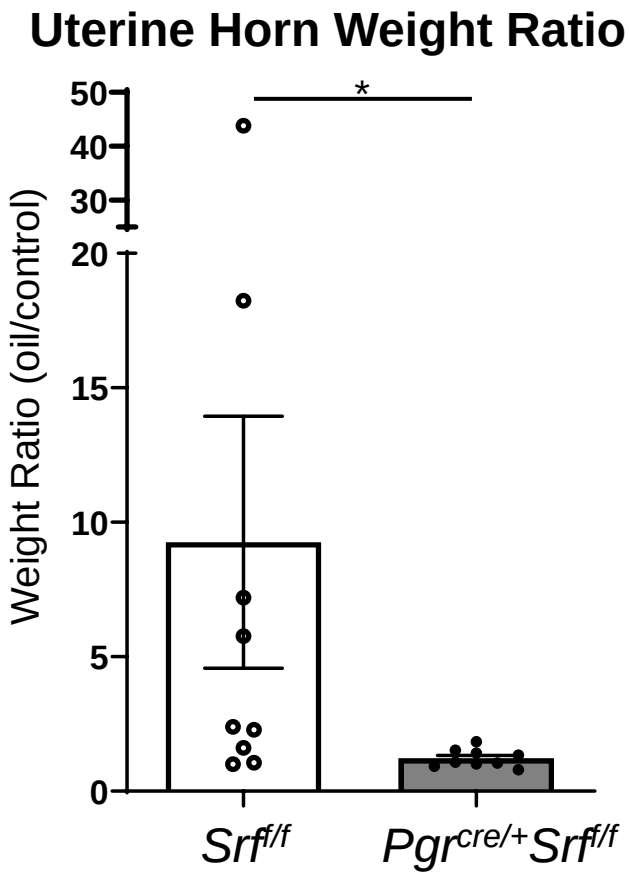
Pgr$^{cre/+}$*Srf*$^{f/f}$ Females are Infertile

Six-Month Fertility Trial



SRF Loss Results in a Decidualization Defect in *Pgr*$^{cre/+}$*Srf*$^{f/f}$ Mice

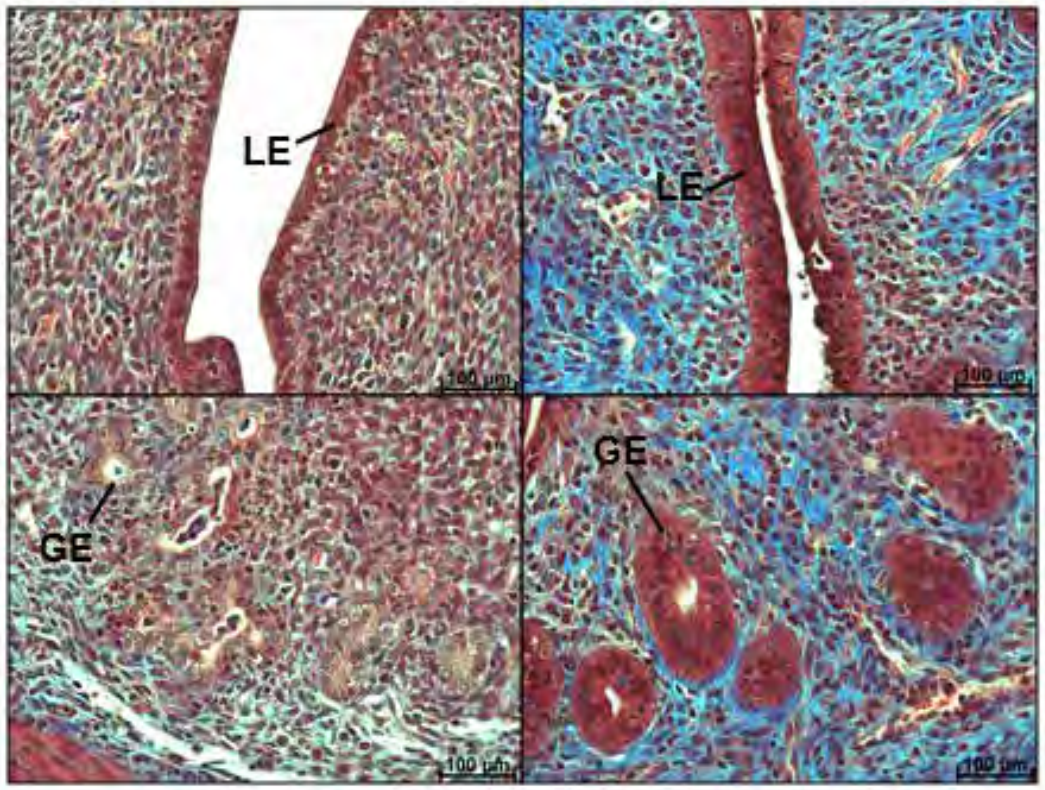




SRF Loss Results in Uterine Fibrosis in *Pgr*$^{cre/+}$*Srf*$^{f/f}$ Mice

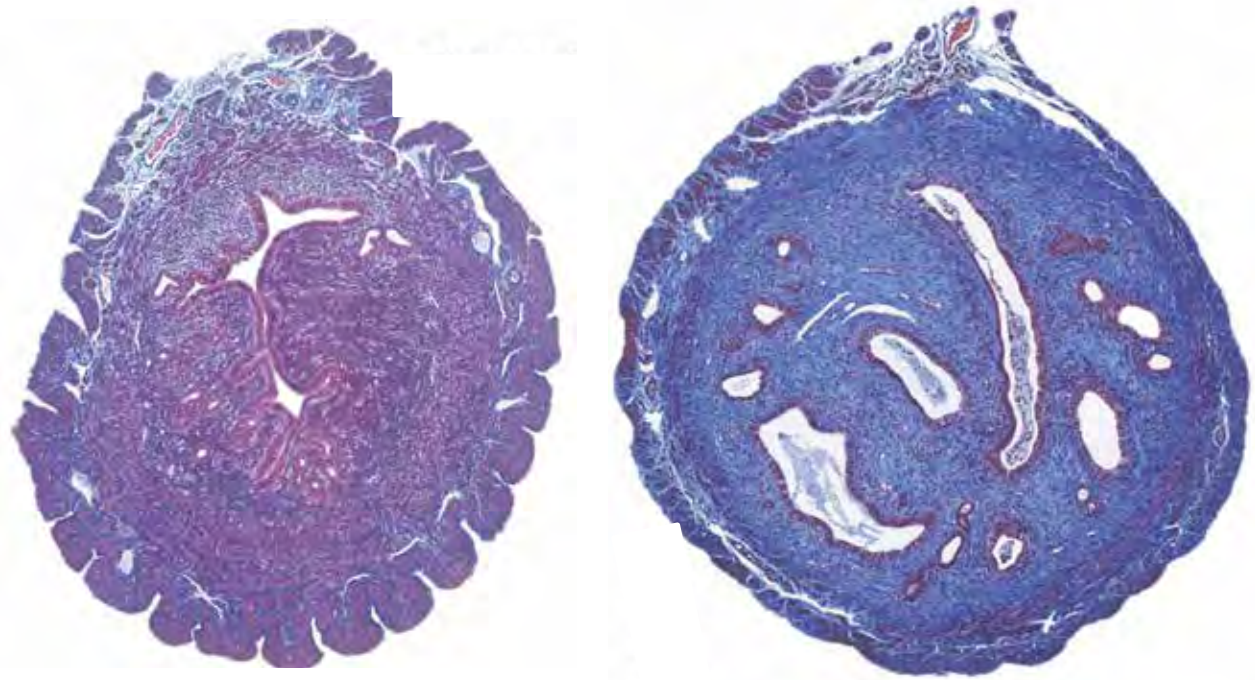
11-Week Old Uterus (Masson Trichrome)

Srf$^{f/f}$ *Pgr*$^{cre/+}$*Srf*$^{f/f}$



Post Breeding Trial Uterus (Masson Trichrome)

Srf$^{f/f}$ *Pgr*$^{cre/+}$*Srf*$^{f/f}$

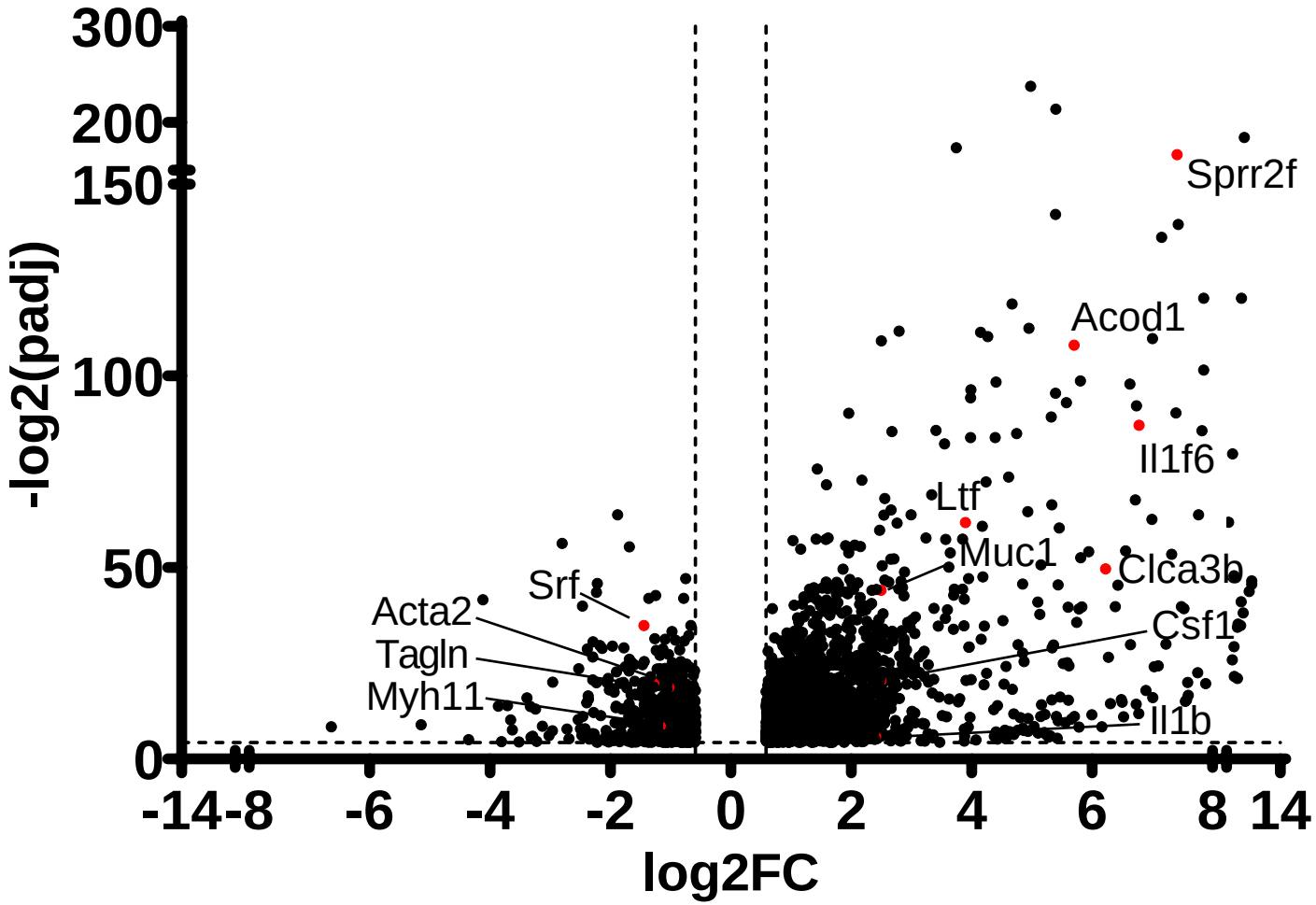


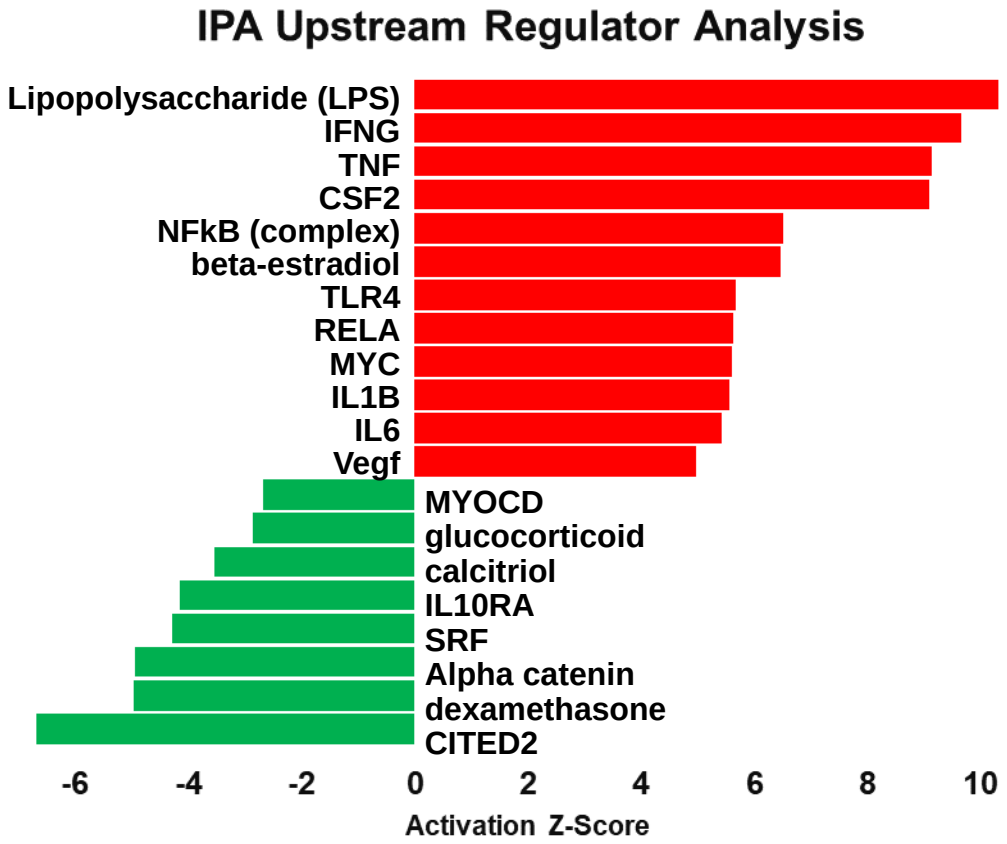
Olivia Emery

Hormone-independent Effects of SRF Loss on Uterus

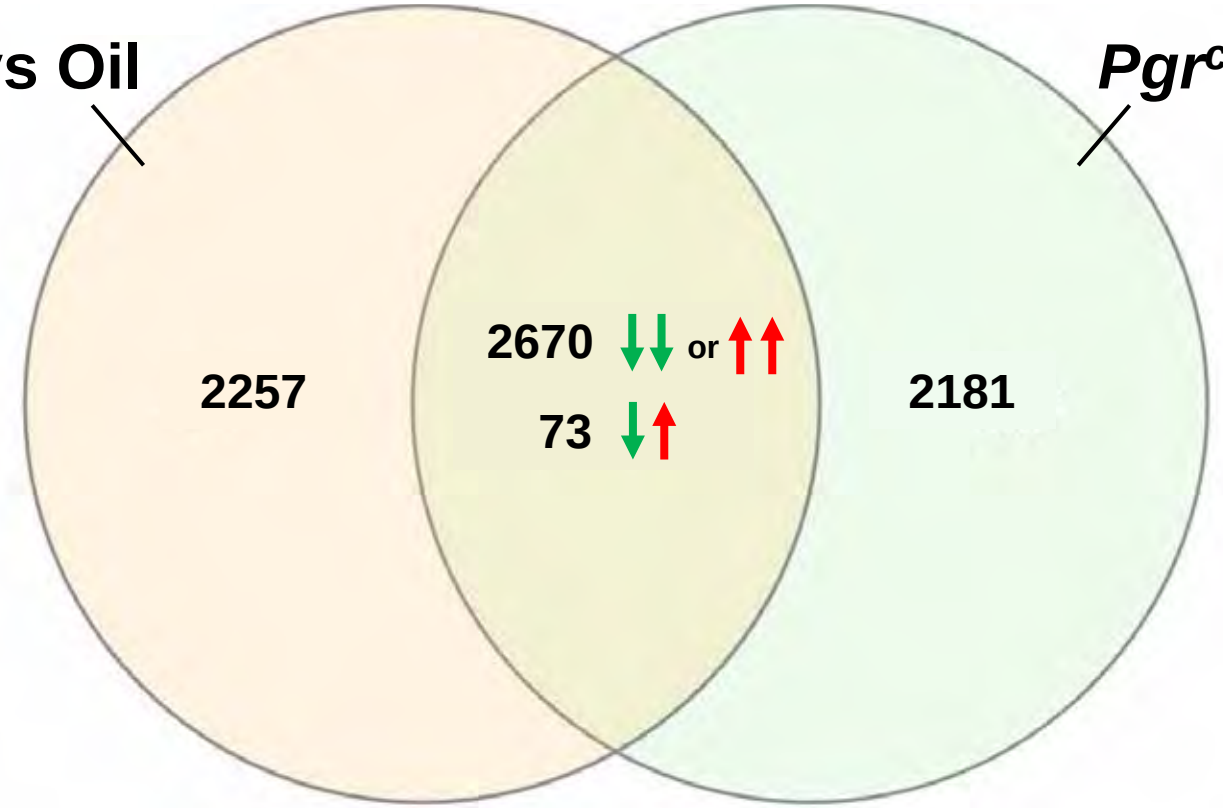
Oil-treated *Pgr*$^{cre/+}$*Srf*$^{f/f}$ vs Oil-treated *Srf*$^{f/f}$

2593 DEG (Padj<0.05; [FC]>1.5): 1635 ↑ 958 ↓



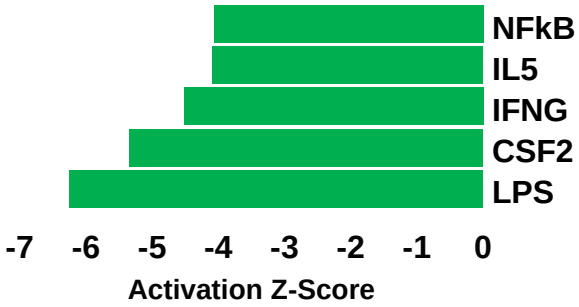


SRF Loss Switches P4 Response to Pro-Inflammatory



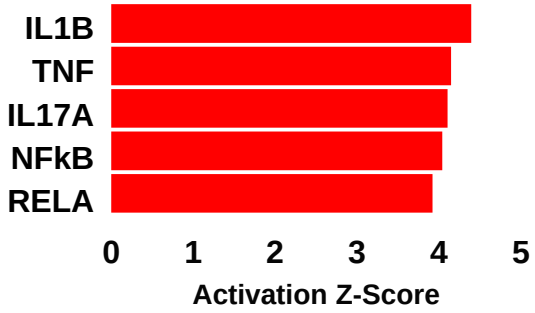
Loss of induction (2257)

Upstream Regulators



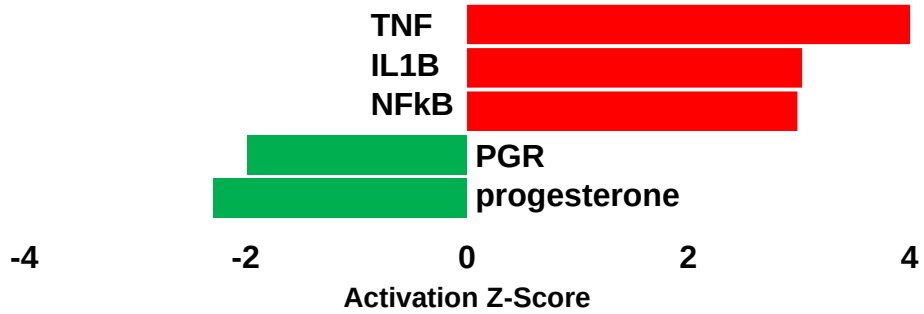
Gain of induction (2181)

Upstream Regulators



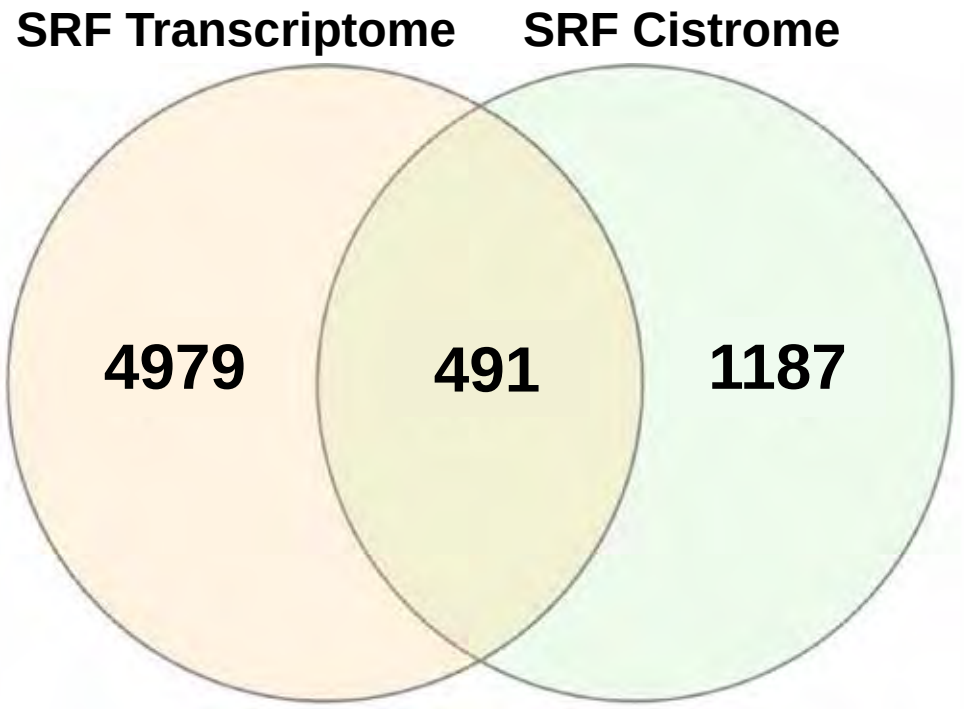
Opposite induction (73)

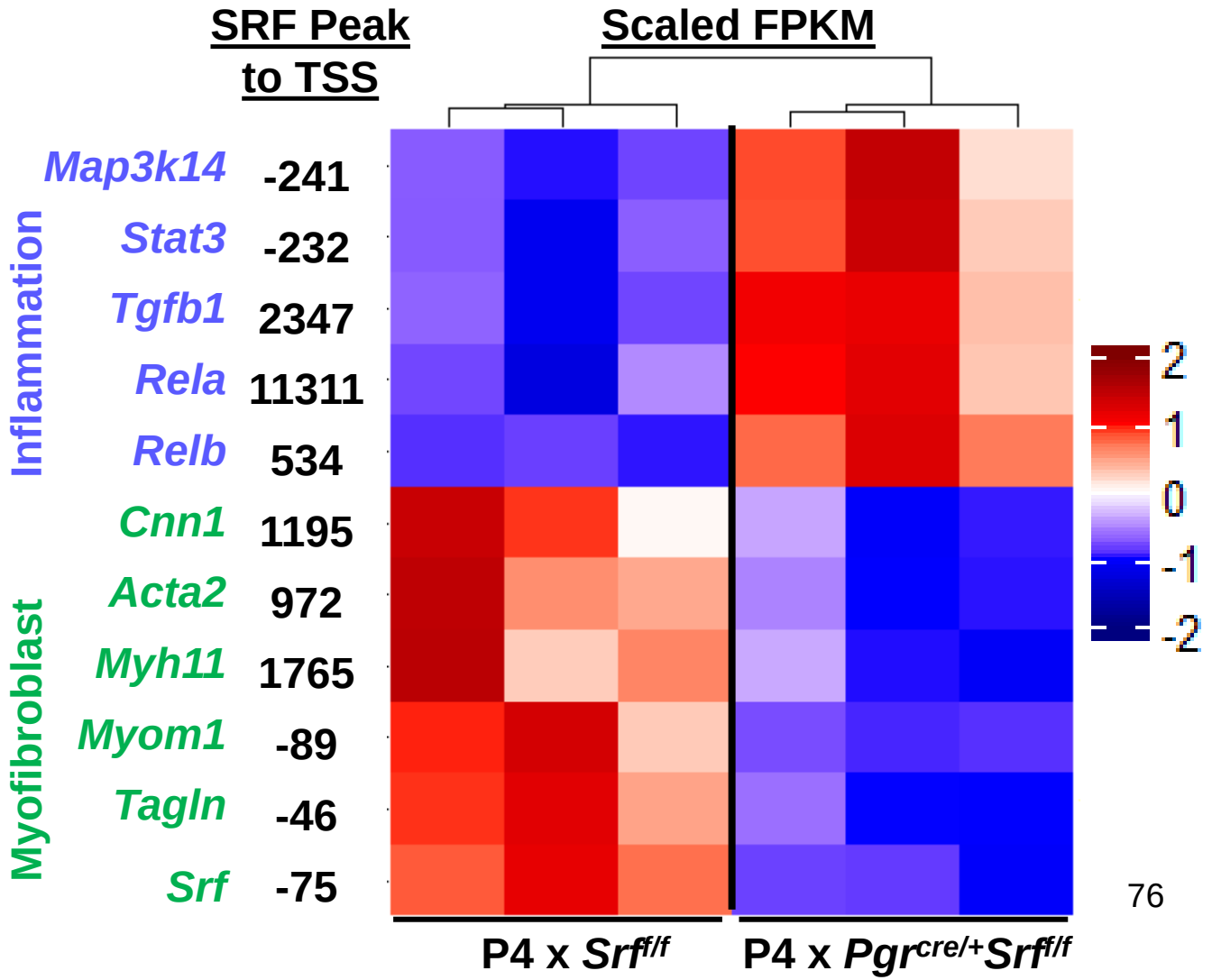
Upstream Regulators



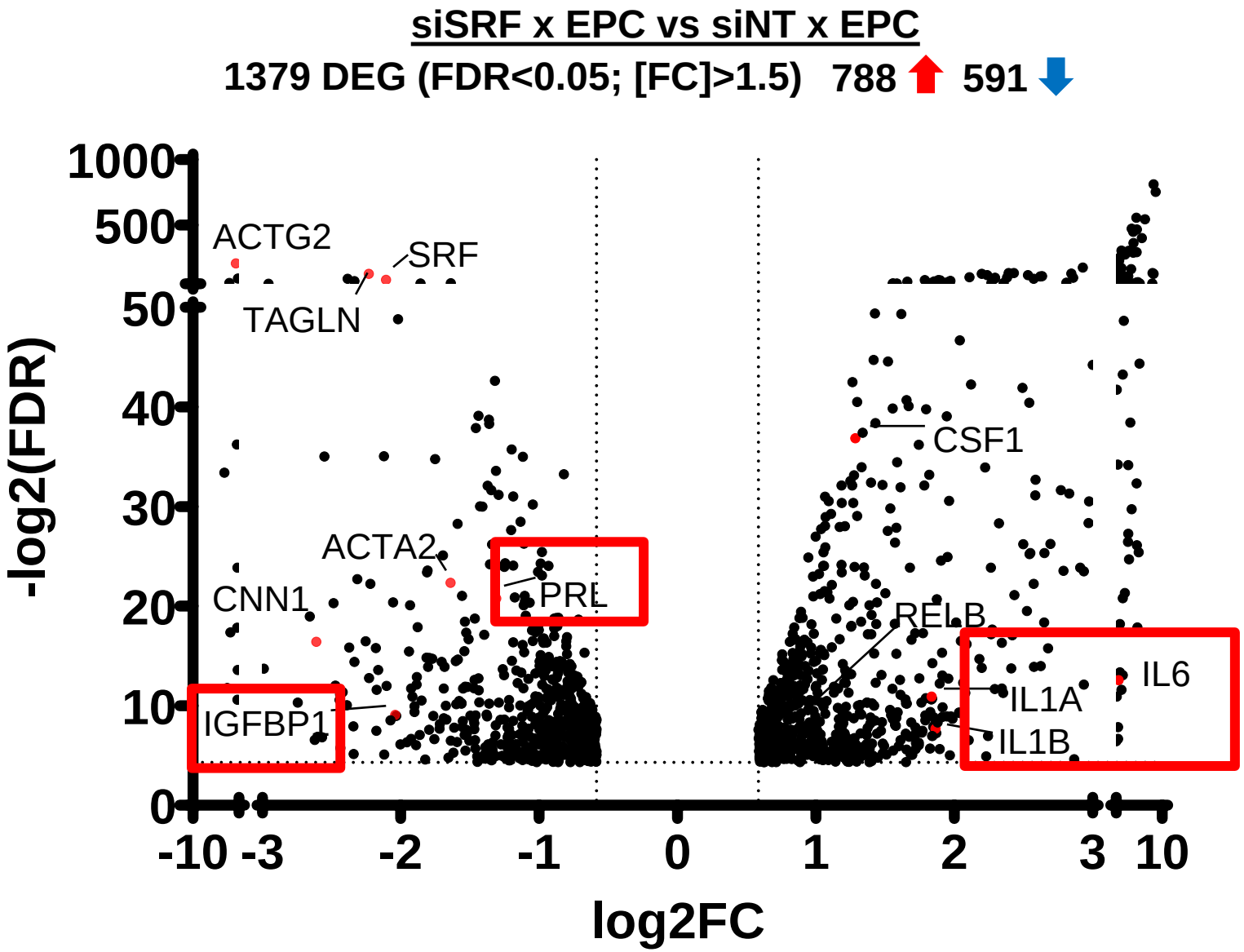
DEG = Padj<0.05; [FC]>1.5

Putative Direct SRF Target Genes in the P4-Treated Uterus





Decidualization Attenuated and Inflammatory Markers Upregulated after *SRF* Knockdown in Primary HESCs



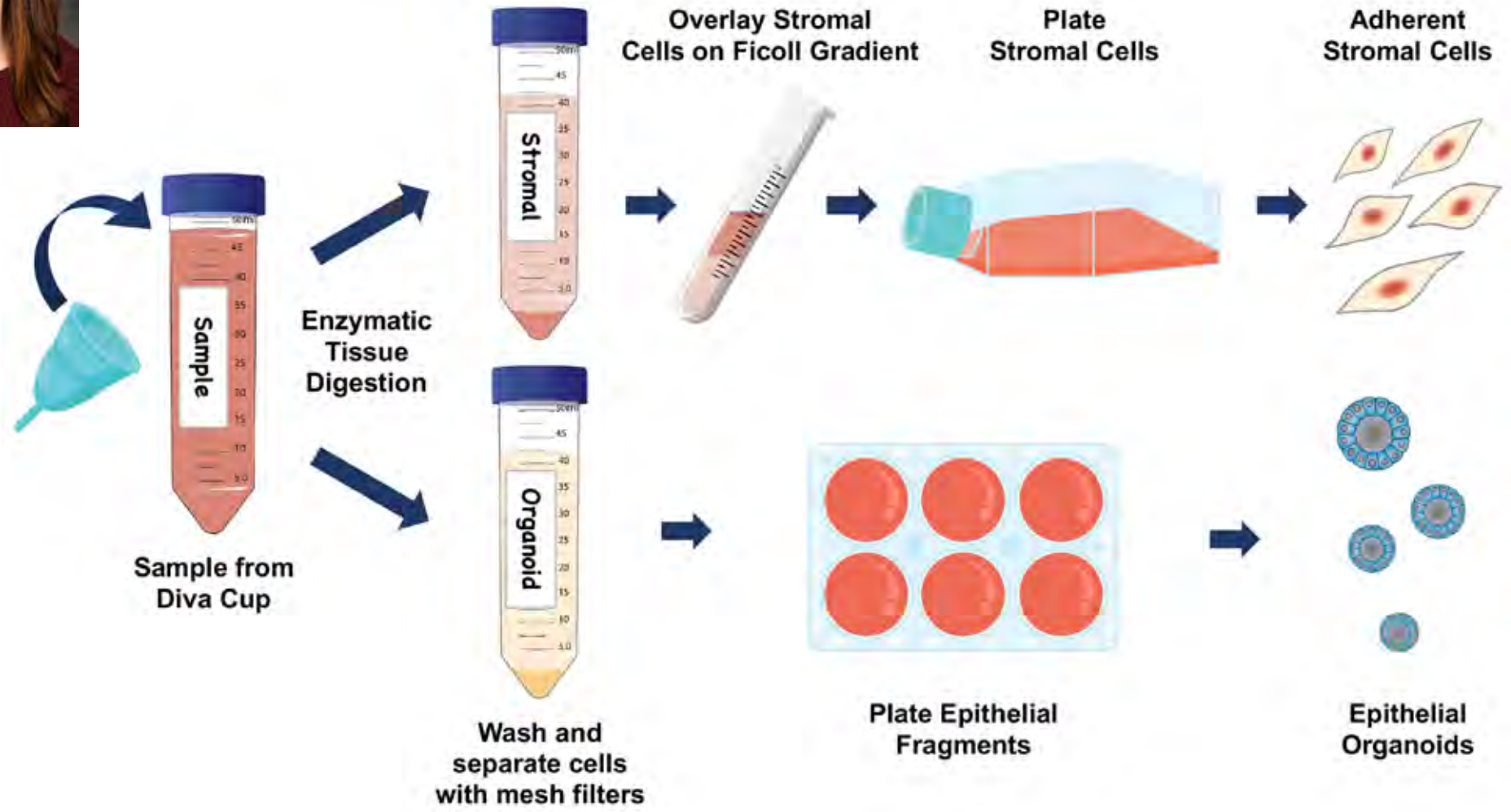
Decidualization Attenuated and Inflammatory Markers Upregulated after *SRF* Knockdown in Primary HESCs

Ingenuity Canonical Pathway	siSRF vs siNT EPC HESC (z-score)	P4 responsive Srf f/f only (z-score)	P4 responsive PRcre/+ Srf f/f only (z-score)	Opposite P4 response in PRcre/+ Srf f/f (z-score)
Pathogen Induced Cytokine Storm Signaling Pathway	4.276	-5.431	2.469	2.646
Macrophage Classical Activation Signaling Pathway	3.053	--	1.528	2.236
Crosstalk between Dendritic Cells and Natural Killer Cells	2.333	-3.207	--	--
IL-17 Signaling	2.236	--	2.558	2.236
TNFR1 Signaling	1.633	--	2.121	--
TNFR2 Signaling	1.633	--	2.236	--
Acute Phase Response Signaling	1.279	-2.683	3.71	2.236
Toll-like Receptor Signaling	1.155	-1.265	2.121	--
IL-8 Signaling	1.069	-2.121	1.807	--
IL-6 Signaling	0.894	--	3.9	--
STAT3 Pathway	0.632	-2.673	--	--
Macrophage Alternative Activation Signaling Pathway	0.471	-3.812	0.408	0.447
IL-10 Signaling	-2.065	0.816	-2.065	-0.816

Summary

- SRF is for female mouse fertility.
- SRF regulates the hormone regulated uterine decidual response.
- SRF loss results in uterine fibrosis.
- SRF is critical for human endometrial stroma decidualization.
- SRF loss promotes inflammatory pathways in mouse and humans.

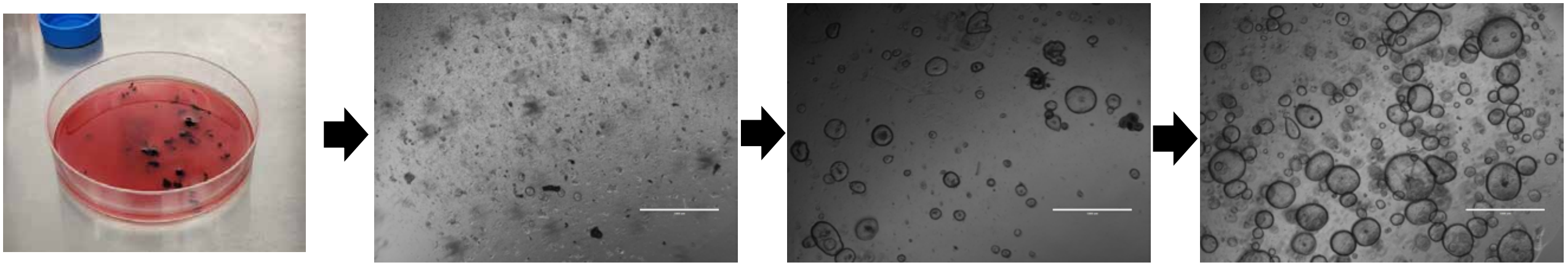
Isolation of Endometrial Epithelial Organoids and Stromal Cells from Menstrual Fluid



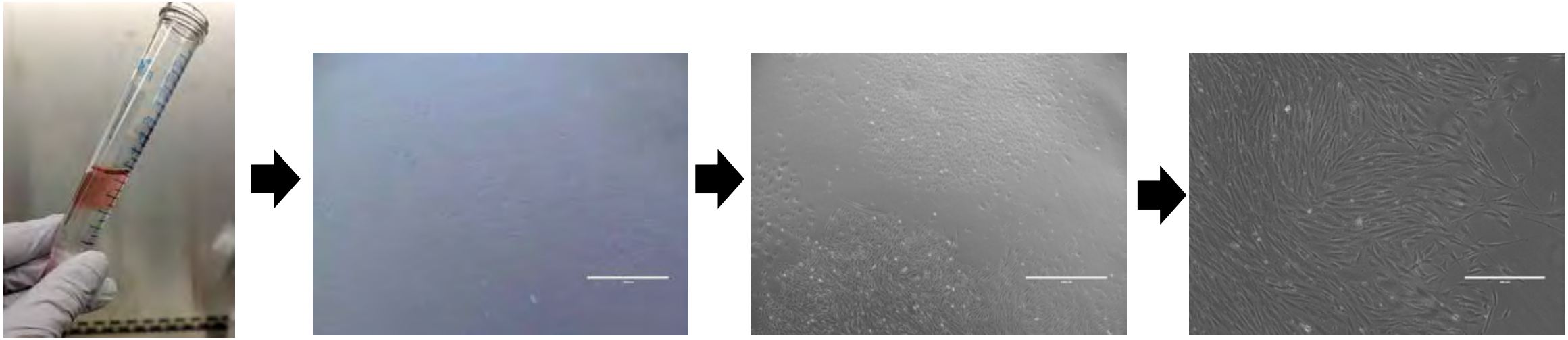
n = 5 per cell type; 4 paired

S. Hewitt & M. Dickson

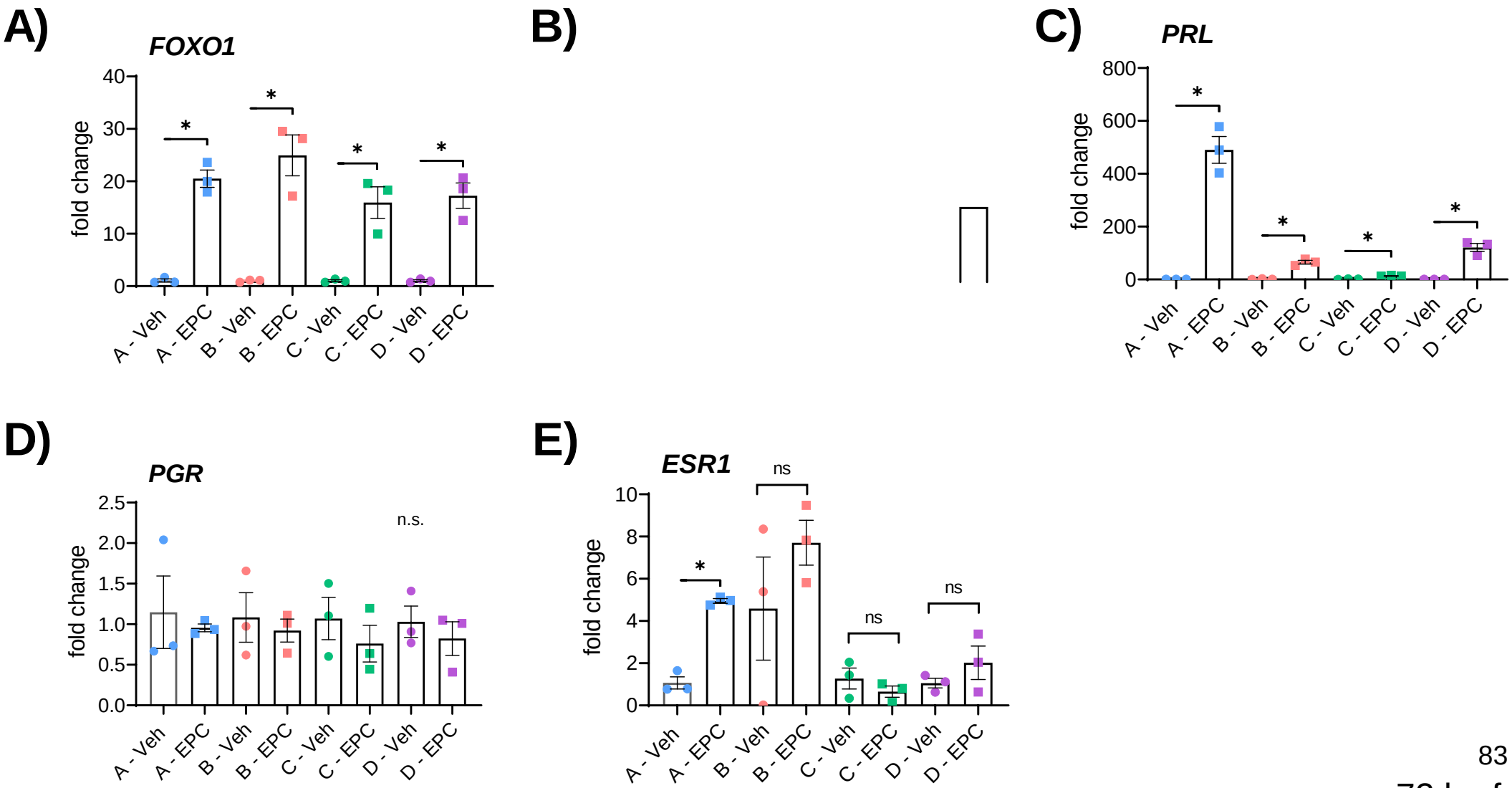
Organoids from Menstrual Fluid



Stromal Cells from Menstrual Fluid



Stromal Cell Decidualization

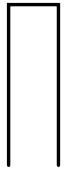


* $P < 0.05$

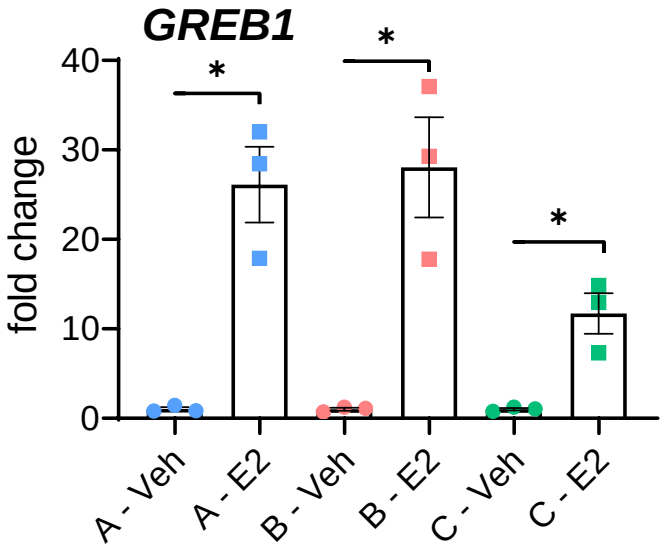
72 h of EPC

Estrogen Responsiveness

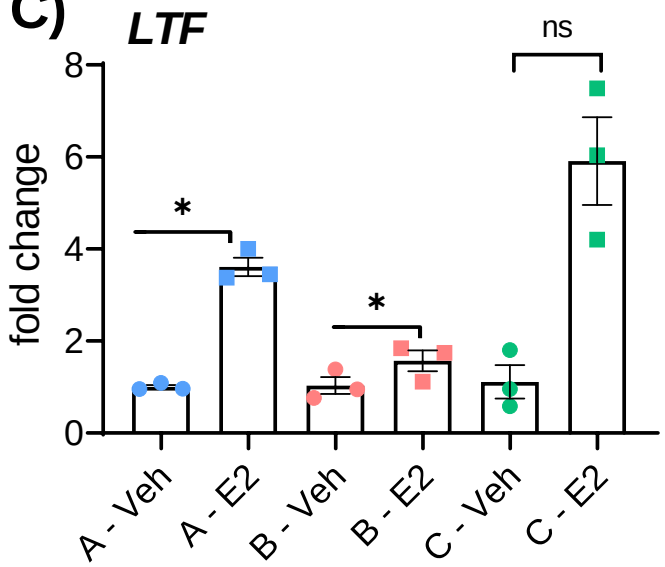
A)



B)



C)



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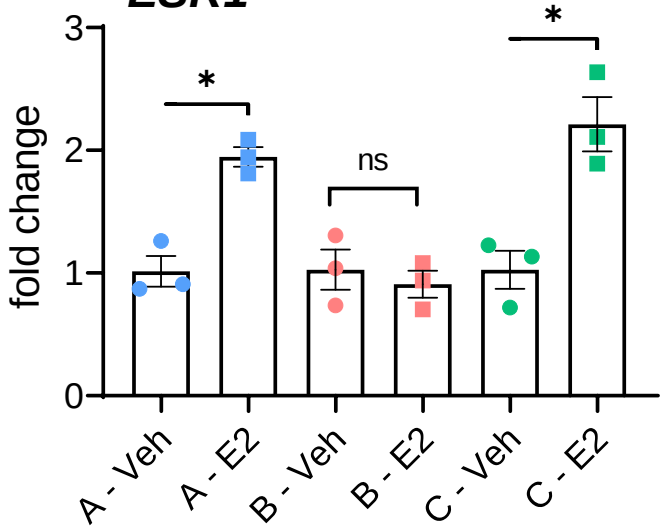

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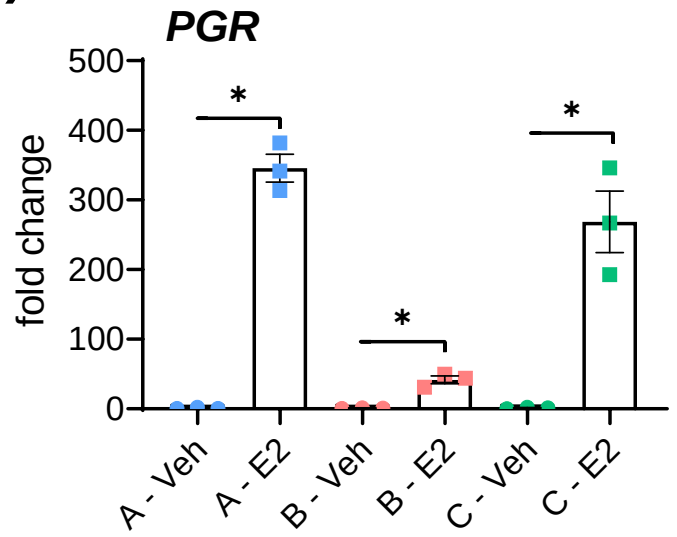
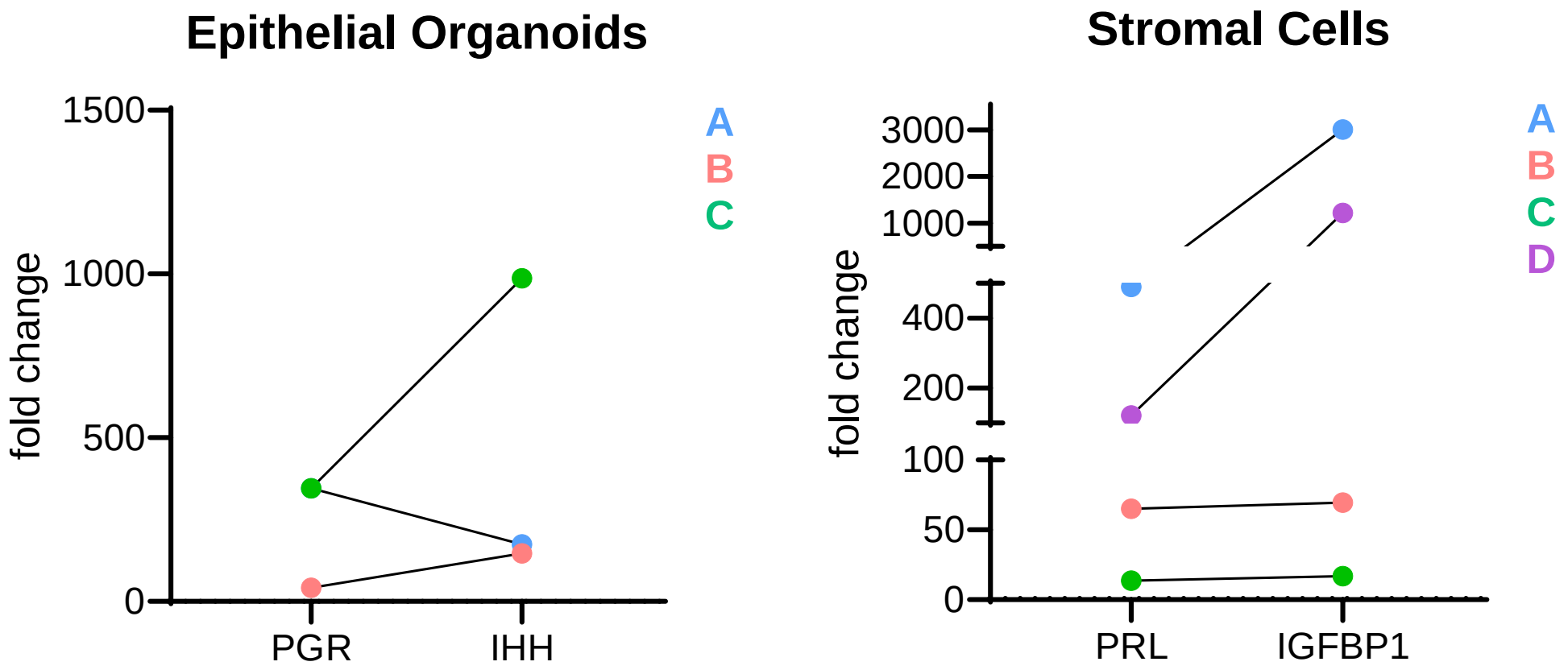


* $P < 0.05$

Relative Responsiveness of Samples from Menstrual Fluid



Advantage of Menstrual Tissue

- Noninvasive means of collecting primary epithelial and stroma cells.
- Can match patient epithelial organoids and stroma in potential coculture experiments
- Can conduct age/environmental effects on epigenome of uterine tissue.

Acknowledgments

Reproductive and Developmental Biology Laboratory
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Dr. Steve Wu
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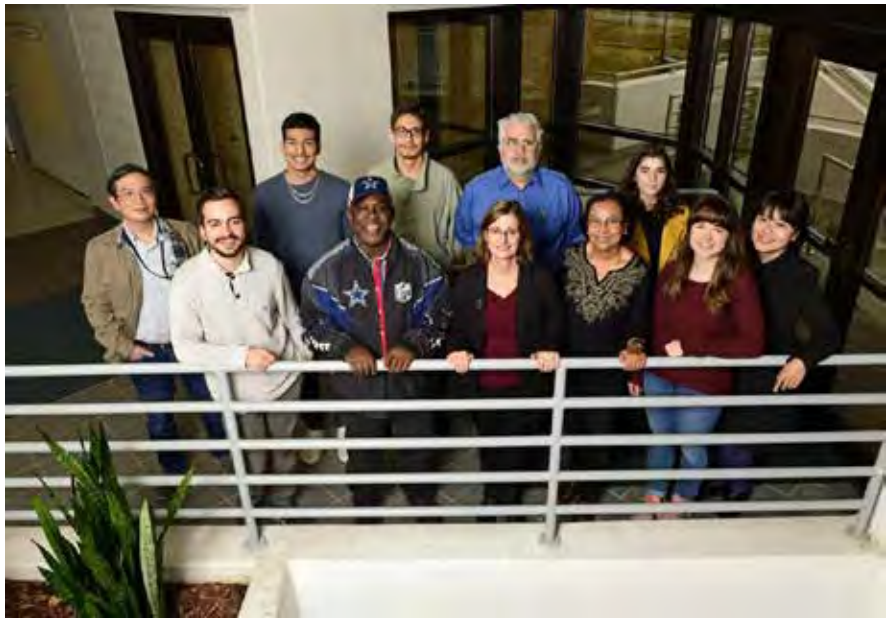
Dr. Rong Li
Dr. Wan-Ning ‘Chloe’ Li
Dr. Ru-Pin ‘Alicia’ Chi
Olivia Emery
Yassmin Medina Laver



Epidemiology Branch
Fertility and Reproductive Health Group
Dr. Anne Marie Jukic

Comparative Medicine Branch
Veterinary Medicine Section

Epigenomics and DNA Sequencing Core

Knockout Mouse Core

NIH Research Panel

Carmen Williams, M.D., Ph.D.

Deputy Chief, Reproductive and Developmental Biology Laboratory

National Institute for Environmental Health Sciences

Cellular confusion in the female reproductive tract: A consequence of estrogenic endocrine disruption during development

Carmen J. Williams, MD, PhD

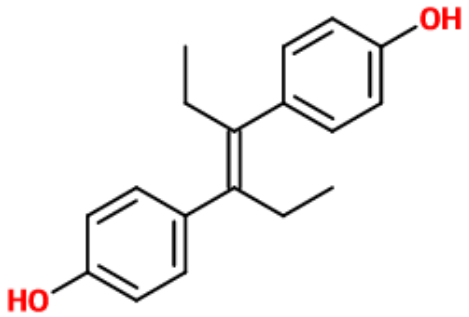
Endocrine Disrupting Chemicals and Women's Health Symposium
July 18-19, 2023

THE ENVIRONMENT INFLUENCES DEVELOPMENT

- Development is genetically and epigenetically programmed
- Environmental cues provide opportunities for adapting development to improve adult fitness
- Altered development can also reduce adult fitness

EXPOSURES DURING HUMAN FETAL DEVELOPMENT CAN AFFECT ADULT HEALTH

- Diethylstilbestrol (DES) is a synthetic estrogen
- Prescribed to 5-10 million pregnant women in the US from 1938-1972; also used internationally
- Heavily advertised to Ob/Gyn physicians

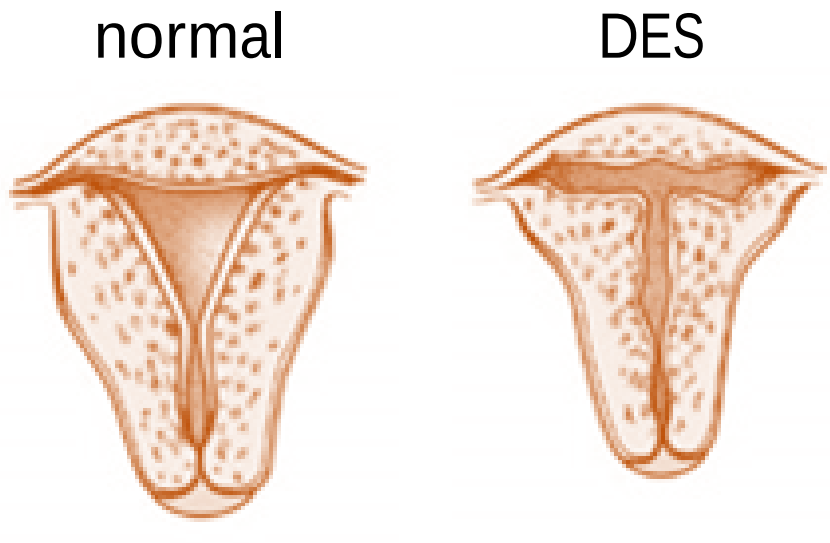


DES

Am J Obstet Gynecol 1957

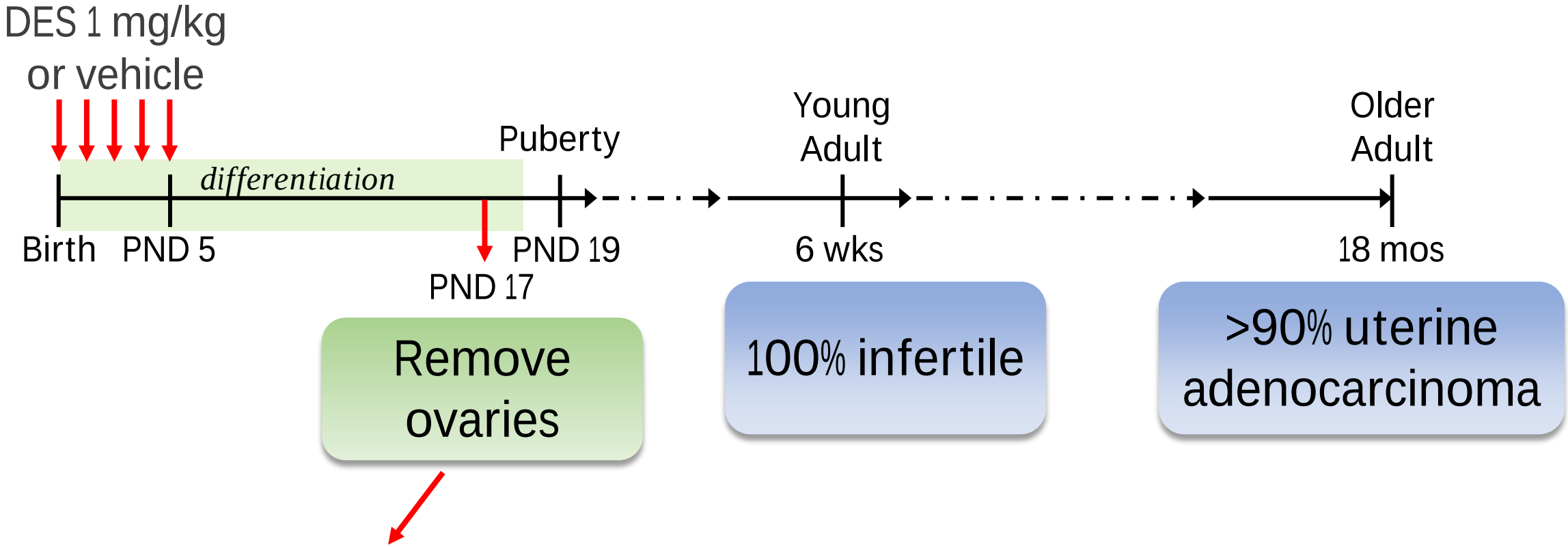
PRENATAL DES ALTERS DEVELOPMENT AND INCREASES CANCER RISK

- Alters developmental patterning of the female reproductive tract
- Causes infertility and pregnancy complications
- Increases the incidence of cancer in adult women
    - Breast cancer
    - Vaginal cancer



MOUSE MODEL OF NEONATAL DES EXPOSURE

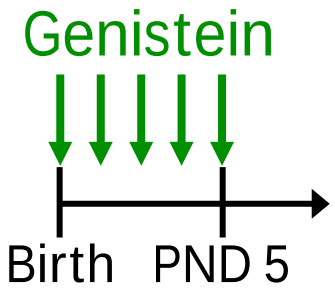
Retha Newbold & John McLachlan

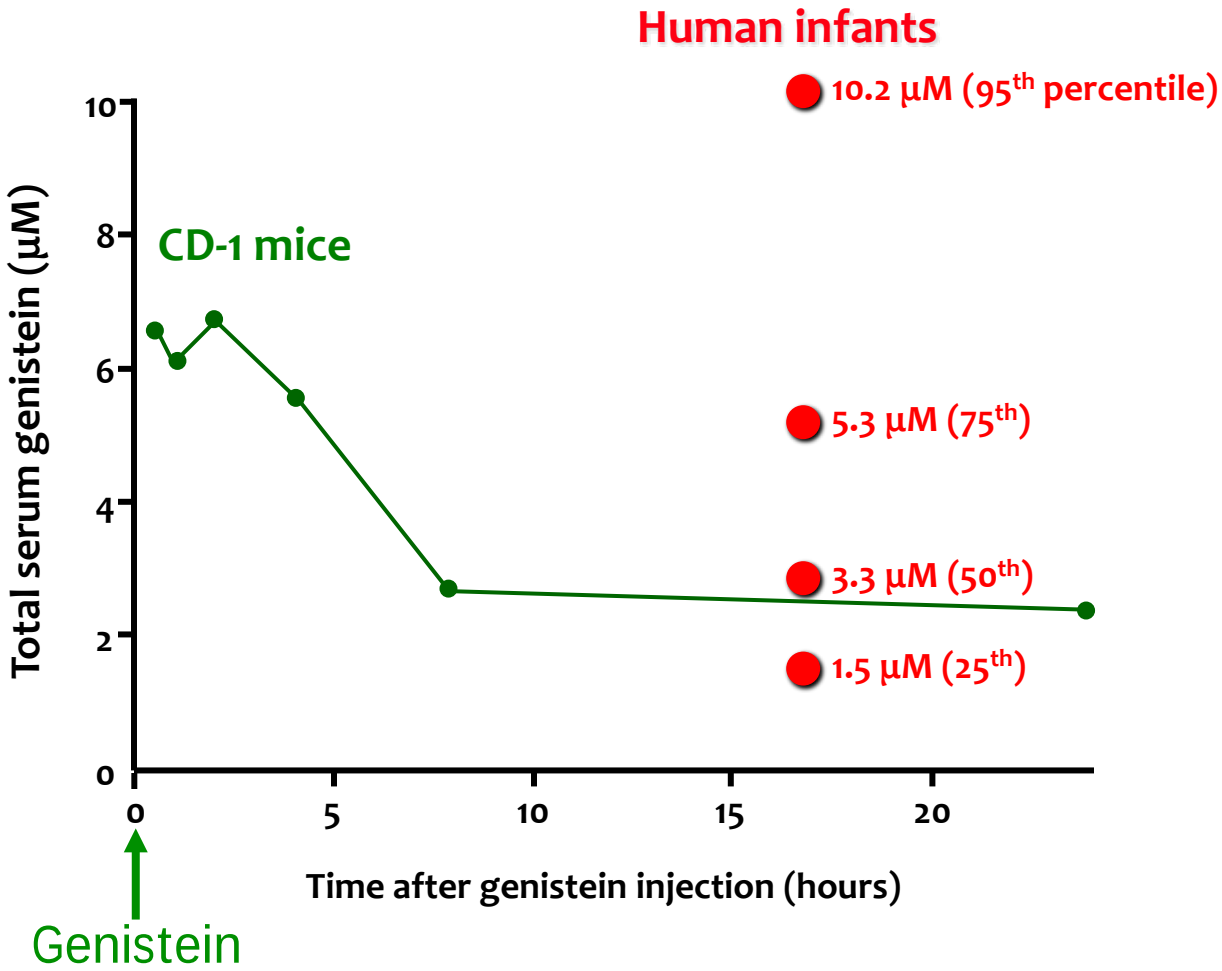


- No cancer develops
- DES-induced cancer requires second 'hit' of estrogen

Newbold et al., Cancer Res (1990)

GENISTEIN DOSING TO MODEL HUMAN INFANT EXPOSURE TO SOY FORMULA

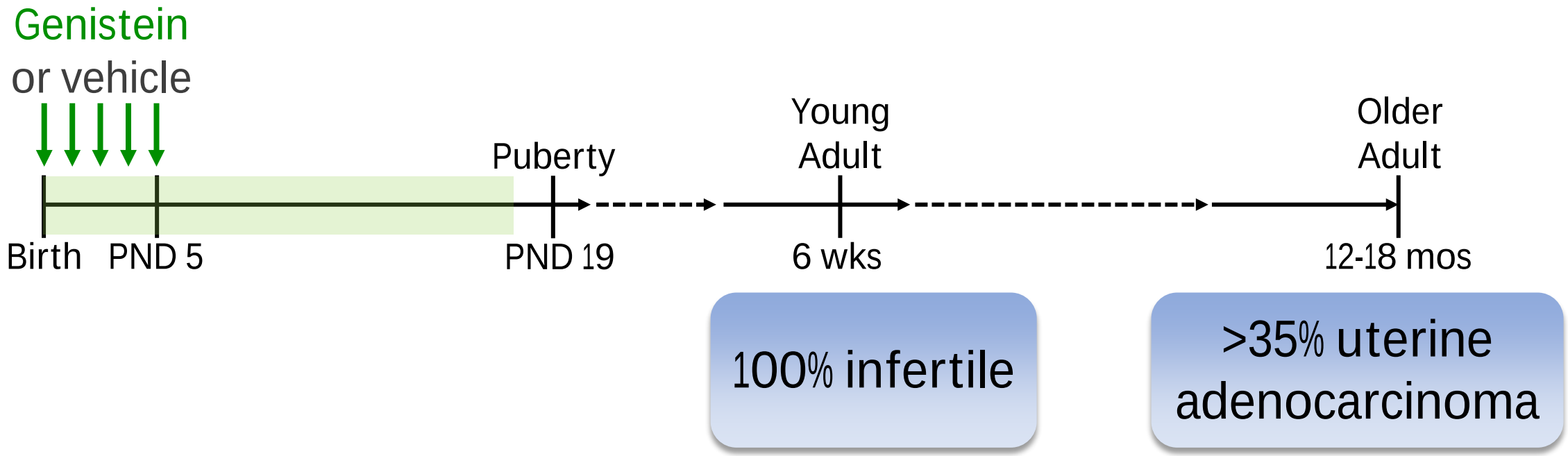




Doerge et al. (2002) Cancer Lett 184:21
Cao et al. (2009) J Expo Sci Environ Epidemiol 19:223

Dosing strategy results in human-relevant serum phytoestrogen levels

EFFECTS OF NEONATAL GENISTEIN EXPOSURE ON FEMALE REPRODUCTIVE FUNCTION

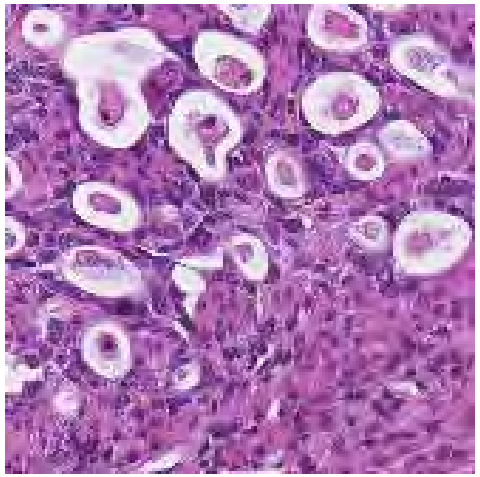


Newbold et al. Cancer Res (2001)

HOW DOES A BRIEF EXPOSURE DURING DEVELOPMENT TO ESTROGENIC CHEMICAL STIMULATION CAUSE ADULT LATE ONSET CANCER?

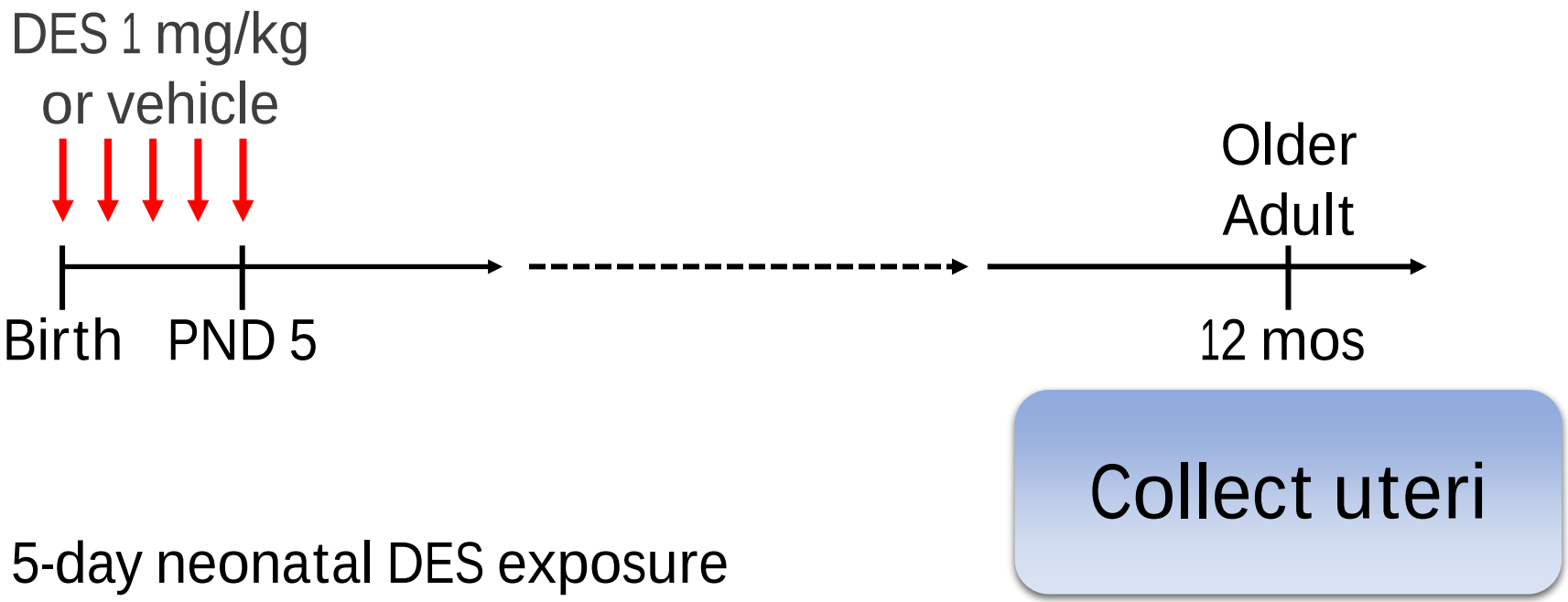
- No mutations in oncogenes or tumor suppressors are found
- There are permanent changes in epigenetic marks, but it is not clear how these changes impact cancer development

Adenocarcinoma



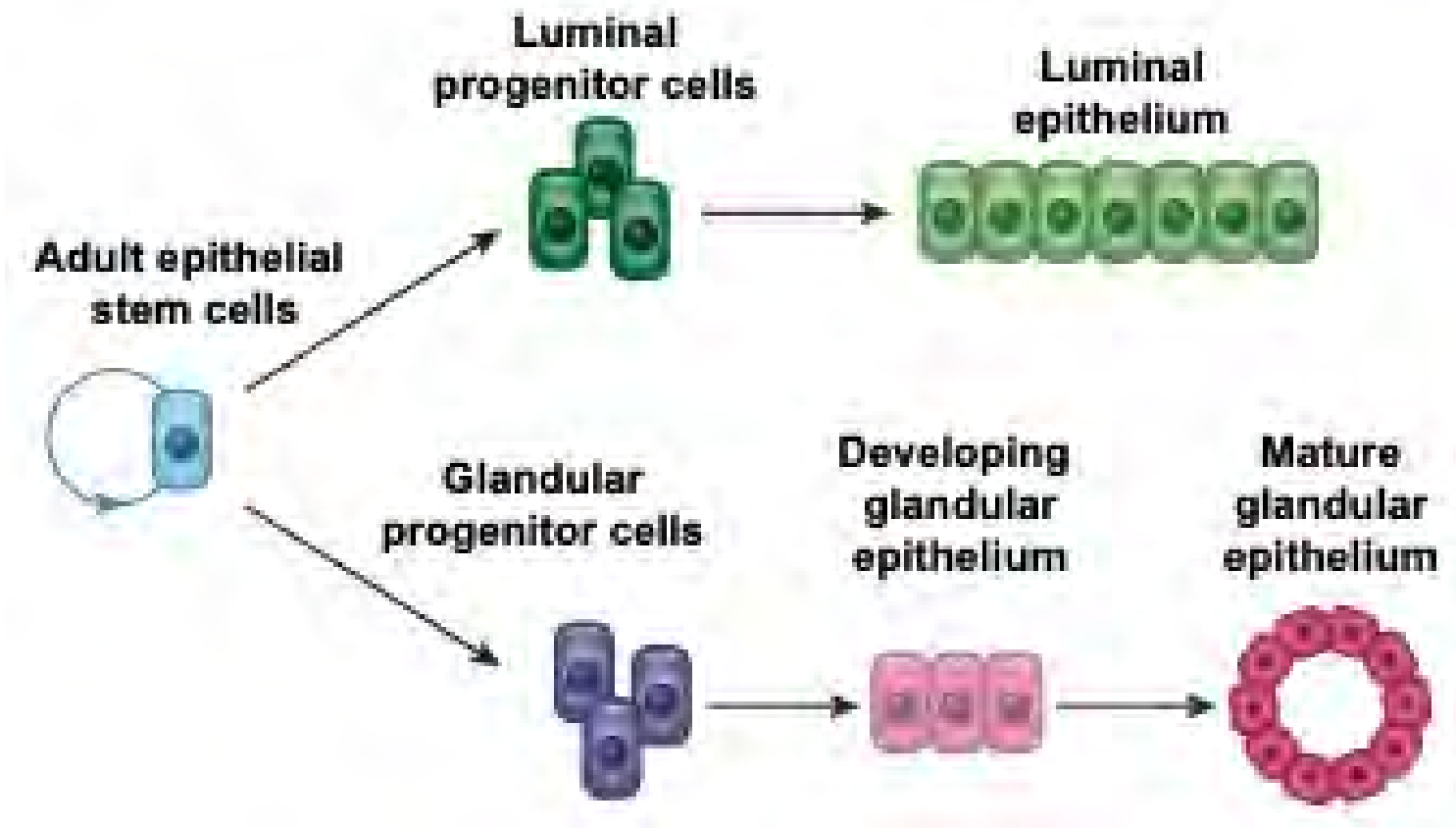
- Experimental approach: Use single cell RNA sequencing to determine how DES exposure alters transcripts in the different uterine cell types, including cancer cells

EXPERIMENTAL DESIGN

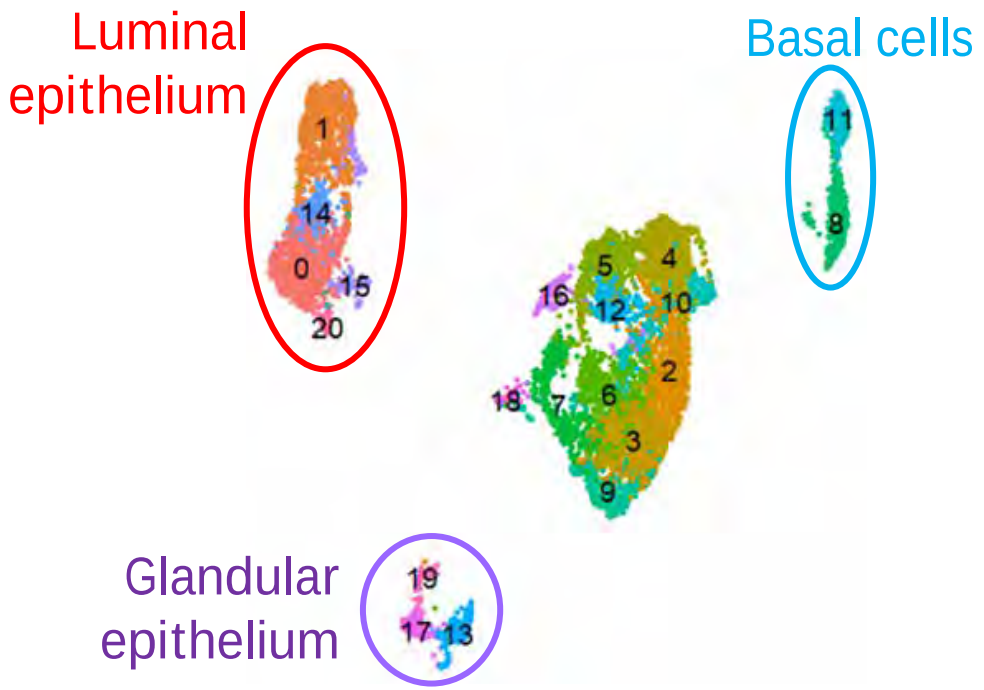


- 5-day neonatal DES exposure
- Collect uteri at 12 months of age
- Isolate living uterine cells for single cell RNAseq analysis

NORMAL UTERINE EPITHELIAL CELL TYPES

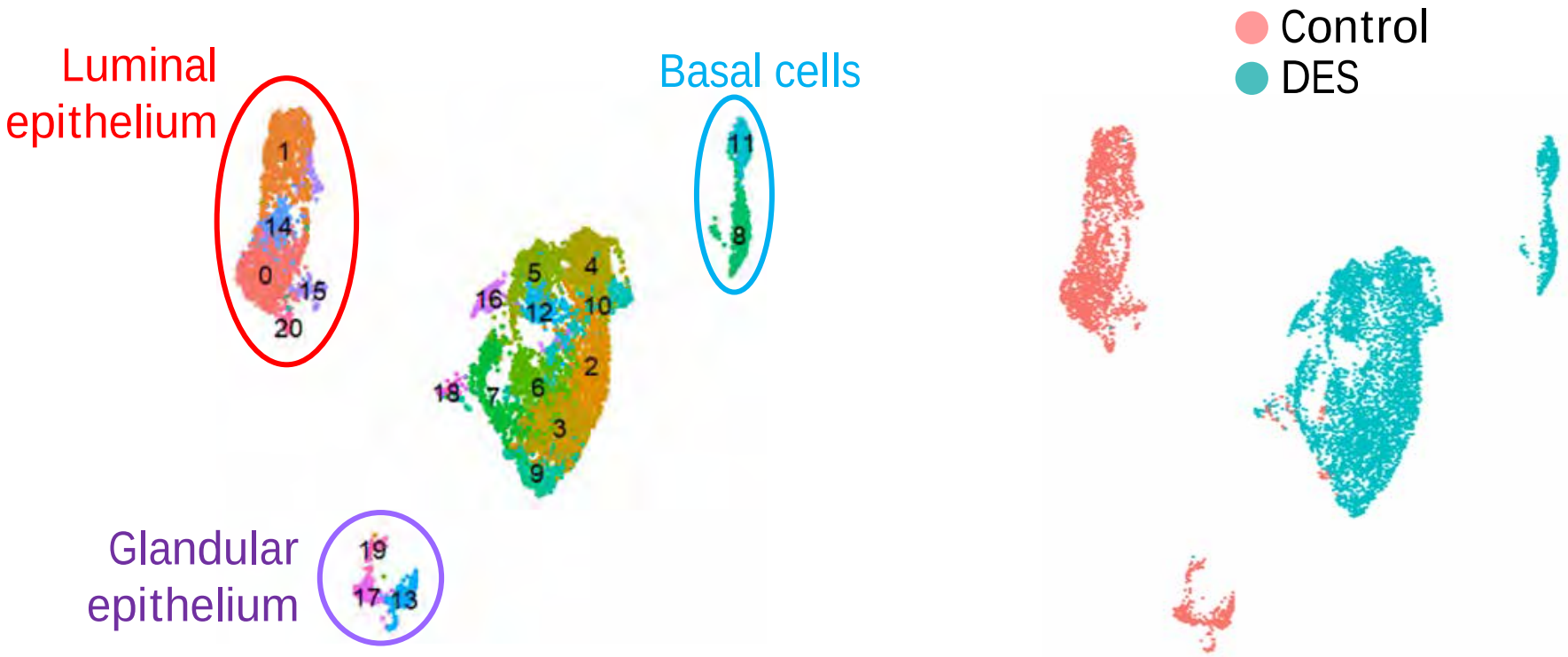


CELL TYPES IN CONTROL AND DES UTERINE EPITHELIAL CELLS



- Luminal epithelium, glandular epithelium, and basal cells were identified using differentially expressed markers
- One large unidentified set of cell clusters was present

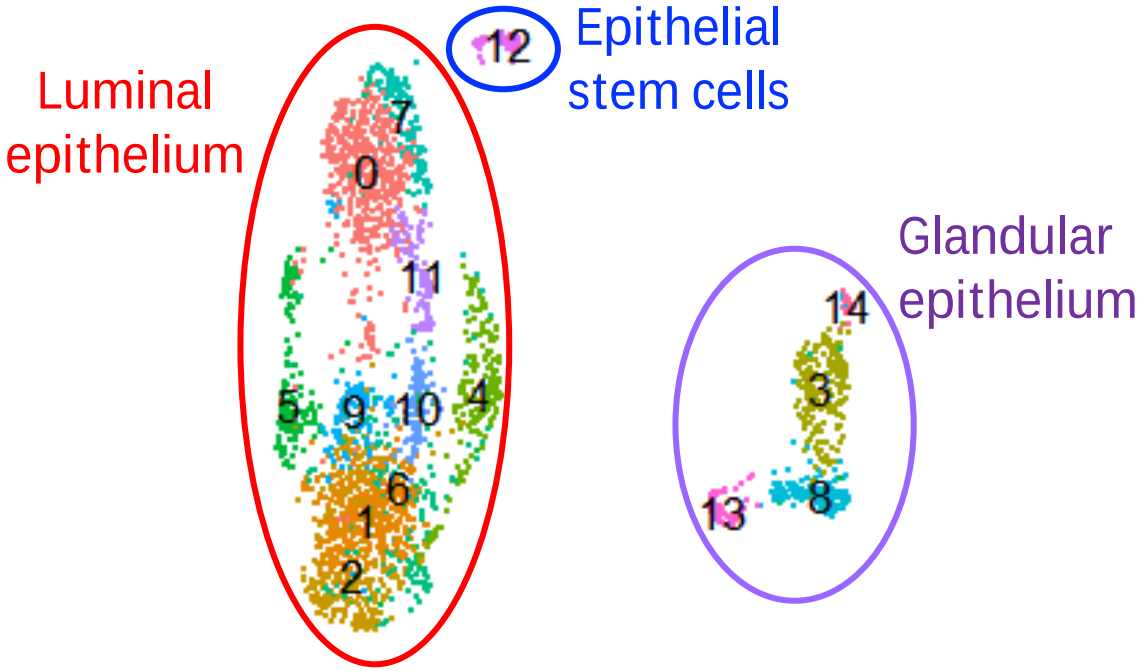
DES-EXPOSED EPITHELIAL CELLS LACK LUMINAL OR GLANDULAR CHARACTERISTICS



- Only CON cells were clearly identifiable as luminal or glandular epithelium
- Basal cells and the uncharacterized large group of clusters almost exclusively derived from DES cells

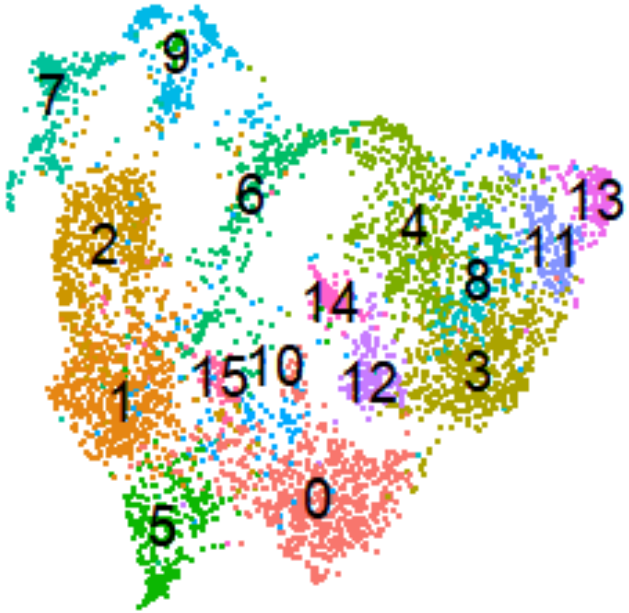
SEPARATE ANALYSIS OF CON AND DES EPITHELIAL CELLS

CON



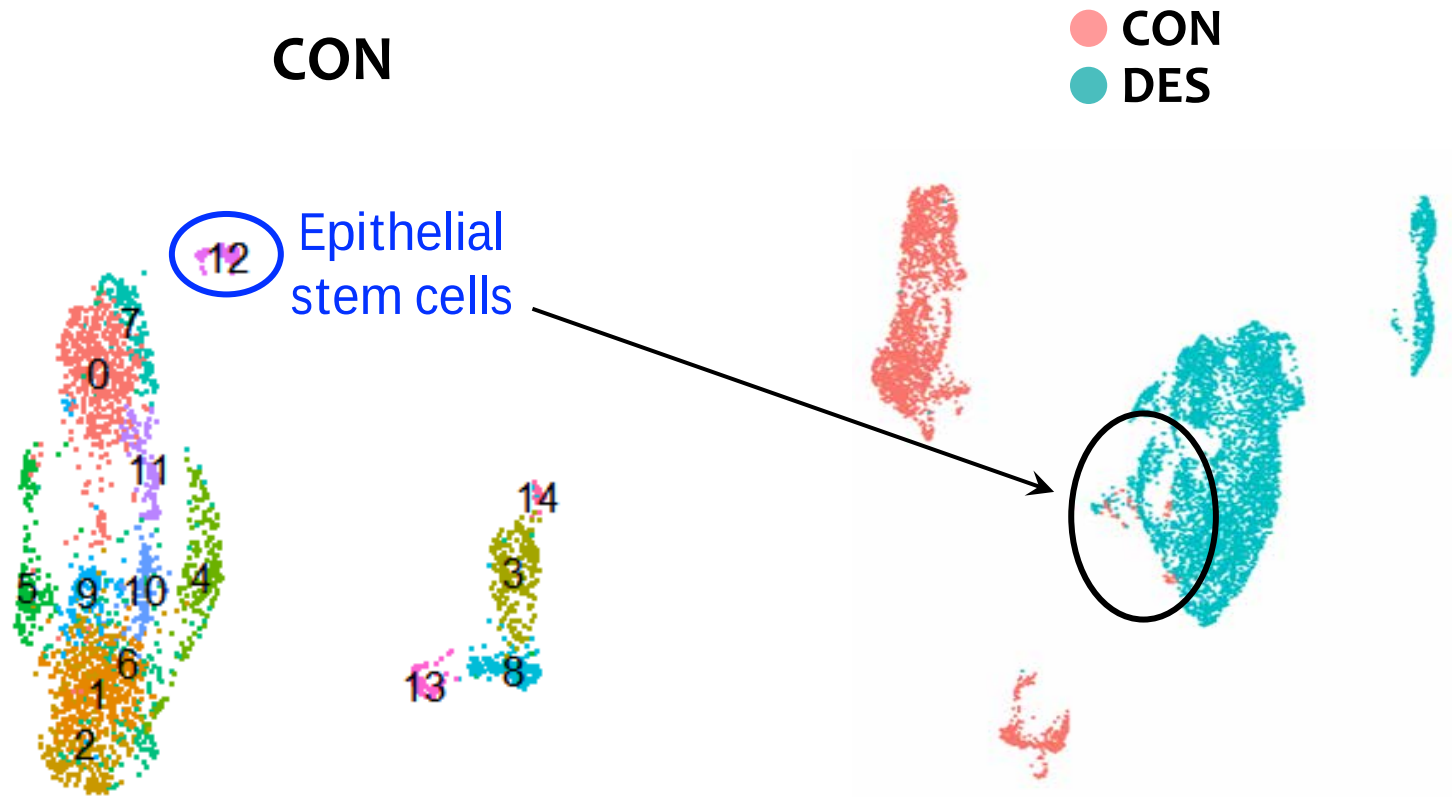
- Three distinct cluster groupings – luminal, glandular, and epithelial stem cells

DES

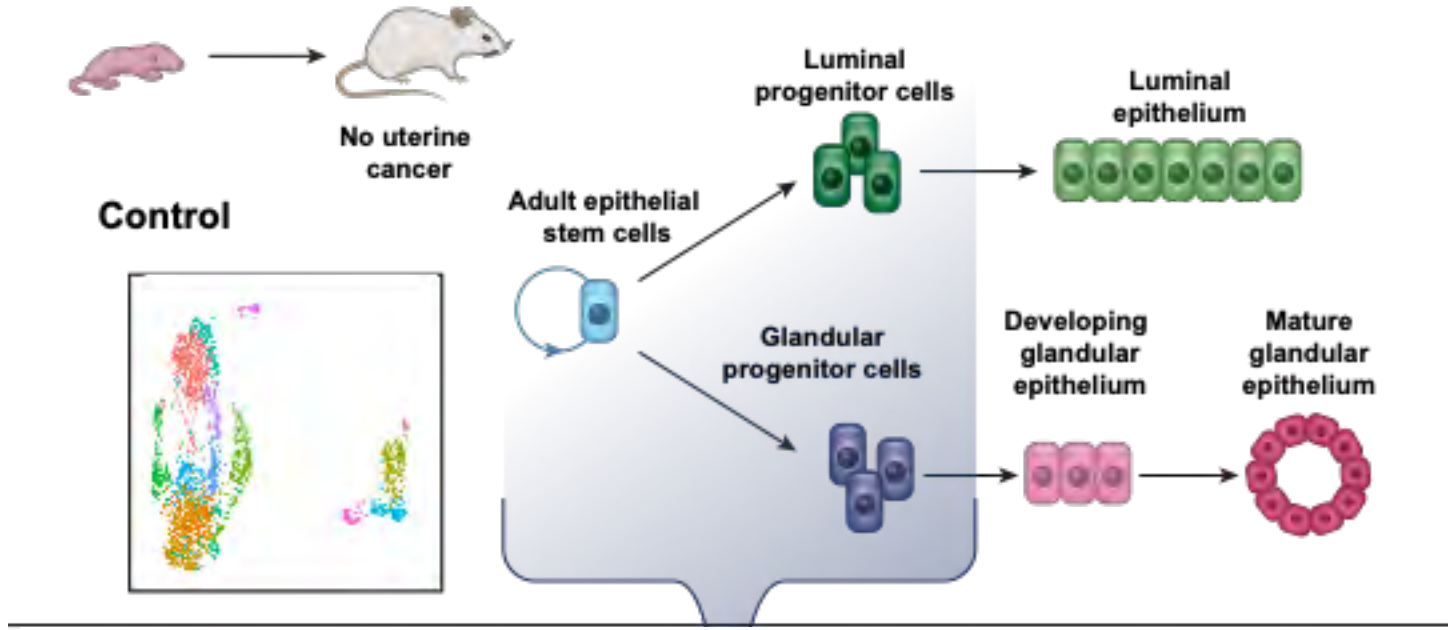


- Basal cells removed from analysis
- One cluster grouping remains – no separation of luminal and glandular cell clusters

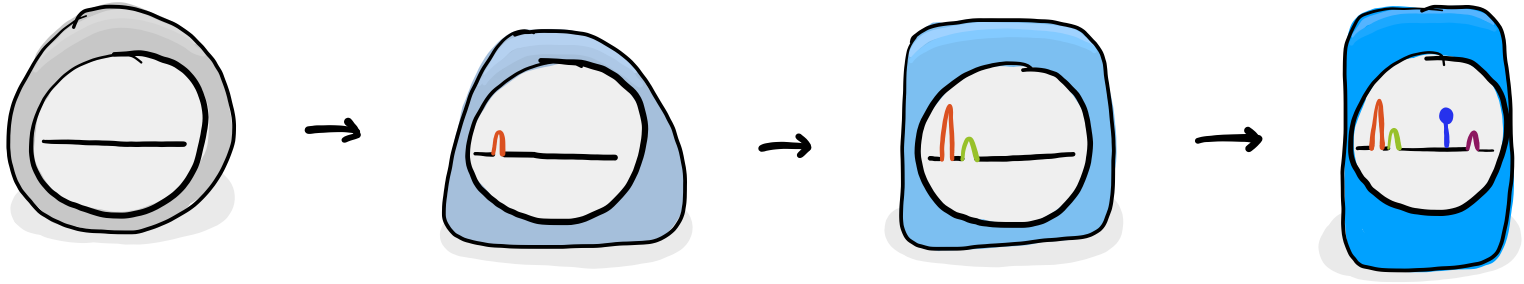
DES EPITHELIAL CELLS HAVE STEM CELL CHARACTERISTICS



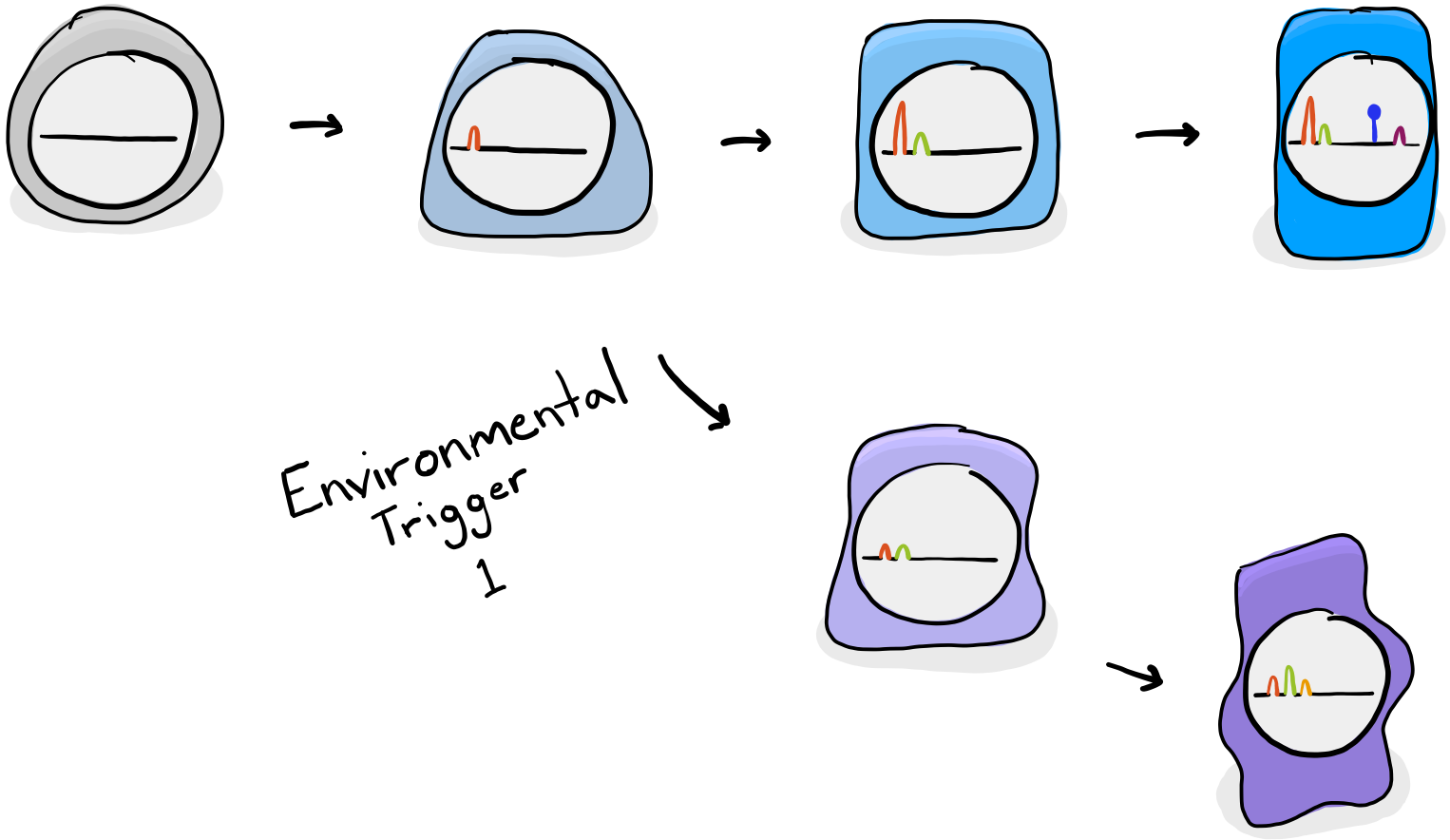
- CON epithelial stem cells cluster with large DES epithelial cell cluster


No uterine cancer
Control
Adult epithelial stem cells
Luminal progenitor cells
Luminal epithelium
Glandular progenitor cells
Developing glandular epithelium
Mature glandular epithelium

CONFUSION OF CELLULAR IDENTITY SENSITIZES CELLS TO ADDITIONAL ENVIRONMENTAL TRIGGERS



CONFUSION OF CELLULAR IDENTITY SENSITIZES CELLS TO ADDITIONAL ENVIRONMENTAL TRIGGERS



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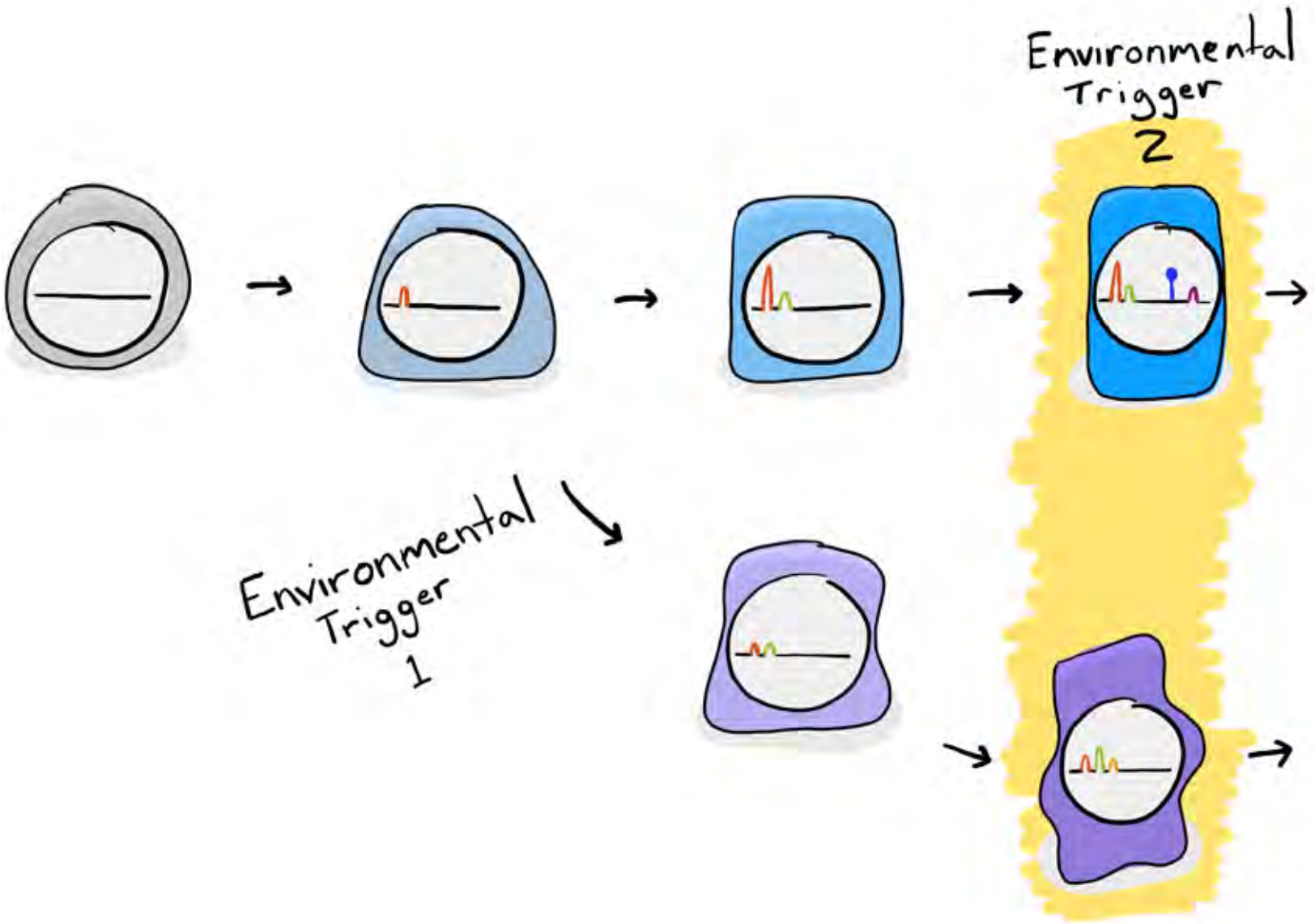

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Williams group

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Lenka Radonova
Rajan Iyyappan

Collaborators

Brian Papas (NIEHS)
Xin Xu (NIEHS)

NIEHS Cores

Epigenomics and DNA Sequencing Core
Integrative Bioinformatics Support Group

NIH Research Panel

Janet Hall, M.D., M.S.

Chief, Clinical Research Branch

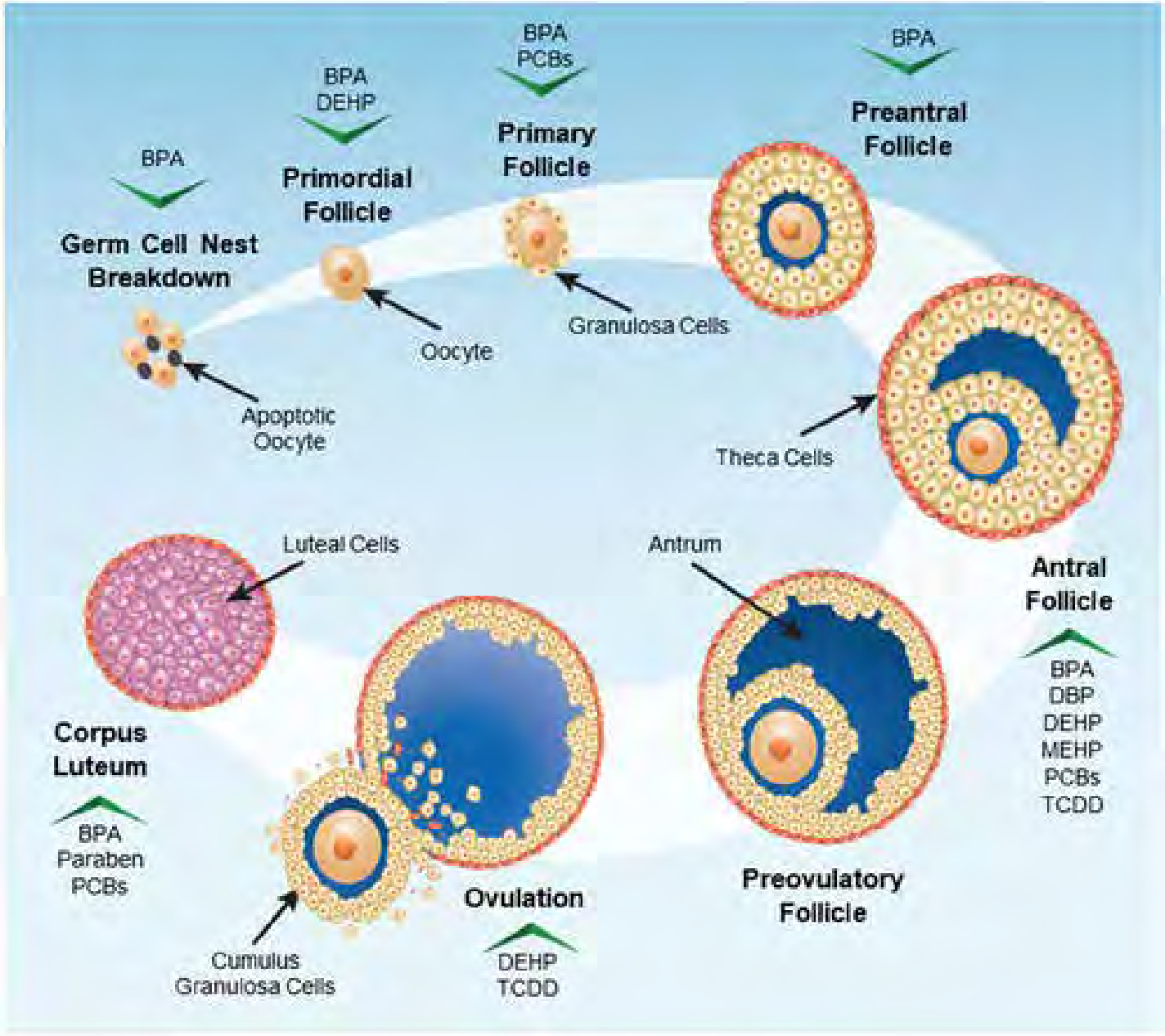
National Institute of Environmental Health Sciences

HHS EDC Symposium
7/17/2023

Does the Environment Affect the Menopause?

Janet E. Hall, MD

Sites of Action of EDCs in the Ovary



The “Bad Actors”

- Persistent Organic Pollutants
- Pesticides
- PFAS
- Phthalates
- BPA
- Metals

Persistent Organic Pollutants and Pesticides

- Highly carcinogenic chemical compounds - electrical equipment like capacitors and transformers, hydraulic fluids
- Banned in the U.S. in 1979 and internationally 2004; still present in pre-1979 products and in landfills
- Forever chemicals that accumulate in food chains, present in soil, air, dust

	Earlier Menopause	POI	Increased FSH	Increased LH	Decreased AMH
Polychlorinated Biphenyls (PCBs)	YES/no	X		X	X
Dioxin	yes/no	X			
Polyaromatic Hydrocarbons (PAHs)		X	X	X	X
Select Pesticides	X	X			

Peri- and Polyfluoroalkyl Substances (PFAS)

- Found in consumer goods such as carpet, leather, apparel, textiles, paper and packaging, coatings, rubber and plastics
- Exposure through contaminated soil, drinking water, food packaging and air
- NHANES – PFAS detected in a high percentage of the population; phase out programs are in effect

Association of PFAS with Earlier Menopause

NHANES 1999-2000 *(Taylor KW, 2014)*

- Women with higher PFAS had earlier age at menopause – consistent across PFOA, PFOS, PFNA, PFHxS
- Could this be reverse causation?

SWAN *(Ding N et al, 2020) (Harlow SD et al, 2021*

- 1,120 mid-life women followed longitudinally to final menstrual period
- Higher PFOS, PFOA associated with shorter time to menopause, particularly in white women;
- Earlier time to menopause associated with an increase in FSH
- PFOA and PFOS were positively associated with FSH; PFOS and PFNA were inversely associated with estradiol
- Prospective design removes concerns of reverse causation

Phthalates

- Used in the manufacture of plastics (increase their flexibility, transparency, durability and longevity), solvents, and personal care products
- Colorless, odorless, oily liquids - do not evaporate easily and do not chemically bind to the material they are added to
- Persist in the environment and have long-term effects through epigenetic effects
- Banned in the EU, Argentina, Japan and other countries; restricted but not completely banned in the US

Association of Phthalates with Earlier Menopause

NHANES 1999-2008 *(Grindler NM et al, 2015)*

- Women with the highest levels of DEHP experienced menopause 3.2-3.8 years earlier than those with low levels

Midlife Women's Health Study 2006-2025

- Baseline analysis from a longitudinal study of 45-54 yo women à 18% increase in odds of recent HF and 38% increase in frequency per doubling of summary measure
- Smaller cohort – high concentrations from use of personal care products à 45% increased odds of experiencing a HF
- Longitudinal analysis found individual metabolites to associate with HF

Association of Phthalates with POI and Poor IVF Outcomes

EARTH Study *(Hauser R et al, 2016)*

- Women undergoing ART (n=256)
- Urinary concentrations of DEHP metabolites were inversely associated with oocyte yield, clinical pregnancy, and live birth following ART

Bisphenol A (BPA)

- Used in food packaging, to line the interiors of food and beverage cans, water pipes, dental fillings
- Repetitive exposure of certain BPA-containing products to light and heat, contact with cleaning agents, and aging of the product may result in increased leaching of BPA into food or beverages
- Human exposure is widespread detected urine, serum, saliva, follicular fluid, breast milk, umbilical cord blood, and amniotic fluid – 93% in NHANES
- A ‘safe’ level has not been established

BPA and Reproductive Aging

Diminished Ovarian Reserve (Cao y et al, 2018)

- BPA levels in the follicular fluid (FF) of patients with diminished ovarian reserve (DOR) were higher than in non-DOR patients while FF AMH and E2 were lower
- FF BPA concentration was inversely correlated with AMH and E2 levels
- Administration of BPA to rodents resulted in decreased AMH and E2

EARTH Study *(Souter I et al, 2015)*

- Prospective cohort of women undergoing infertility treatments
- BPA, detected in >80% of women,
- Average decrease in AFC of 12%, 22% and 17% in the 2nd, 3rd, and 4th BPA quartiles compared to the 1st quartile (p-trend: <0.001)
- No association of BPA with FSH

Metals and Earlier Menopause: Lead

Nurses Health Study *(Mendola P et al, 2014)*

- Bone lead concentration (K-shell X-ray fluorescence) is a marker of cumulative lead exposure (n=434)
- Higher tibial lead concentration was a marker of earlier age at menopause
- Odds ratio for menopause < 43 was 5.3 for women in the highest vs lowest tertile of lead exposure

NHANES 1999-2010 *(Mendola P et al, 2012)*

- U.S. women aged 45-55
- Higher lead levels in menopausal women vs women who were still cycling
- Increased odds of menopause was linearly associated with increasing lead levels (1.7 à 4.2)
  - This relationship persisted even with correction for markers of bone turnover

Endocrine Disrupting Chemicals and Reproductive Aging

Earlier menopause

- ü Persistent Organic Pollutants
- ü Pesticides
- ü PFAS
- ü Phthalates
- q BPA
- ü Metals

Early Reproductive Aging

- ü Persistent Organic Pollutants
- ü Pesticides
- ü PFAS
- ü Phthalates
- ü BPA
- q Metals

You can’t change your Genes…
but you CAN change your Environment!!!

Break

We will resume in Gather.Town at 11:15AM

Virtual Poster Session

Gather.Town

11:15AM – 12:15PM

Join us in Gather.Town for the Virtual Poster Session

How to Join Gather.Town

- Please join our sessions **currently in progress** in Gather.Town
- **The Gather.Town link is available in the chat box as well as on the** website.
- Detailed Gather.Town instructions can also be found on the website.
- Abstracts and posters are viewable on the symposium website and in Gather.Town.
- For assistance, please send a direct message or email to the contracting team:
  - Damon (RLA), damon.kane@roseliassociates.com
  - Sofia (RLA), sofia.jones@roseliassociates.com

Symposium Website

Upcoming Agenda

11:15 – 12:15 PM Virtual Poster Session in Gather.Town

12:15 – 1:00 PM Lunch

Lunch

We will resume at 1PM

Hiding In Plain Sight

Jodi Flaws, Ph.D., Natasha Mesinkovska, Ph.D., M.D.

Moderated by Deb Kilday

Hiding In Plain Sight

Jodi Flaws, Ph.D.

Professor in Comparative Biosciences

University of Illinois Urbana-Champaign

Hiding in Plain Sight (Water Disinfection Byproducts)

JODI A. FLAWS, PH.D.

DEPARTMENT OF COMPARATIVE BIOSCIENCES

UNIVERSITY OF ILLINOIS URBANA-CHAMPAIGN

Overview

- Background
  - Water disinfection by-products
    - Iodoacetic Acid (IAA)
  - Female reproduction
    - Ovary
- Effects of water disinfection by-products on female reproduction
  - Hypothesis
  - Specific Aims
  - Results
- Summary/Conclusions

Water Disinfection

- A major public health achievement of the last century
- Decreased the incidence of waterborne diseases
  - Cholera
  - Typhoid
  - Amoebic dysentery
- Increased life expectancy

Water Disinfection Byproducts

- The reaction between disinfectants and organic matter form **water disinfection byproducts (DBPs)**

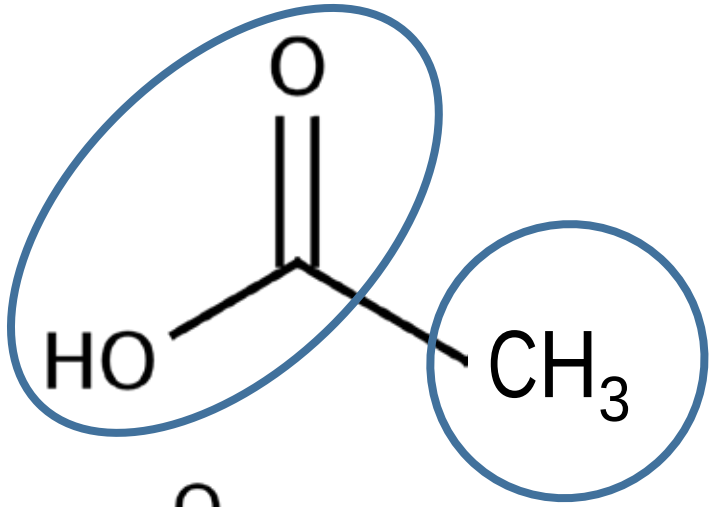
- More than 700 DBPs have been identified in drinking water

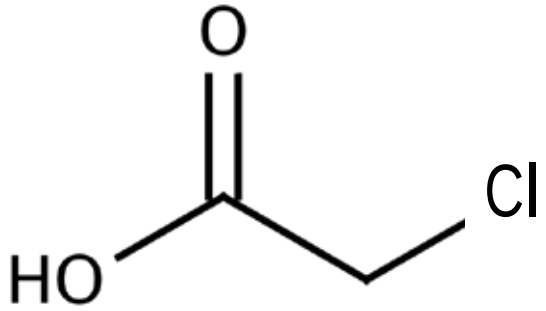
- Trihalomethanes and **haloacetic acids** (HAAs) are the two major classes of DBPs

Haloacetic Acids

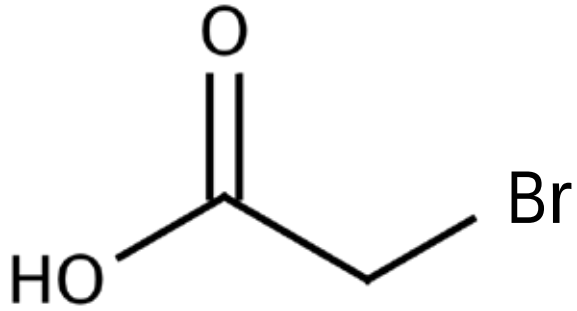
- Haloacetic acids share a common structure with acetic acid

- These molecules consist of two carbons, including carboxylic acid and an alpha carbon

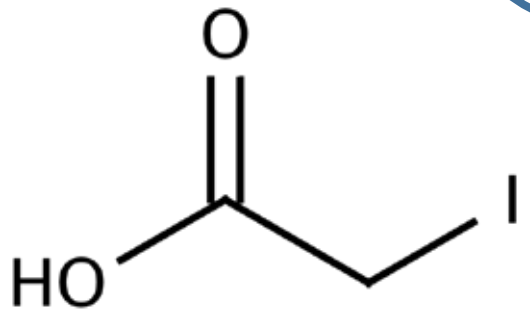




Chloroacetic acid



Bromoacetic acid



Iodoacetic acid

Haloacetic Acid Regulation

- So far, 13 haloacetic acids have been identified in drinking water
- USEPA 1998 - HAA5: chloroacetic acid, bromoacetic acid, dichloroacetic acid, dibromoacetic acid, and trichloroacetic acid

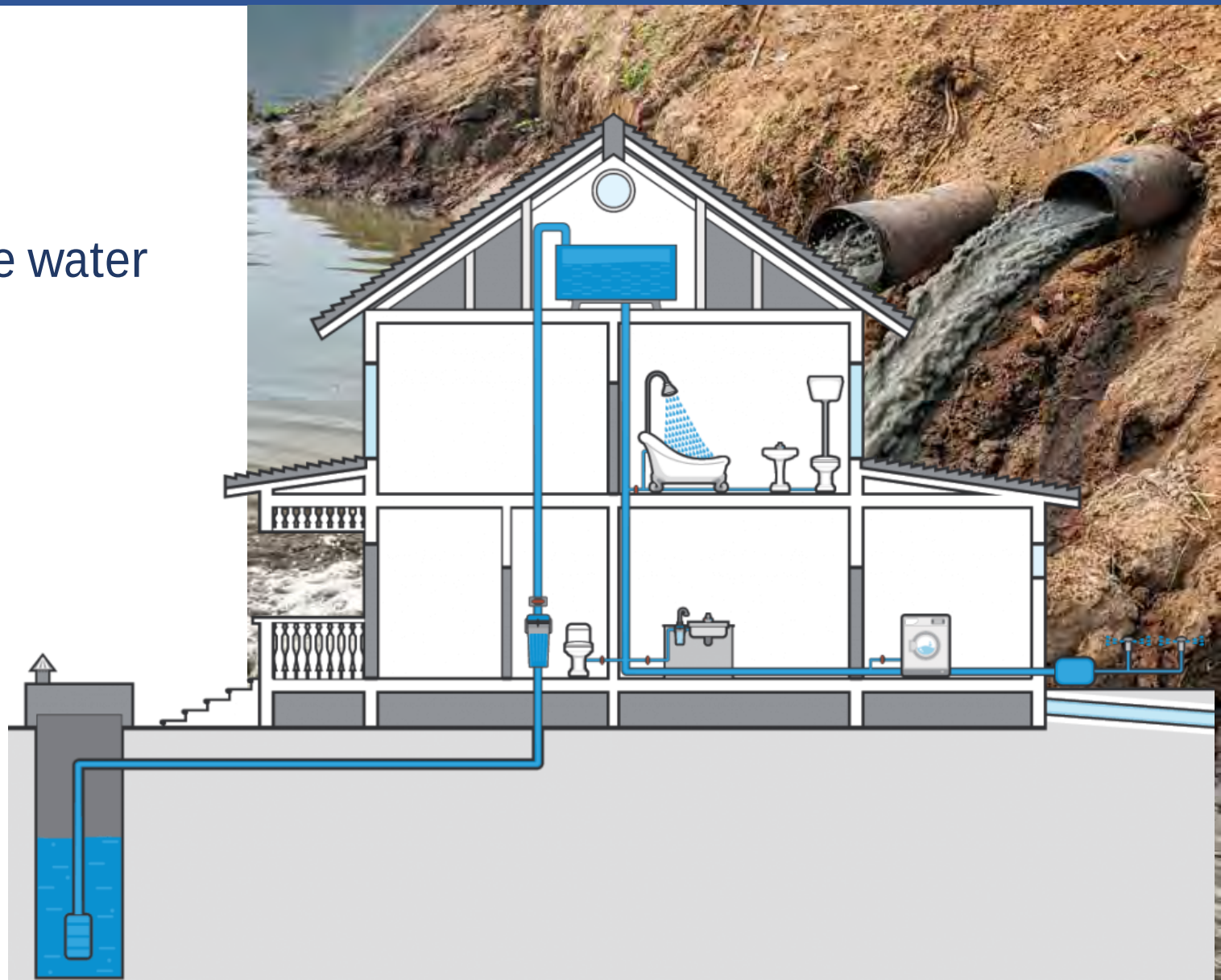
- USEPA 2016 - HAA9: bromochloroacetic acid, bromodichloroacetic acid, chlorodibromoacetic acid, and tribromoacetic acid

Unregulated HAAs?

Iodoacetic acid

Levels of DBPs in Drinking Water are Variable

- Concentration of organic matter
- Chemical composition of the source water
- pH
- Temperature
- Type of the disinfectant
- Concentration of the disinfectant

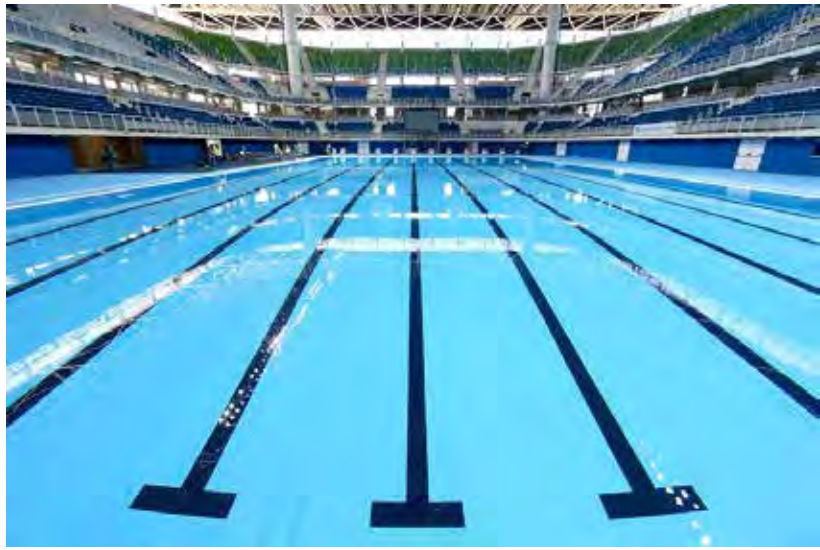


Routes of Exposure

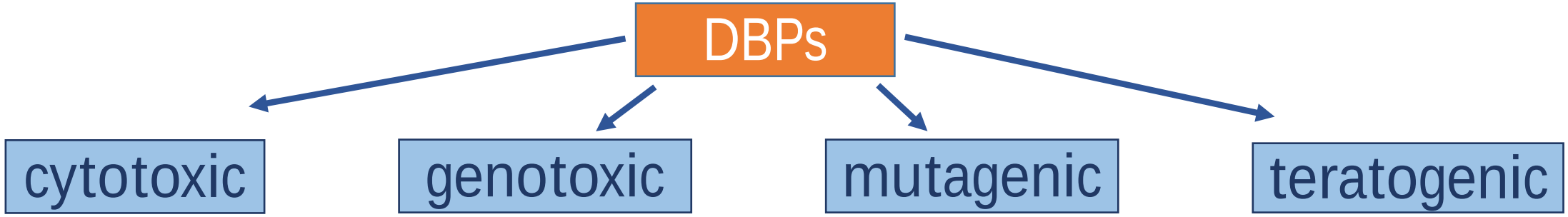
- Ingestion
  - drinking water
  - beverages
  - food

Routes of Exposure

- Inhalation and dermal absorption
  - showers, bathtubs
  - swimming pools, steam rooms



Health Concerns



- HAAs modulate gene expression (Attene-Ramos *et al*., 2010; Muellner *et al*., 2010)
  - stress response to DNA damage
  - cell cycle regulation
  - reactive oxygen species
  - apoptosis

Reproductive Health Concerns

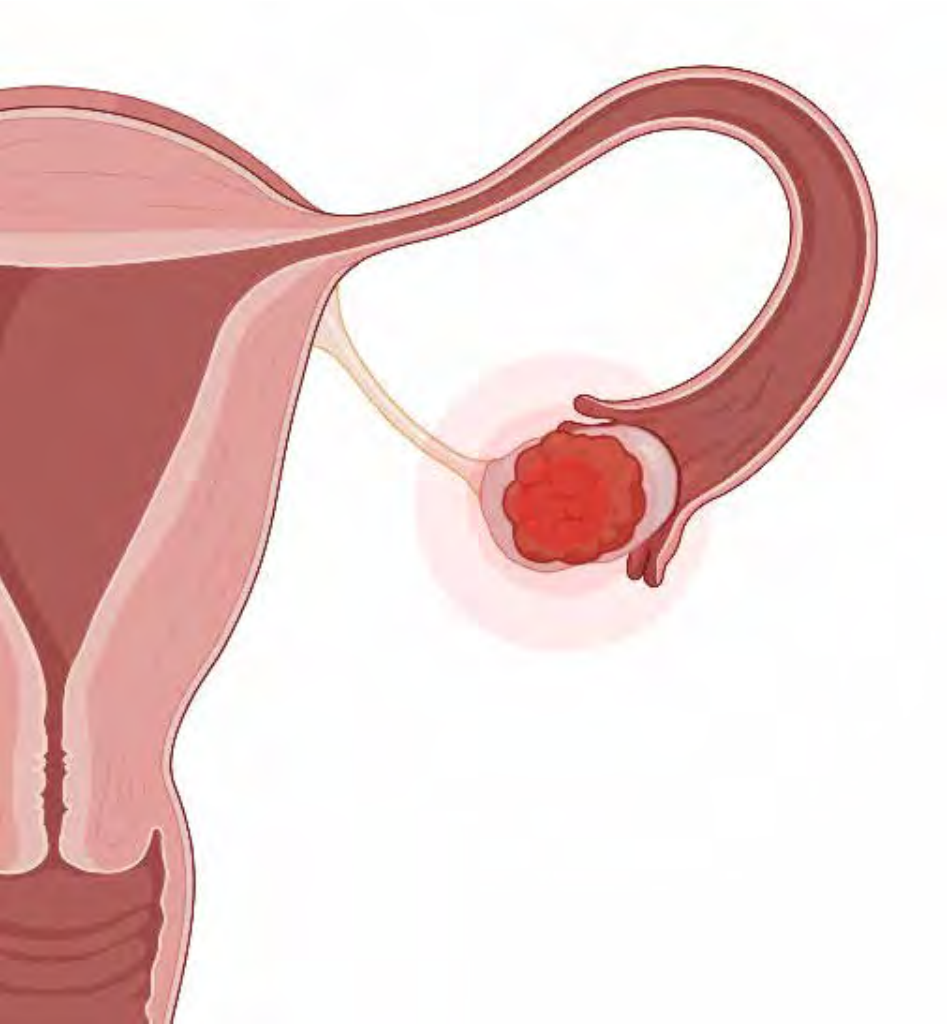
- HAAs disrupt estrous cyclicity and suppress estradiol catabolism, which leads to alterations in steroid production in female rats (Goldmann and Murr, 2003)

- Gestational exposure of a mixture of regulated HAAs results in pregnancy loss and eye malformation in rats (Narotsky *et al*., 2011)

- Adverse pregnancy outcomes (Nieuwenhuijsen *et al.*, 2000; Chisholm *et al.*, 2008; Hwang *et al.*, 2008; Rivera-Nunez *et al*., 2013)
  - low birth weight
  - small-for-gestational age
  - still birth
  - birth defects

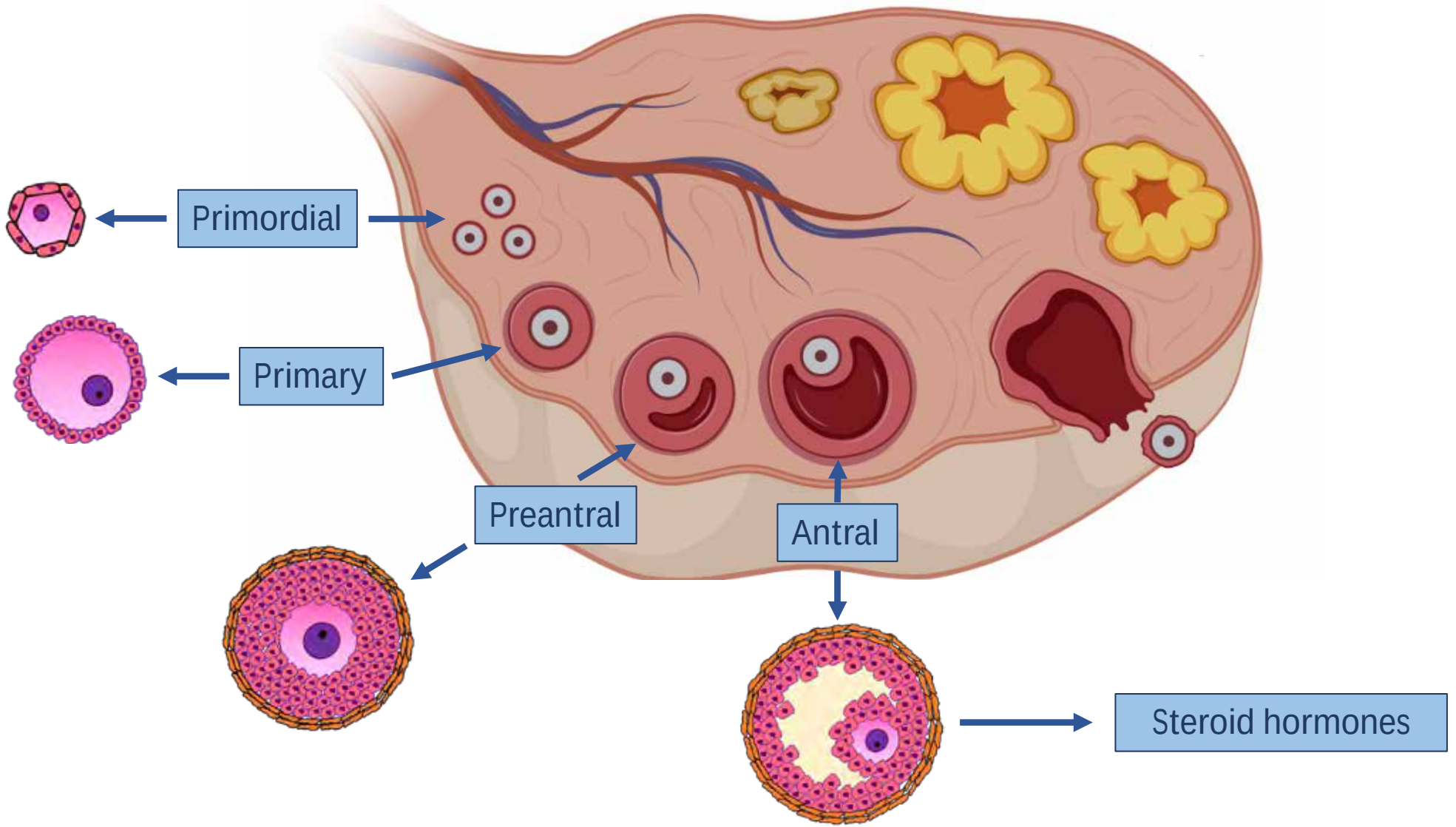
The effects of the HAAs on the ovary are largely unknown

Importance of the Ovaries



- Produce oocytes (folliculogenesis)
  - fertility
- Synthesize/secrete hormones (steroidogenesis)
  - development of eggs
  - estrous cyclicity
  - maintenance of reproductive tract
  - fertility
  - non-reproductive functions
    - cardiovascular, brain, bones

The follicle is the functional unit of the ovary

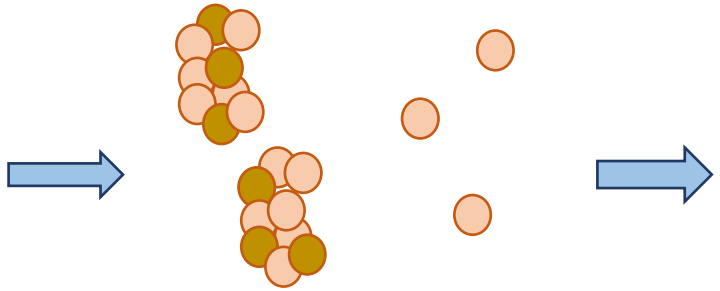


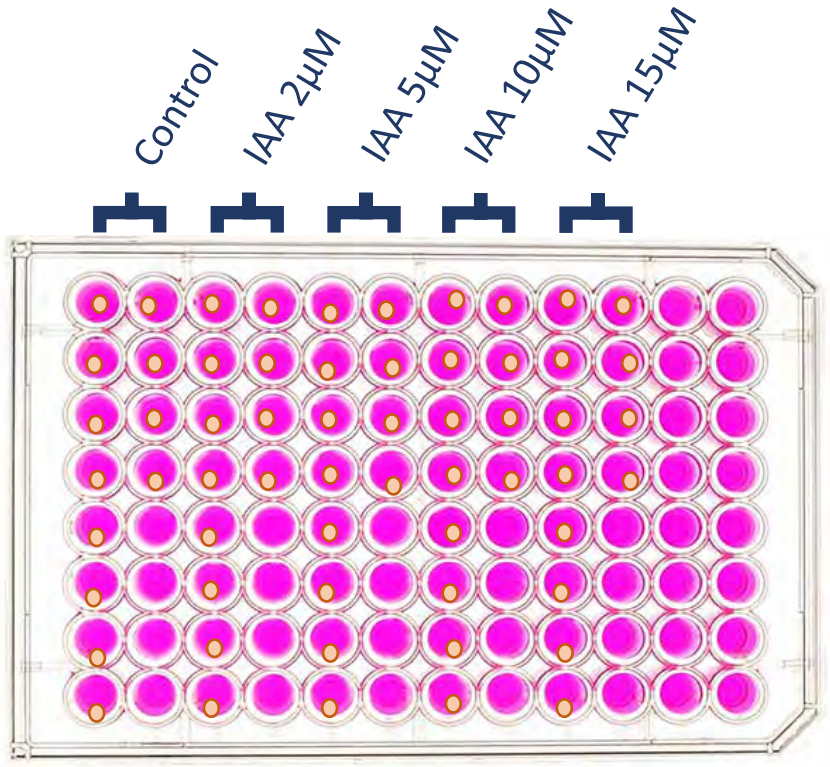
Hypothesis

Iodoacetic acid exposure inhibits antral follicle growth and steroidogenesis

Experimental Design

Follicle isolation



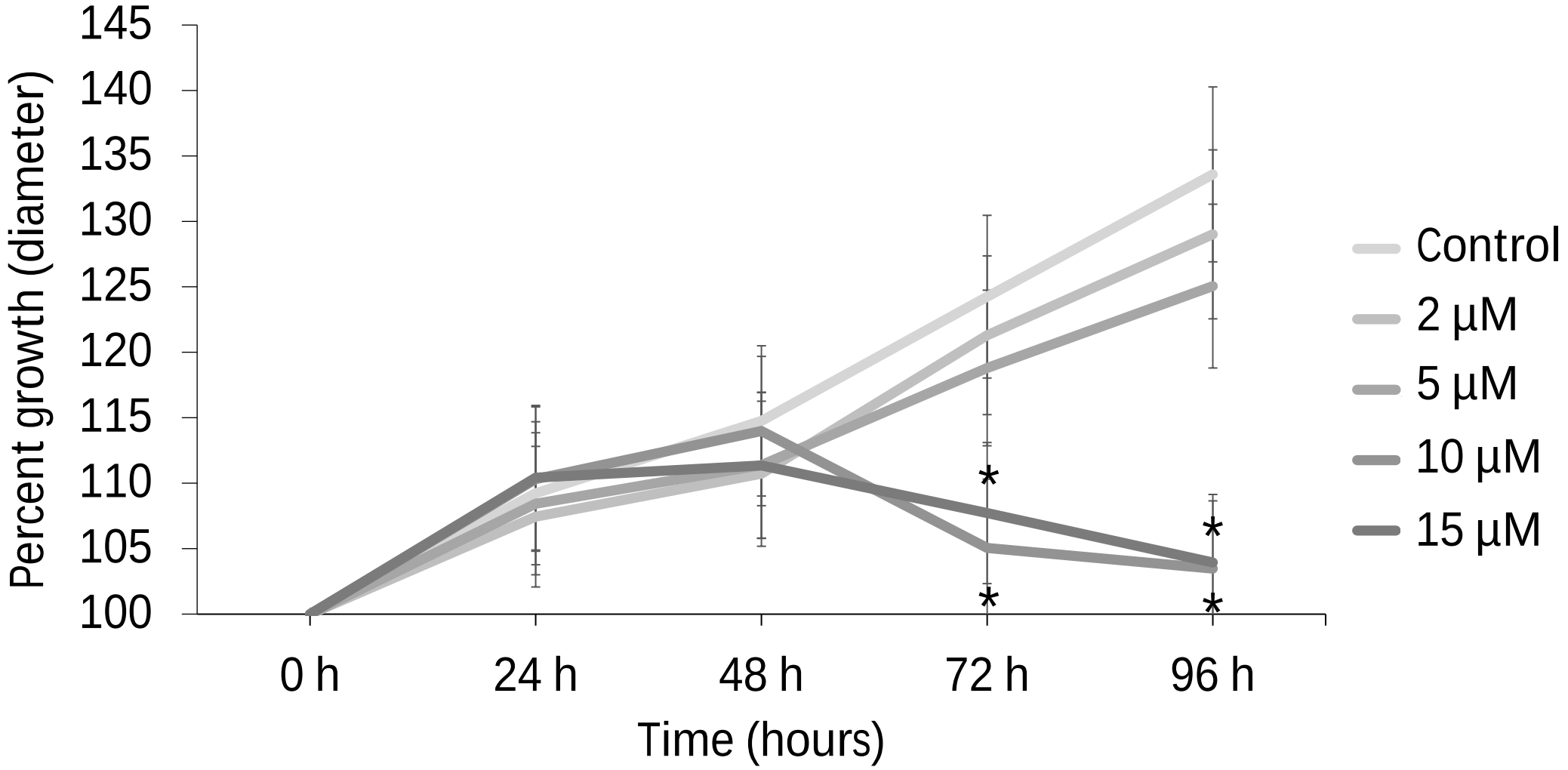


- Ovaries were collected from adult CD-1 mice (32 to 42 days old)

- Antral follicles were dissected from the ovaries and placed individually in 96-well culture plates

- Follicle growth
- Follicular gene expression
- Hormone levels in supernatant

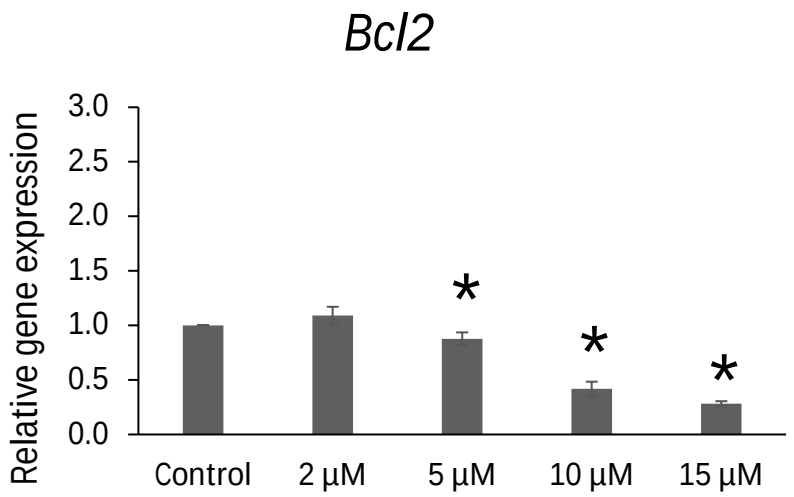
IAA decreases follicle growth in vitro

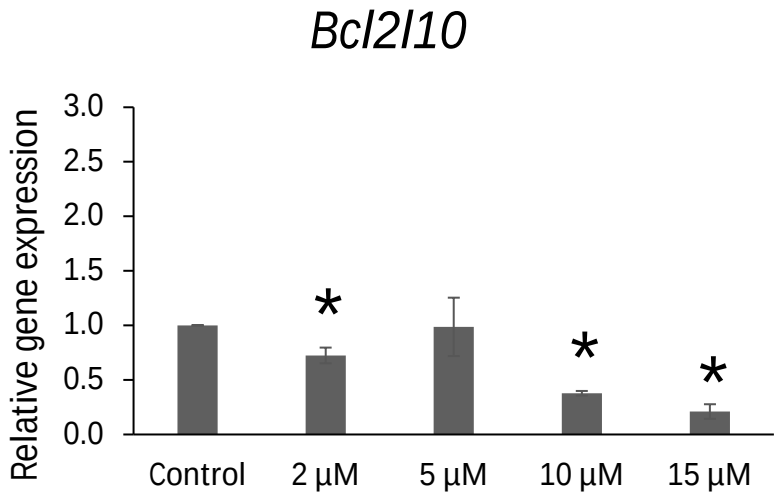


* $p \leq 0.05$

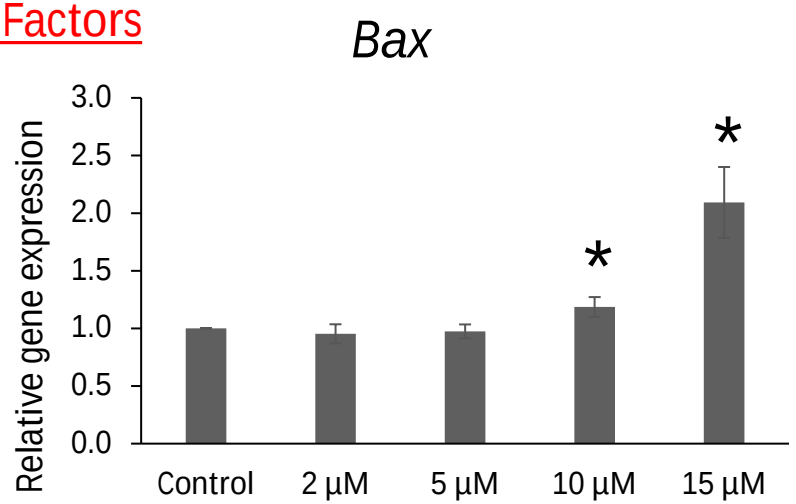
IAA alters expression of apoptotic factors

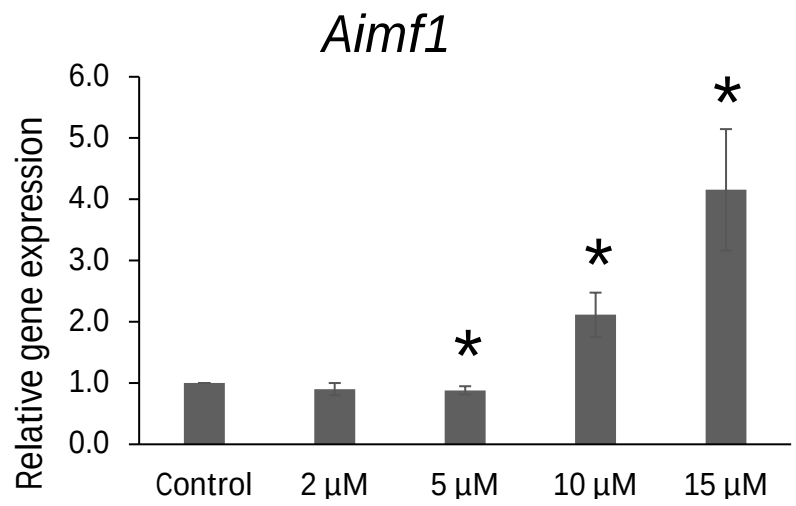
Anti-apoptotic Factors





Pro-apoptotic Factors

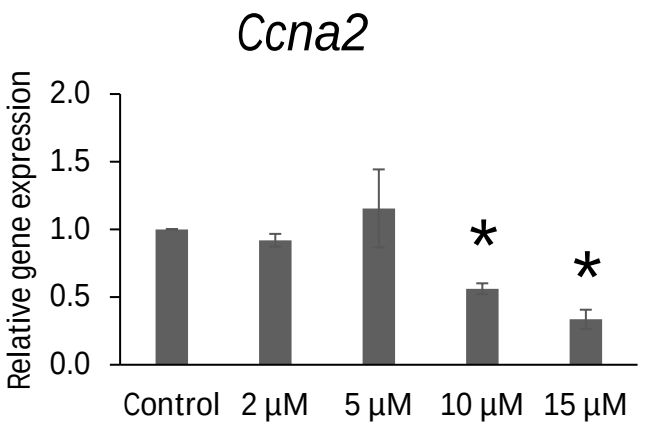


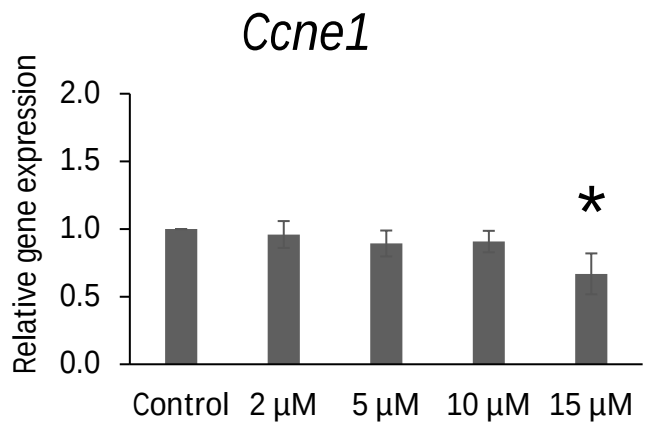


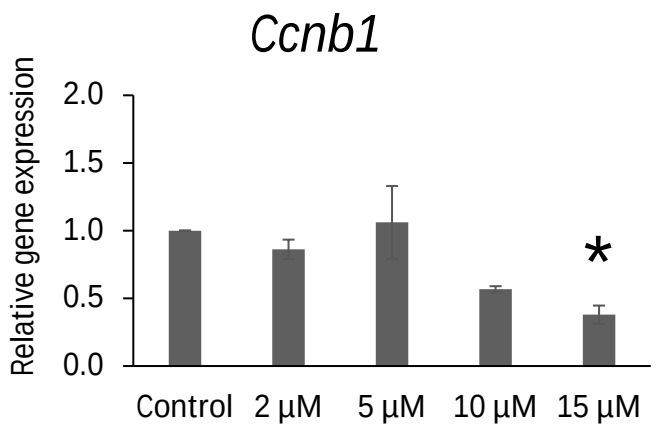
* $p \leq 0.05$

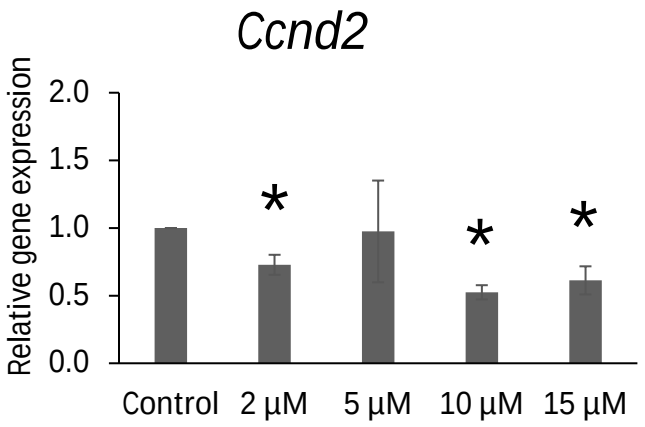
IAA alters expression of cell cycle regulators

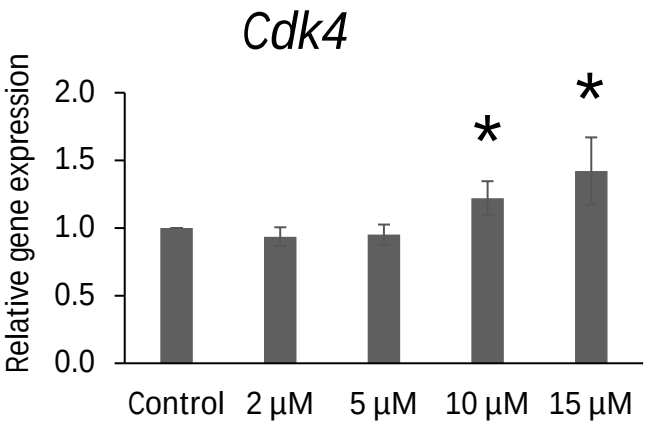
Promoters



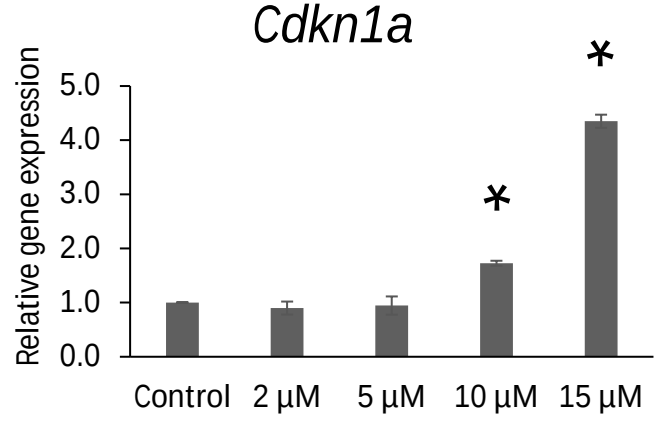






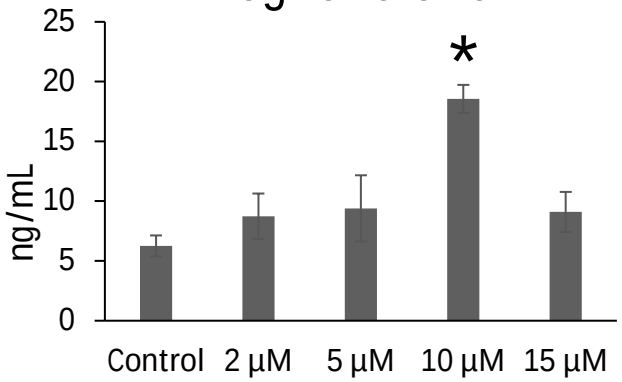


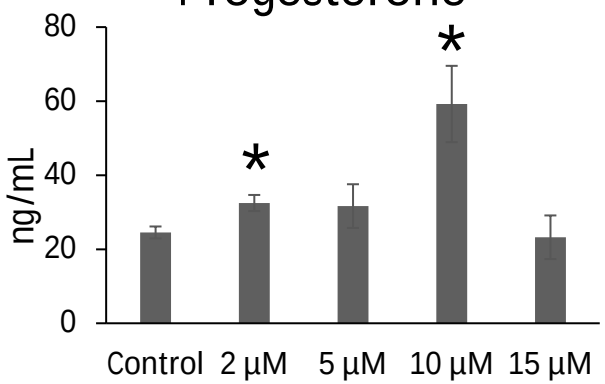
Inhibitor

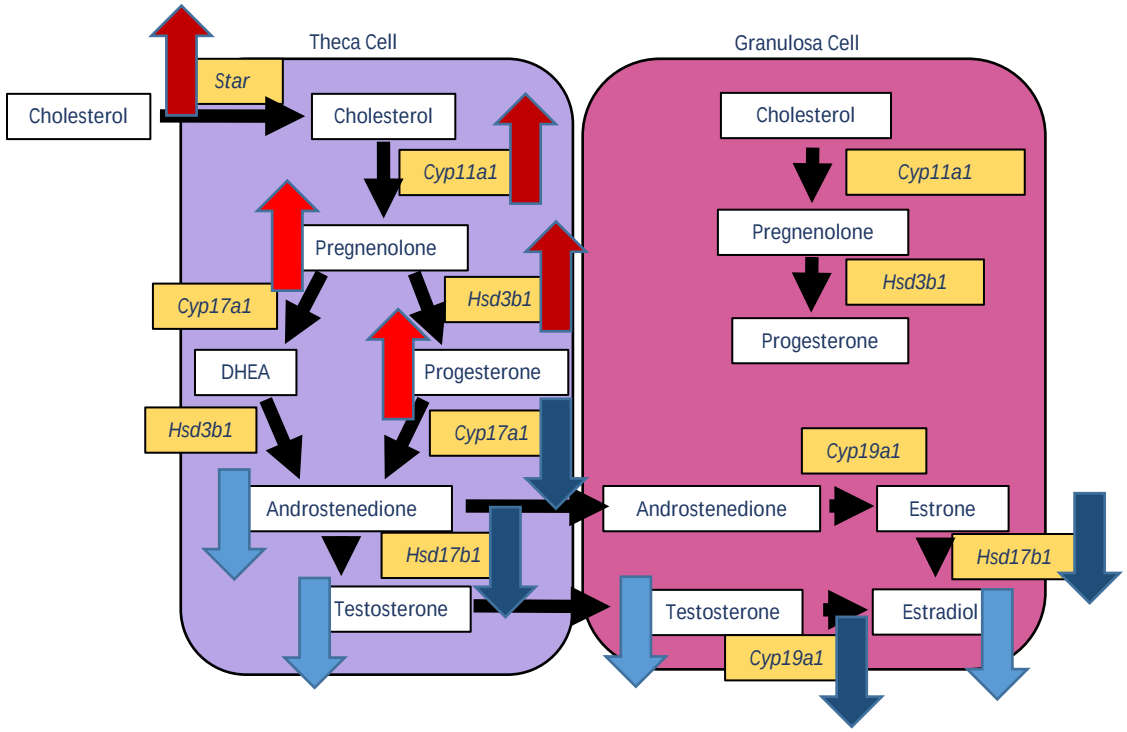


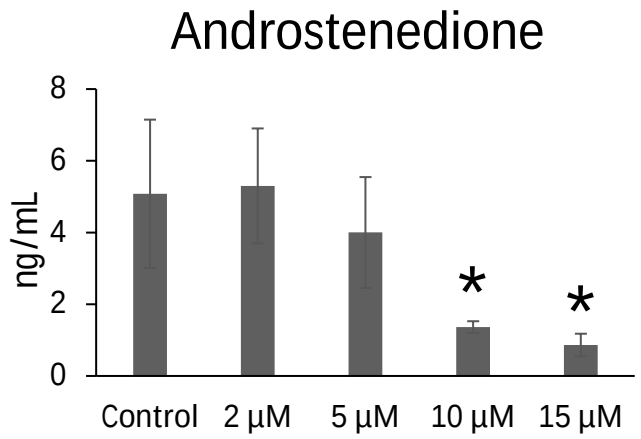
* $p \leq 0.05$

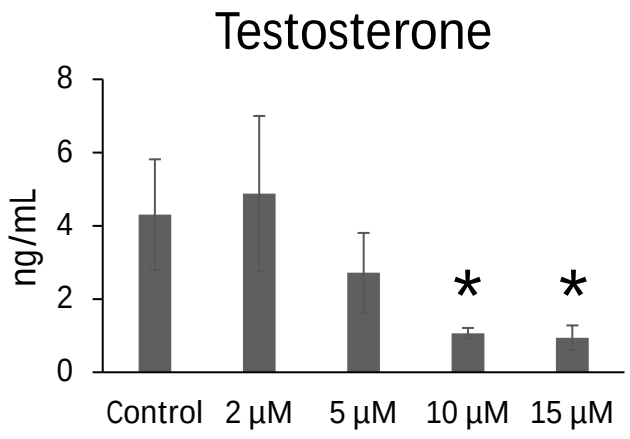
IAA alters sex steroid hormone levels

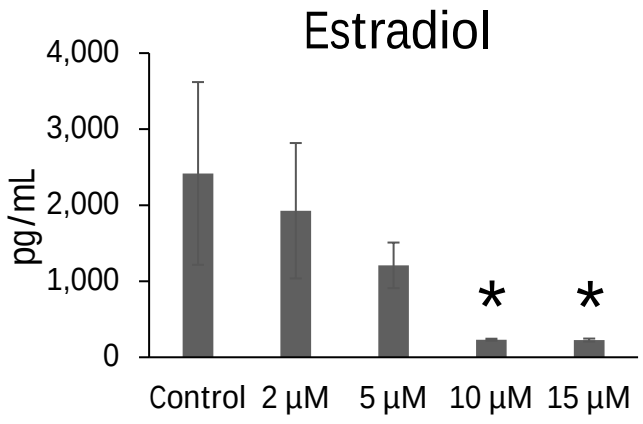






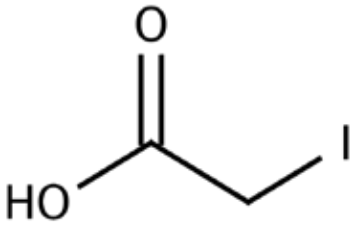






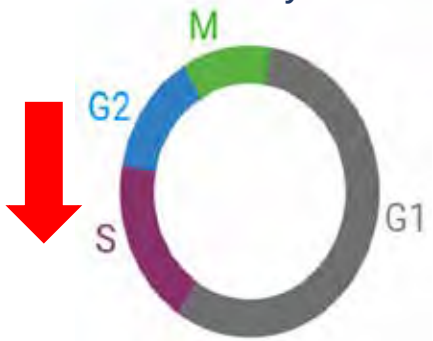
* $p \leq 0.05$

Summary (In Vitro)

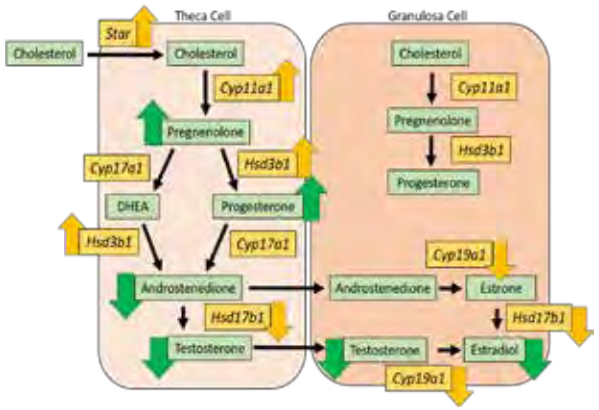


Apoptosis

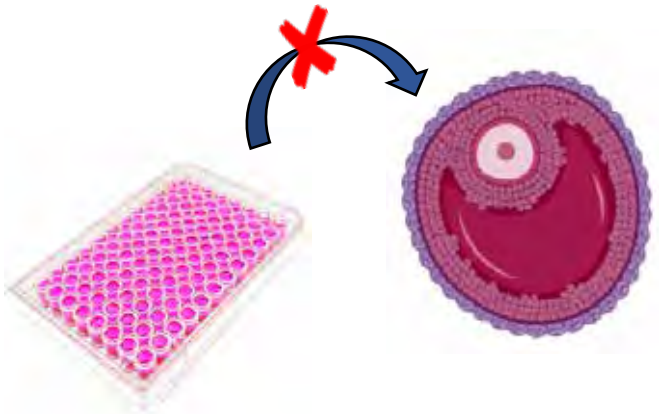
Cell Cycle



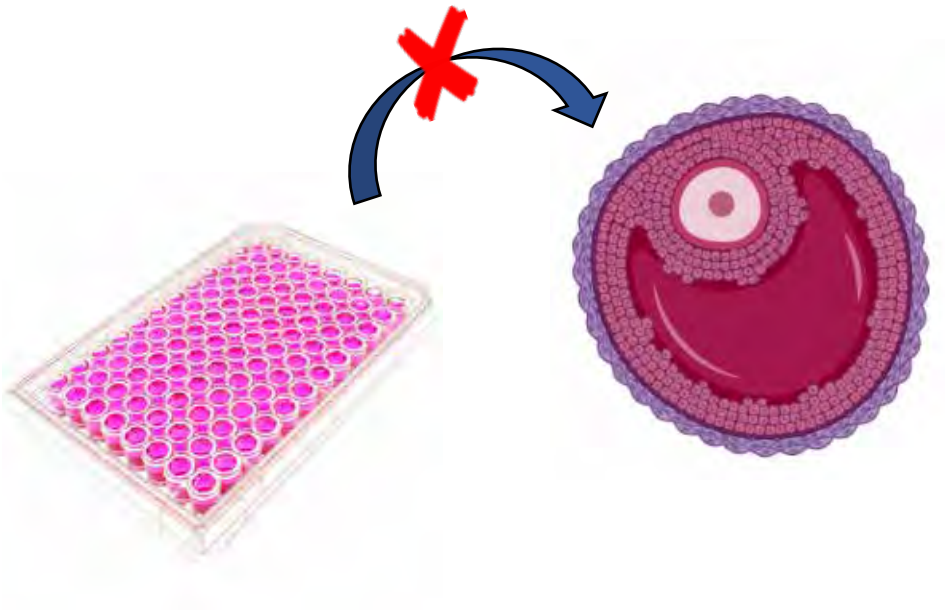
Steroidogenesis



Follicle growth



What about in vivo?



vs.

Hypothesis

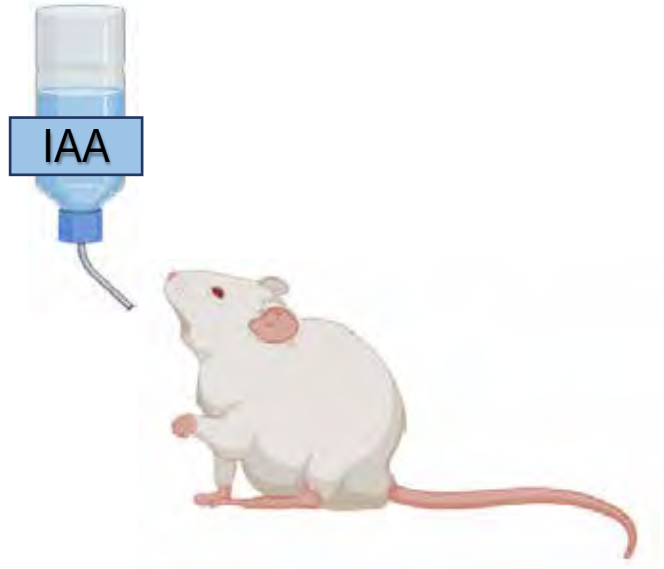
Iodoacetic acid exposure affects female reproductive outcomes in vivo

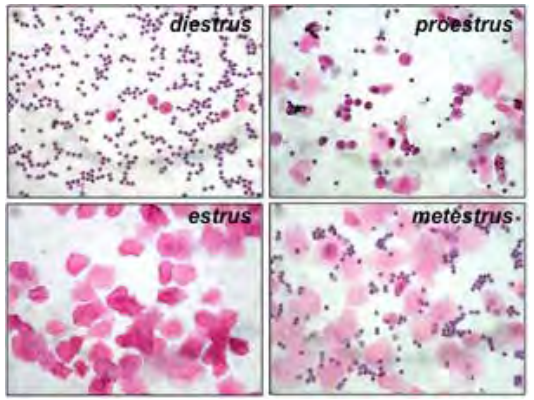
Experimental Design

- Female CD-1 mice were dosed with IAA in the drinking water for 35 days

- Control: only water
- 0.5 mg/L IAA
- 10 mg/L IAA
- 100 mg/L IAA
- 500 mg/L IAA

  n= 12 per group



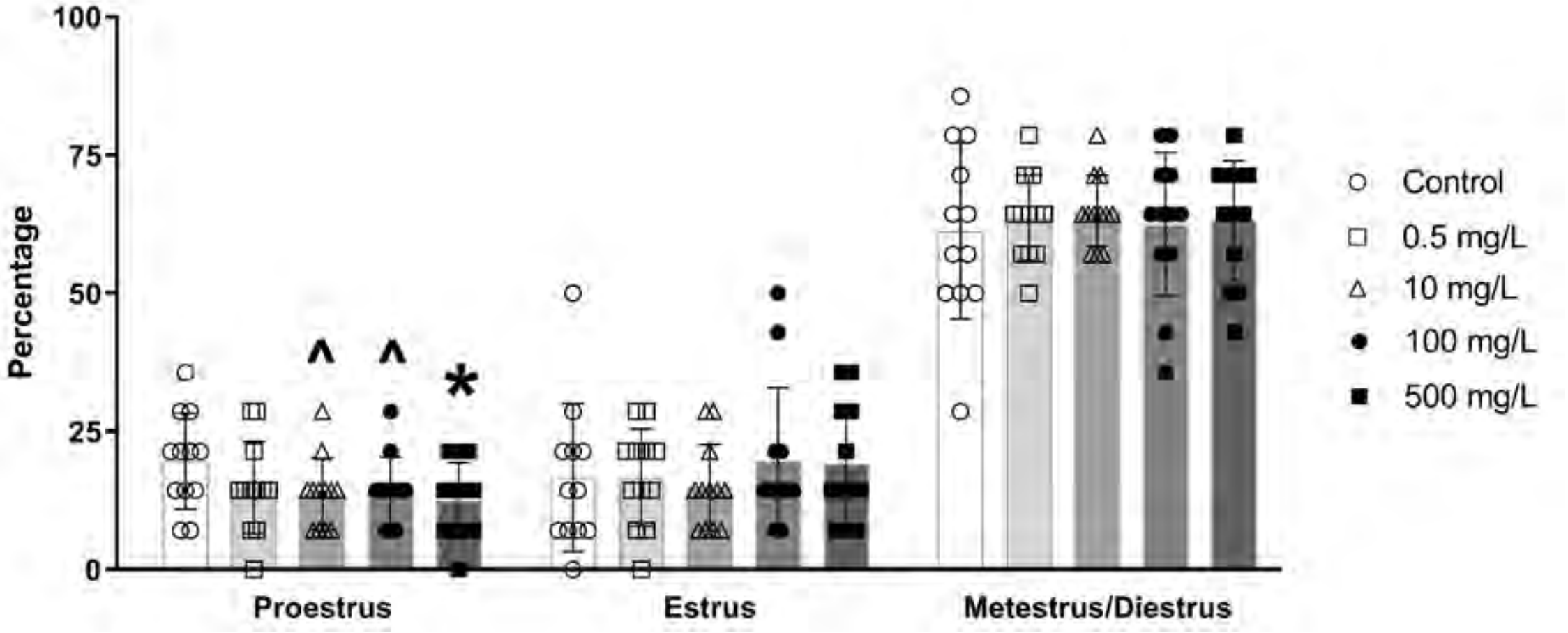


Estrous cyclicity

Ovaries for gene expression analyses

Serum for hormone analyses

IAA exposure affects estrous cyclicity

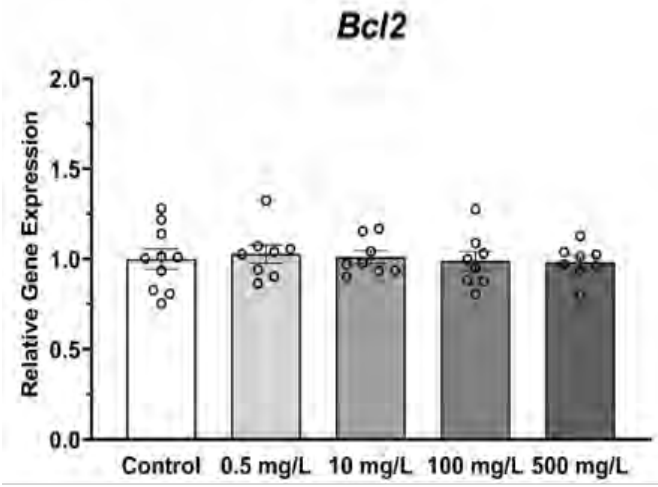


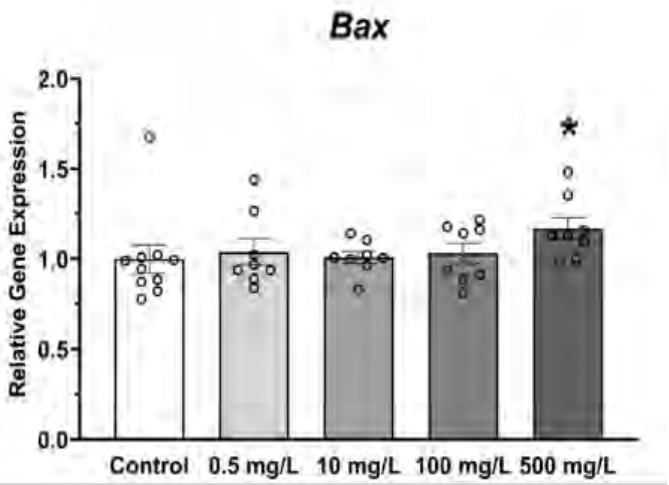
n=12, * $p \leq 0.05$, ^ $p \leq 0.096$

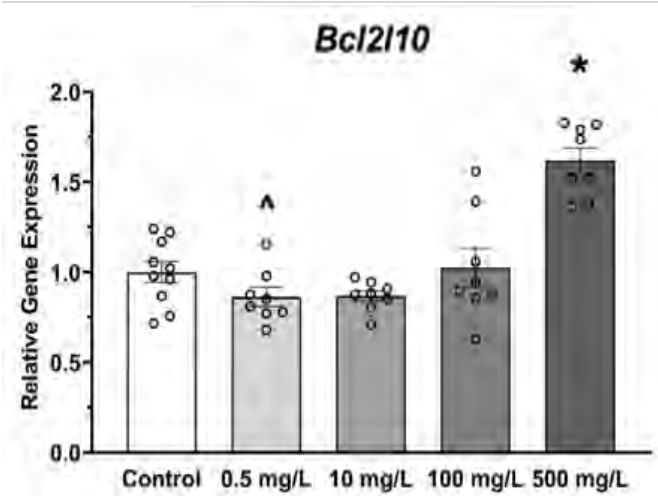
IAA exposure affects expression of apoptotic factors

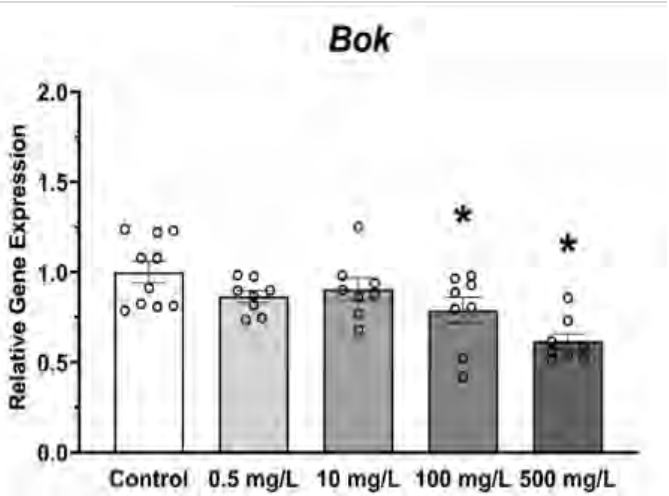
Anti-apoptotic Factors

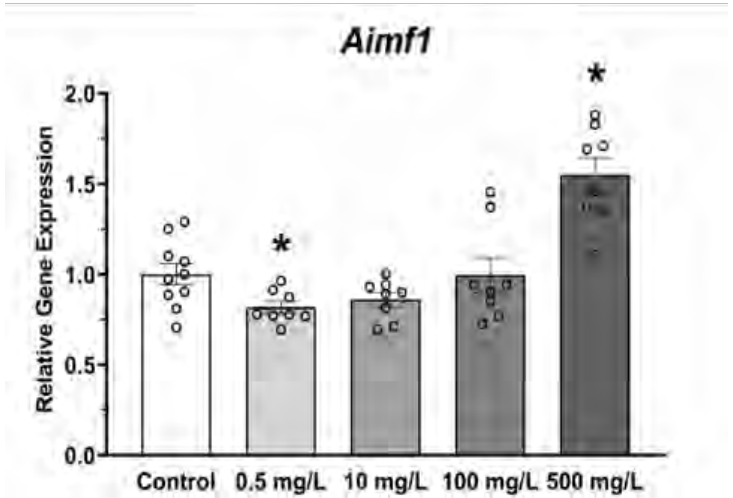
Pro-apoptotic Factors







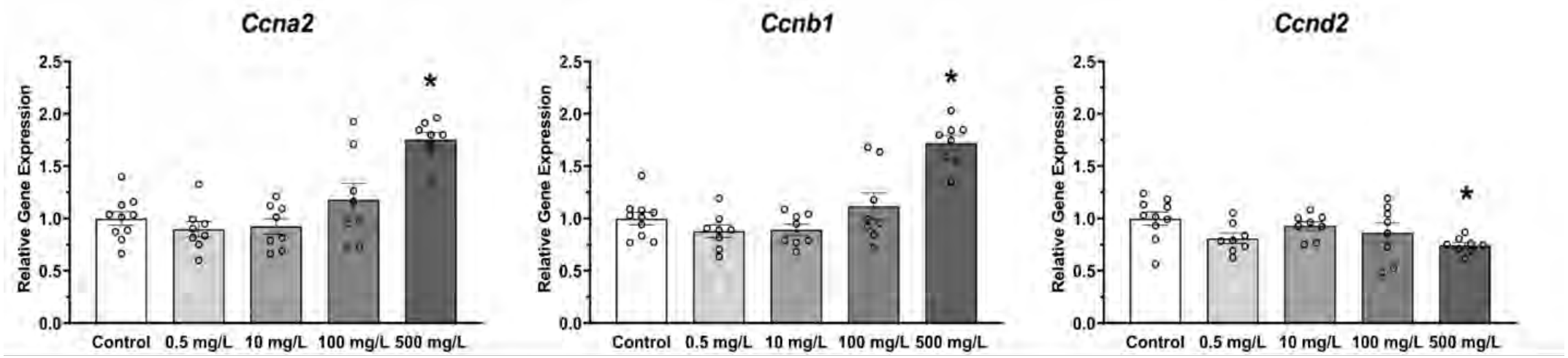


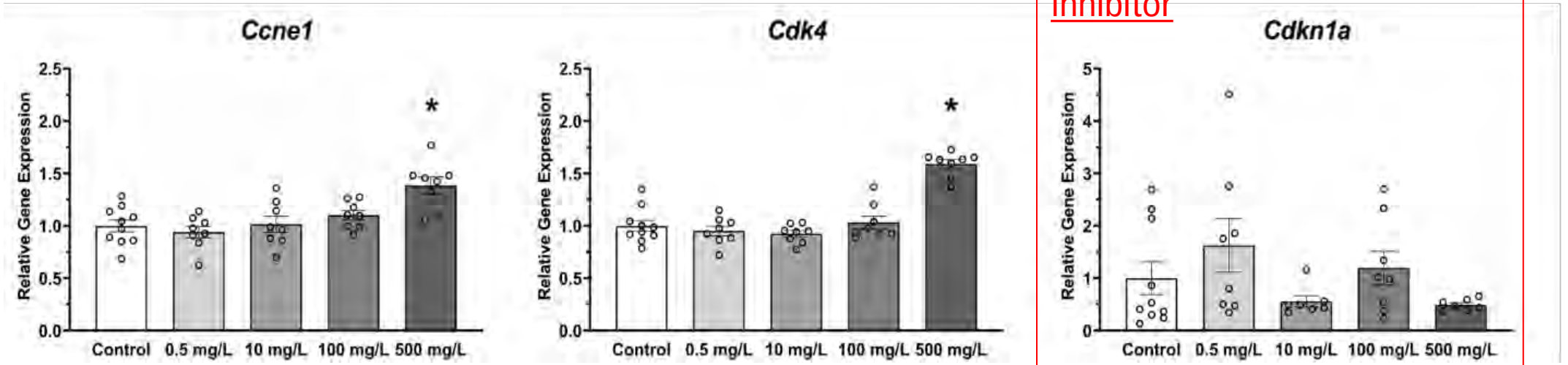


n=10-12, * $p \leq 0.05$, ^ $p \leq 0.076$

IAA exposure affects expression of cell cycle regulators

Promoters

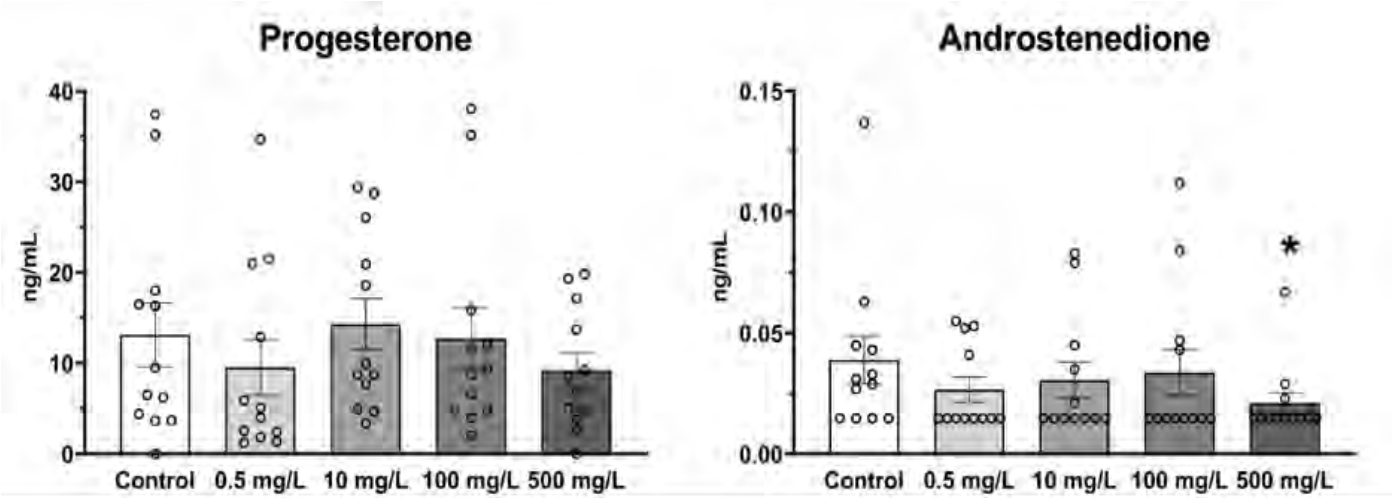


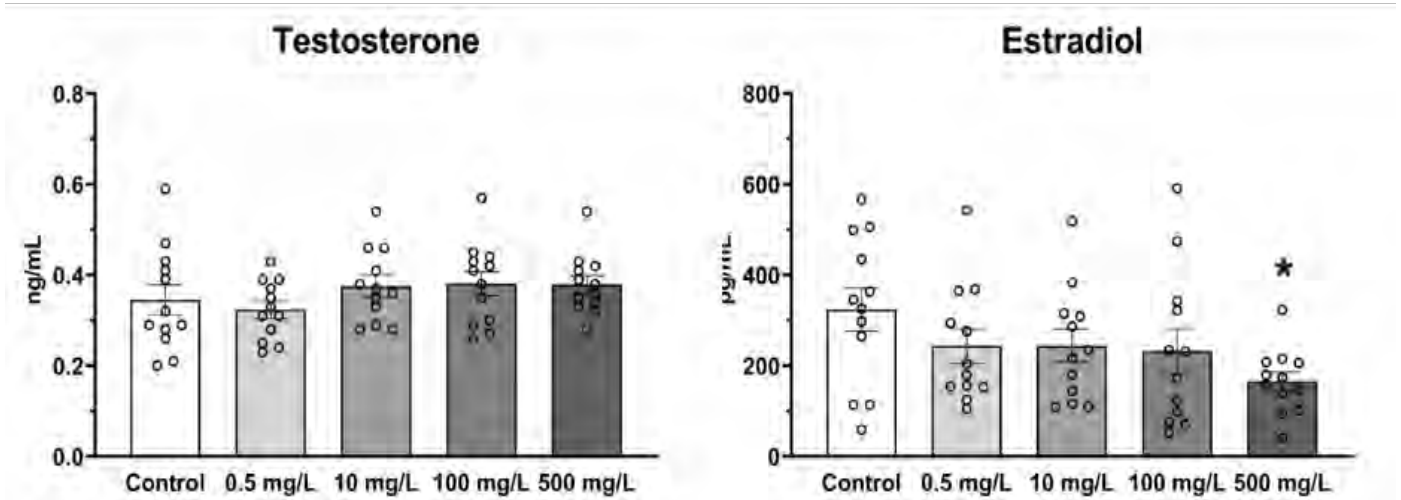


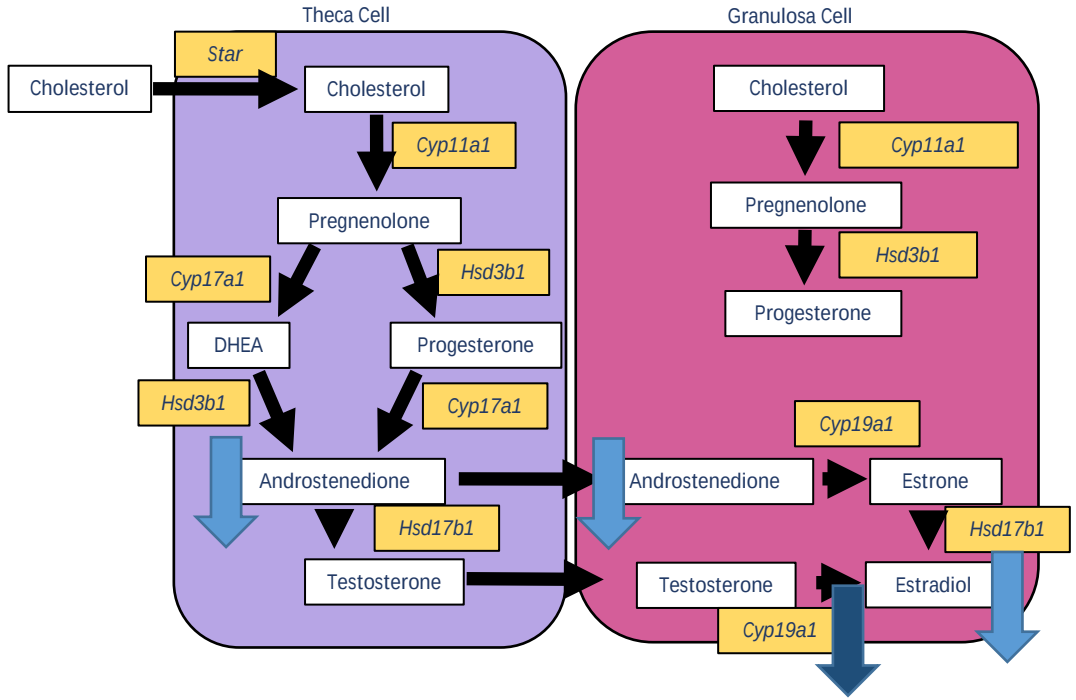
Inhibitor

n=10-12, * $p \leq 0.05$

IAA exposure affects estradiol levels







n=12, * $p \leq 0.05$

Comparison of gene expression in vitro vs. in vivo

	Gene	Antral follicles in vitro	Whole ovaries in vivo
Anti-apoptotic Factors	*Bcl2*	↓	↔
	Bcl2l10	↓	↑ ↓
Pro-apoptotic Factors	*Bax*	↑	↑
	Bok	**No Data**	↓
	Aimf1	↑	↑ ↓
Cell Cycle Promoters	*Ccna2*	↓	↑
	Ccnb1	↓	↑
	Ccnd2	↓	↓
	Ccne1	↓	↑
	Cdk4	↑	↑
Cell Cycle Inhibitor	*Cdkn1a*	↑	↔

Comparison of gene expression in vitro vs. in vivo

	Gene	Antral follicles in vitro	Whole ovaries in vivo
Steroidogenic Factors	*Star*	↑	⇔
	Cyp11a1	↑	⇔
	Hsd3b1	↑	⇔
	Hsd17b1	↓	⇔
	Cyp17a1	↓	⇔
	Cyp19a1	↓	↓

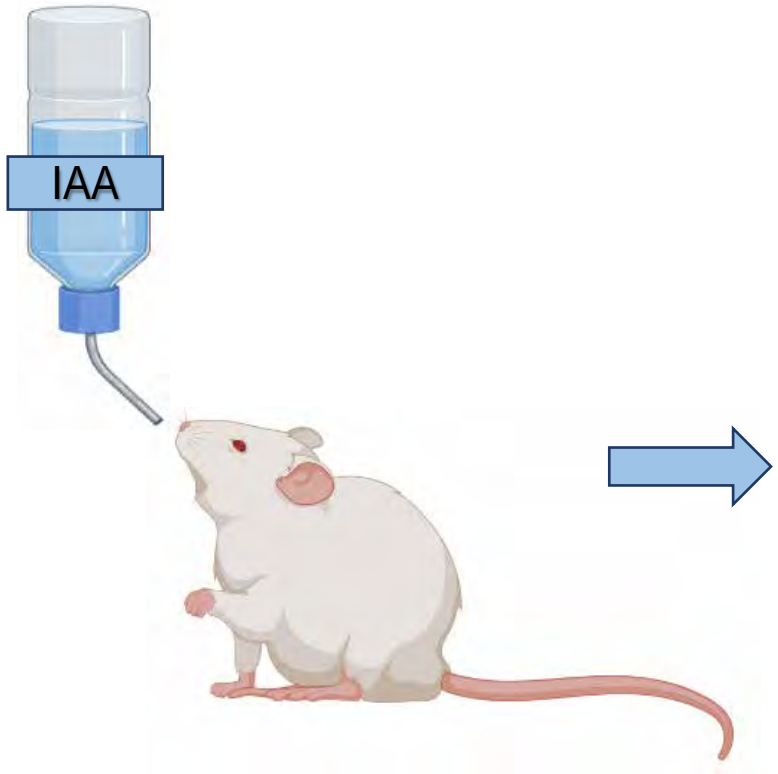
Comparison of hormone levels in vitro vs. in vivo

	Hormone	Antral Follicles In Vitro	In Vivo
Sex Steroid Hormones	*Pregnenolone*	↑	↔
	Progesterone	↑	↔
	Androstenedione	↓	↓
	Testosterone	↓	↔
	Estradiol	↓	↓

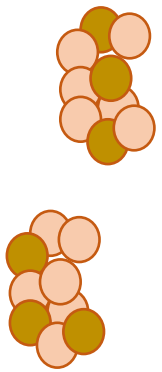
Hypothesis

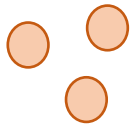
Iodoacetic acid exposure affects the transcriptome in ovarian antral follicles

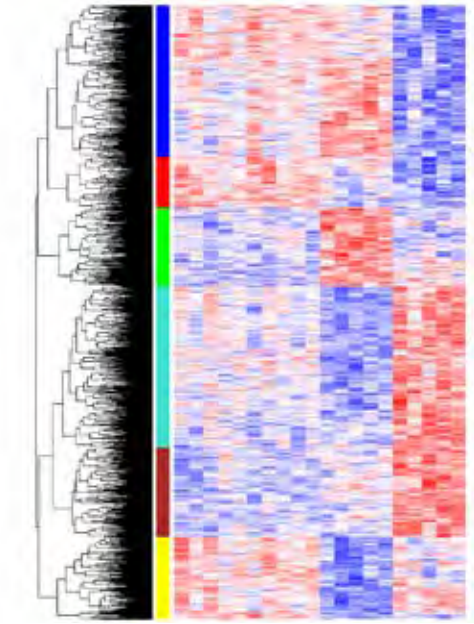
Experimental Design



Isolated antral follicles







- Female CD-1 mice were dosed with IAA in the drinking water for 35 days

- Control: only water
- 10 mg/L IAA
- 500 mg/L IAA

n= 12 per group

- RNA sequencing analysis

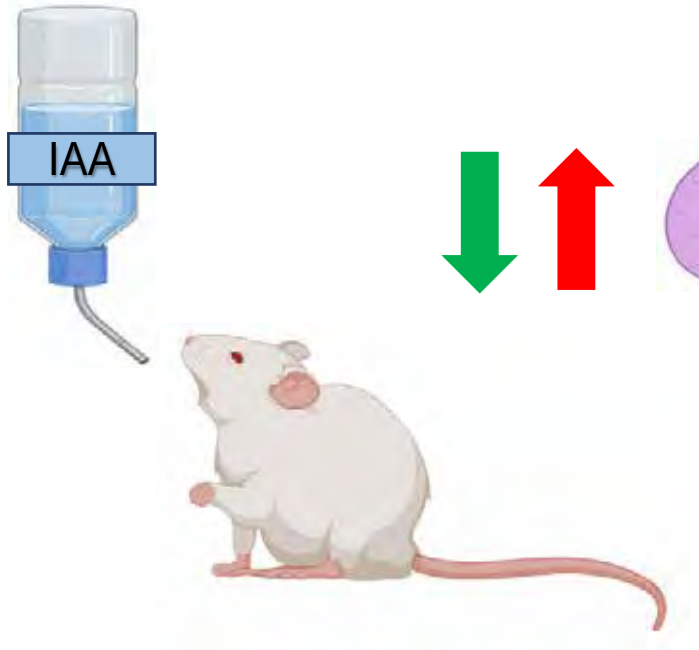
- Enrichment annotation analysis

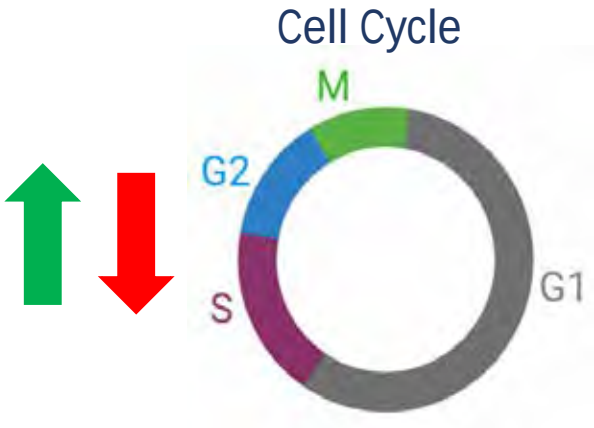
Summary

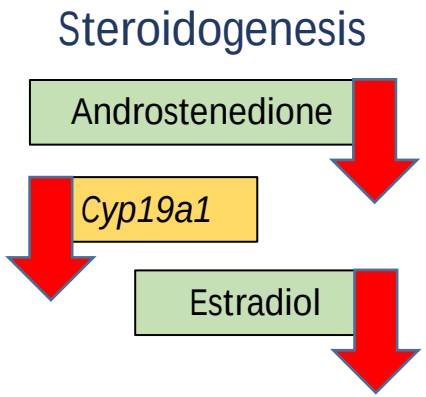
IAA exposure altered expression of genes involved with:

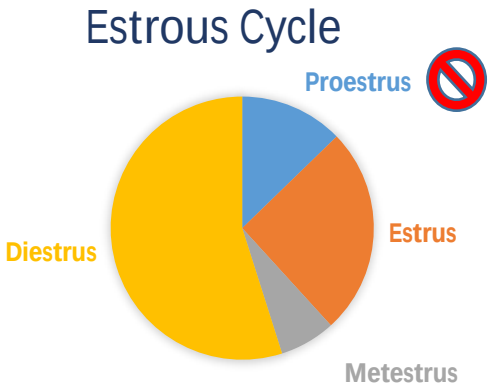
- RNA processing
- regulation of angiogenesis
- cell cycle
- mitotic cellular division
- cell division
- the PI3K-Akt signaling pathway
- the estrogen signaling pathway
- the GnRH signaling pathway
- the insulin signaling pathway
- the oxytocin signaling pathway

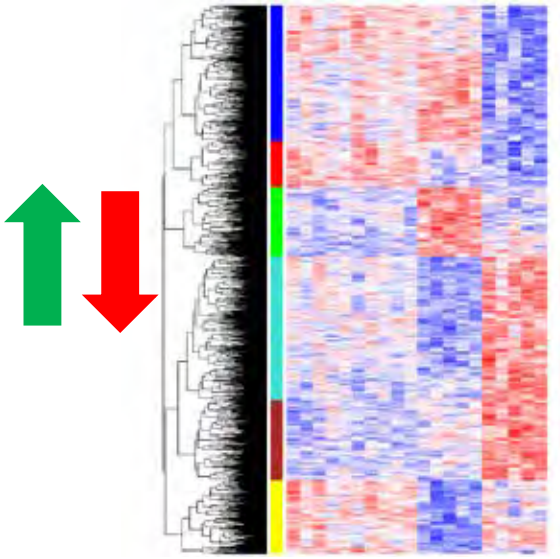
Summary (In Vivo)



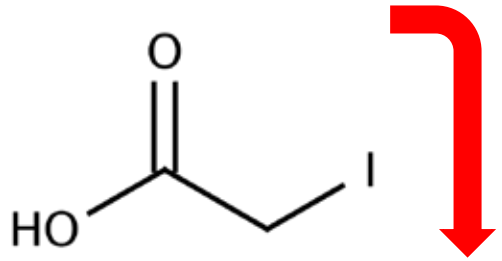




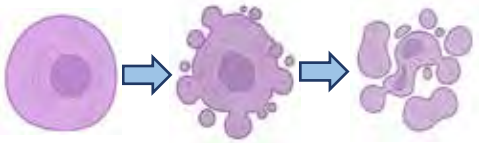


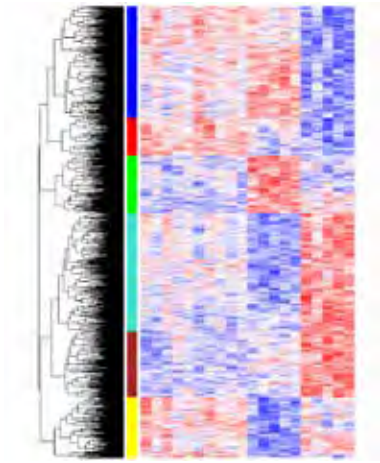


Conclusions

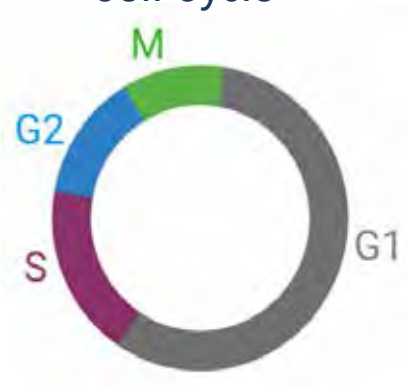


Apoptosis

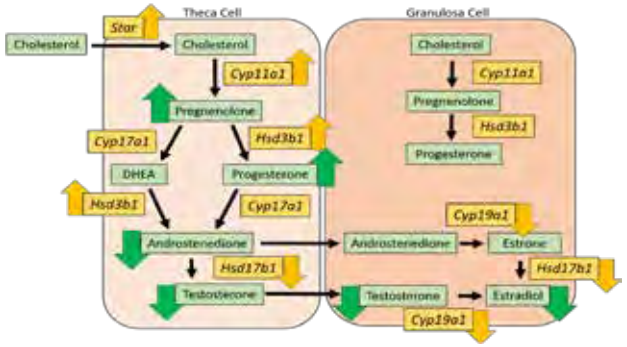




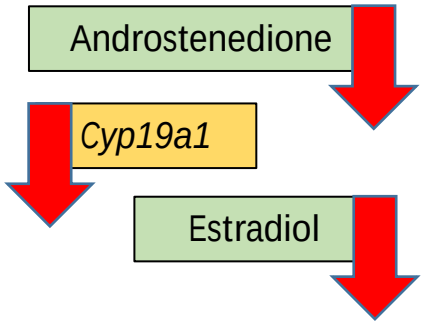
Cell Cycle



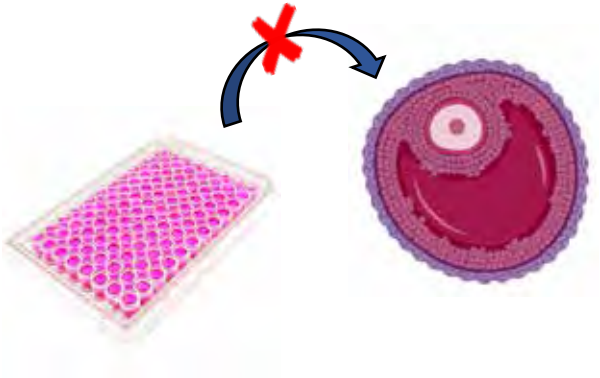
Disrupts steroidogenesis (In Vitro)



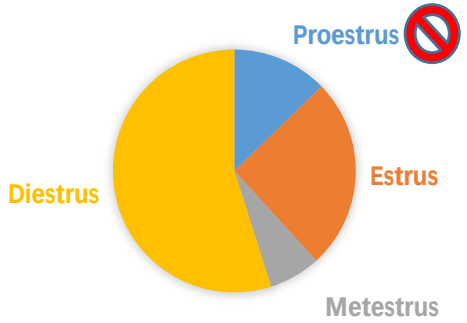
Disrupts steroidogenesis (In Vivo)



Impairs follicle growth



Disrupts estrous cycle



Acknowledgements

- Dr. Michael Plewa
- Flaws laboratory members

- Funding
  - NIH R21 ES028963
  - NIH T32 ES007326
  - Toxicology Scholarship

Questions?

Hiding In Plain Sight

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Breast Cancer Risk

Elizabeth Boham, M.D., Michele La Merrill, Ph.D., Lindsey Treviño, Ph.D.

Moderated by Deb Kilday

Elizabeth Boham, M.D.

Medical Director

The UltraWellness Center

Endocrine Disrupting Chemicals and Breast Cancer Risk

U.S. Dept of Health and Human Services

Endocrine Disrupting Chemicals and Women's Health Symposium

July 18th 2023

Outline

- Extent of the problem
- What are endocrine disrupting chemicals
- Mechanism of action
- How to avoid
- Prevention
- Phytonutrients
- What can YOU do
- What can WE do

Breast Cancer Statistics in the US in 2022

- Approximately 13% of women (1 in 8) will be diagnosed with invasive breast cancer, and 3% (1 in 39) will die from the disease in their lifetime
- In 2022 approximately
  - 287,850 new cases of invasive breast cancer
  - 51,400 cases of DCIS
  - 43,250 women die from breast cancer
  - 4.1 million women with a history of breast cancer living in the United States
- Incidence rates continue to increase
- Most common cancer in women, except for skin cancer
- Median age is 62

Giaquinto, A.N., Sung, H., Miller, K.D., Kramer, J.L., Newman, L.A., Minihan, A., Jemal, A. and Siegel, R.L. (2022), Breast Cancer Statistics, 2022. CA A Cancer J Clin, 72: 524-541. https://doi.org/10.3322/caac.21754

Causes of Breast Cancer

- Genetics – high and low penetrance genes
- Reproductive history
- Lifestyle
  - Obesity
  - Diet
  - Lack of physical activity
  - Alcohol intake
- Environmental exposures – Since WWII and increased exposure and increased incidence of breast cancer

Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

Endocrine Disrupting Chemicals What Matters?

Exposure

Timing

Genetics

Lifestyle

Endocrine Disrupting Chemicals What Matters?

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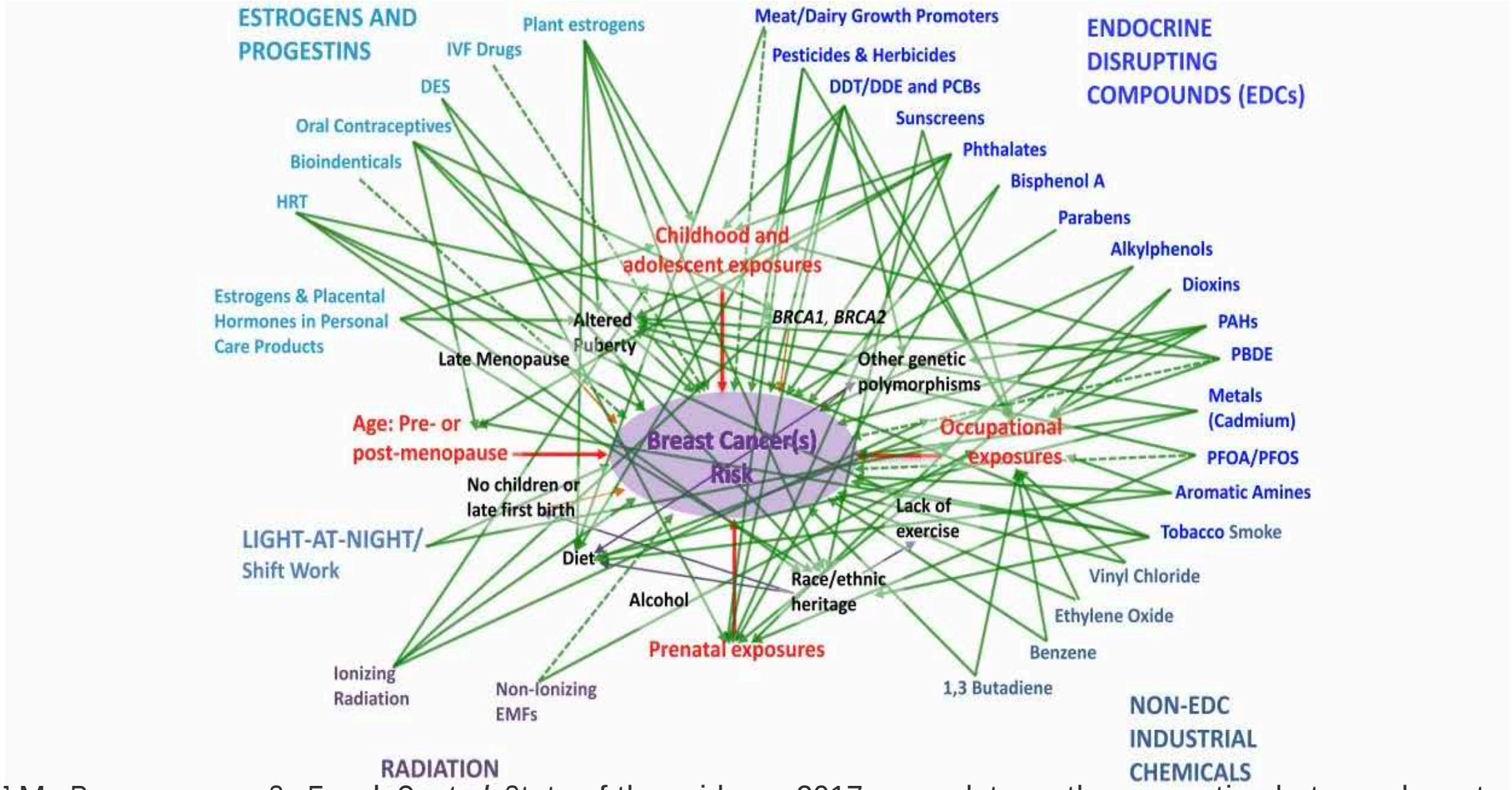
Lifestyle

Complexity of Factors Impacting Risk of Breast Cancer



Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

Endocrine Disrupting Chemicals

An exogenous chemical or mixture of chemicals that interferes with any aspect of hormone action

- BPA – bisphenol A – used in polycarbonate plastic, dental sealants, thermal receipts, food packaging and epoxy resins lining food cans
- Phthalates – fragrance ingredients in personal care and cleaning products, plastics, building materials, insecticides, food packaging, plastic wrap
- Parabens – antimicrobial preservatives in foods, personal care products, cosmetics, moisturizers, soaps, detergents and pharmaceuticals
- Alkylphenols – detergents and cleaning products
- Triclosan and triclocarban – antimicrobials in toothpaste, personal care and household products

Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

BPA

- Prenatal exposure of rats to BPA resulted in increases in the number of pre-cancerous lesions and in situ carcinomas, as well as an increased number of mammary tumors following adult exposures to sub- threshold doses of known **carcinogens**
- Neonatal **exposure** of mice to BPA **increased sensitivity** to estradiol-mediated development of mammary gland structures at puberty
- Effects of BPA on mammary tissue development may also be manifested via **epigenetic mechanisms**, leading to changes in gene regulation across the lifetime
- BPA **reduces the efficacy of common chemotherapy** agents (cisplatin, doxirubicin and vinblastin) in their blocking the proliferation of human breast cancer cells when tested in vitro
- **3-day period of limiting intake of packaged foods** decreased the concentrations of BPA found in urine by an average 65%

Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

Phthalates

- They can **bind to estrogen receptors** induce estrogen-appropriate cellular responses and act additively with estradiol in altering these systems
- Promote cancer stem cell growth
- Phthalates can also induce **proliferation, malignant invasion, and tumor formation** in breast cancer cell lines that are receptor negative, indicating that at least some effects of these compounds are independent of their direct estrogenic or androgenic effects
- Mouse studies – interfere with production of testosterone and estradiol and abnormalities in male offspring exposed prenatally included nipple retention, shortened anogenital distance and increased cryptorchidism
- BBP, DBP and DEHP all significantly increased cell proliferation in MCF-7 breast cancer cells. In addition, these three phthalates **inhibited the anti-tumor action of tamoxifen** in MCF-7 breast cancer cells. BBP also decreased the efficacy of the chemotherapeutic agents, doxorubicin and cyclophosphamide
- A dietary intervention study has demonstrated that just a **3-day period of limiting intake of packaged foods** decreased by half the concentrations of DEHP (phthalates) found in urine

Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

Others

- Forever Chemicals - PFOA / PFOS – Teflon and Gore-tex, carpet and furniture protectants
- Higher levels of these and PCBs are found in women with breast cancer
- EDCs found in sunscreens
- Polycystic Aromatic Hydrocarbons (pyrene and benzene) – byproducts of combustion – coal burners, grilled meats, cigarettes, diesel fuel
- DDE (metabolite of DDT)
  - DDT/DDE – used for malaria control – still used in sub- Sararan Africa
  - Clear association when looking at early life (prenatal and childhood) exposure -
- Triazine herbicides (ie atrazine) –
  - shown to increase aromatase activity
  - exposure in rats during pregnancy let to changes in mammary gland of pups

Endocrine Disrupting Chemicals What Matters?

Exposure

Timing

Genetics

Lifestyle

Timing of Exposure

- We need to think about timing of exposure – ie fetal and adolescent exposure and later developing the disease
- Long latency between exposures and diagnosis
- Earlier developmental exposures can be especially powerful in affecting development of breast cancer, even decades later
- EDC disrupt the endocrine system – does not follow the typical linear dose relationship
- Even at low dose they may impact health at critical periods

Gray, J.M., Rasanayagam, S., Engel, C. *et al.* State of the evidence 2017: an update on the connection between breast cancer and the environment. *Environ Health* **16**, 94 (2017). https://doi.org/10.1186/s12940-017-0287-4

Timing of Exposure – Examples

- For BPA most profound impact is exposure during early development
- Prenatal and neonatal exposure to BPA (bisphenol A) can change mammary tissue development and impact likelihood of development of mammary tumors later in life
- DES (diethylstilbestrol) exposure increased risk of breast cancer in daughters and granddaughters
  - Impacts that mammary gland epigenome through alterations in histone methylation – leading to altered gene expression in puberty and adulthood
  - Epigenetic changes could be the mechanism for trans-generational effects
- Higher maternal DDT (dichlorodiphenyl trichloroethane) levels were associated with 4 x increase risk of breast cancer in daughters by at 52
- Exposure to radiation in childhood and adolescence increases risk for breast cancer later in life

Endocrine Disrupting Chemicals What Matters?

Exposure

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Endocrine Disrupting Chemicals What Matters?

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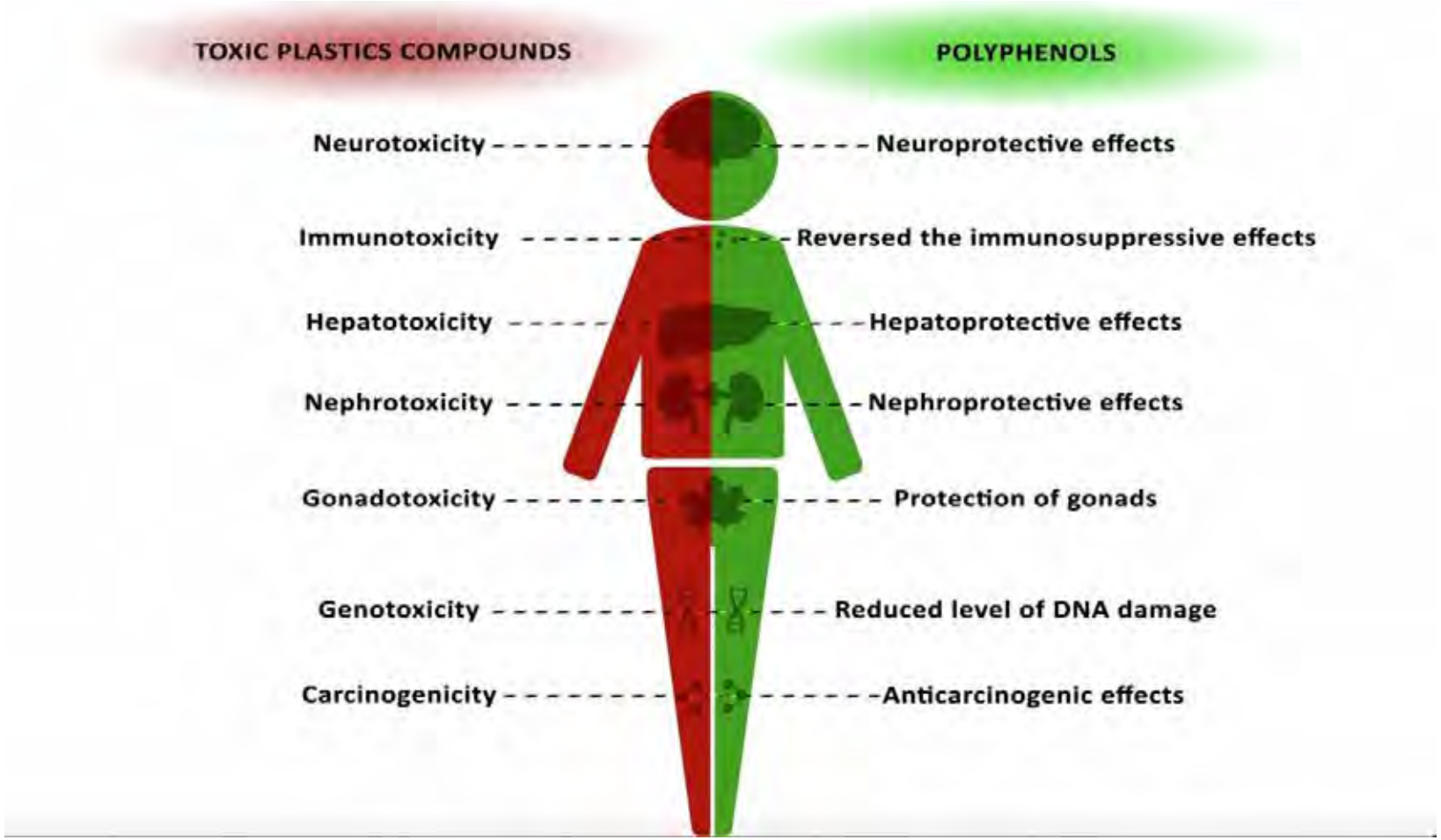
The influence of polyphenols on metabolic disorders caused by compounds released from plastics - Review



Żwierełło, W. (2020). The influence of polyphenols on metabolic disorders caused by compounds released from plastics – Review. *Chemosphere*, 240. https://doi.org/10.1016/j.chemosphere.2019.124901.

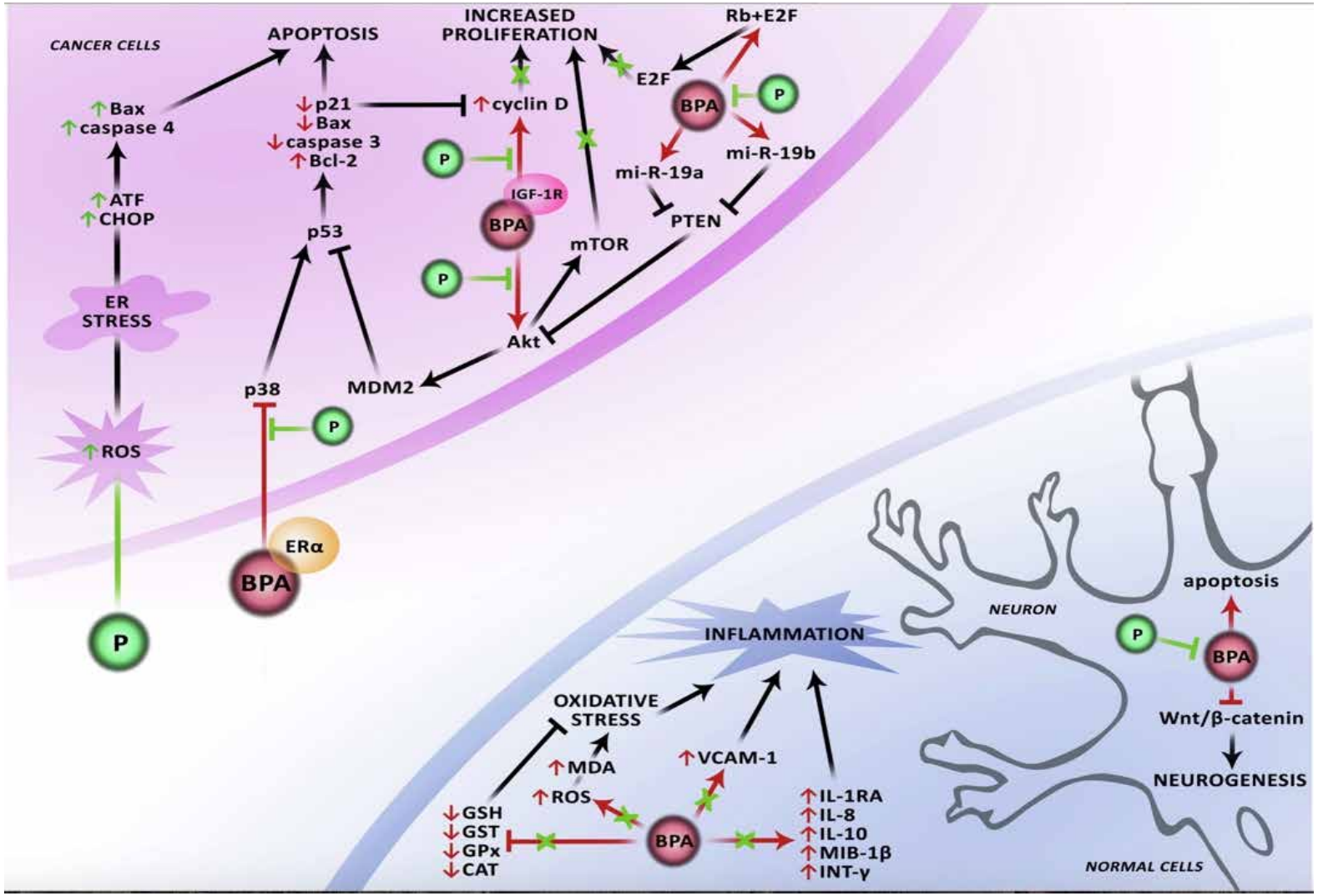
The influence of polyphenols on metabolic disorders caused by compounds released from plastics - Review

Highlights

- Toxic substances released from plastics pose environmental pollution.
- Plastic compounds induce e.g. oxidative stress, apoptosis and inflammation.
- Toxic plastic compounds may promote cancer progression and metastasis.
- Polyphenols exert protective effect against ACN, PCBs, BPA, phthalates and dioxins.
- Polyphenols inhibit cancer progression and metastasis promoted by plastics compounds.

Żwierełło, W. (2020). The influence of polyphenols on metabolic disorders caused by compounds released from plastics – Review. *Chemosphere*, 240. https://doi.org/10.1016/j.chemosphere.2019.124901.

Polyphenols Negate toxicity of BPA



Żwierełło, W. (2020). The influence of polyphenols on metabolic disorders caused by compounds released from plastics – Review. *Chemosphere*, 240. https://doi.org/10.1016/j.chemosphere.2019.124901.

Phytonutrients

Phytonutrients - plant metabolites that defend against microbes

- Increase host defense against DNA damaging molecules. Reduce oncogenic potential of carcinogens.
- Organic has more phytochemicals and mineral content
  - Plant under stress

Czech A, Szmigielski M, Sembratowicz I. Nutritional value and antioxidant capacity of organic and conventional vegetables of the genus Allium. Sci Rep. 2022 Nov 4;12(1):18713. doi: 10.1038/s41598-022-23497-y. PMID: 36333512; PMCID: PMC9636188.

THINK COLOR!

- Chlorophyll – green vegetables
- Glucosinolates – cruciferous vegetables
- Xanthophyll – yellow carotenoid pigment
- Isoflavones – phytoestrogen
- Polyphenols – quercetin, lignan, flavonoids
- Flavonoids – Catechins, ECGC = epigallocatechin
- Carotenoids – yellow / orange
- **8 - 10 ½ cups PER DAY**
- **8 - 12 servings Per Day**

Isoflavones

- Phytoestrogens = Anti-estrogen
- Genistein
- Daidzein
- Equol – secondary metabolite of soy made in gut from healthy microbiota

Soy and Flax

- Phytoestrogens – weak estrogenic activity – block / antagonize the impact of estrogen
- Lignans – seeds and grains - flax, pumpkin, sunflower, poppy, sesame, whole grains (rye, oat, barley) and tofu.
- Association with lignan intake and a lower risk of breast cancer, especially in postmenopausal women on hormone therapy.
- Flax seeds
  - highest concentration of lignans
  - alpha – linolenic acid - rich in omega 3 fats

https://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/lignans#reference9

Wang X.. Exploring the Biological Activity and Mechanism of Xenoestrogens and Phytoestrogens in Cancers: Emerging Methods and Concepts. Int J Mol Sci. 2021 Aug 16;22(16):8798. doi: 10.3390/ijms22168798. PMID: 34445499; PMCID: PMC8395949.

ORIGINAL CONTRIBUTION

The Shanghai Breast Cancer Survival Study

Soy Food Intake and Breast Cancer Survival

Xiao Ou Shu, MD, PhD
Ying Zheng, MD, MSc
Hui Cai, MD, PhD
Kai Gu, MD
Zhi Chen, MD, PhD
Wei Zheng, MD, PhD
Wei Lu, MD, PhD

Among women with breast cancer, soy food consumption was significantly associated with **decreased risk of death and recurrence**.

The inverse association was evident among women with either ER-positive or ER-negative breast cancer and was present in both users and non-users of tamoxifen.

This study suggests that moderate soy food intake is **safe and potentially beneficial** for women with breast cancer.

Shu, X. JAMA, 2009;302:2437-43.

What can you do to lower your toxin load?

- Buy organic
  - Decreased pesticide exposure
  - Decreased growth hormone exposure
- Use glass whenever possible
- Store food in glass
- Do not heat or microwave plastic
- Limit processed / packaged foods
- Cook More
- Improve our diet
  - Increase nutrient dense whole foods, fiber and phytonutrients
- Switch to glass / stainless steel reusable water bottles
- Read labels on personal care products
- Use unscented lotions, laundry detergents and cleaning supplies
- Avoid herbicides / pesticides on your lawn and garden
- Environmental Working Group – www.ewg.org
- Think Dirty App – www.thinkdirtyapp.com

What Can We Do

- Collect data on whole life exposure to EDCs
- Move away from packaging containing EDCs in favor of biodegradable products
- Adopt policy to restrict EDCs
- Transform how federal dollars are used to support food as medicine (foodfix.org)
  - Incentivize Regenerative Organic Agriculture
  - Reimagine agricultural practices, nutrition, and health
  - Improve food supply so more people have access to healthful foods

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Breast Cancer Risk

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The International Agency for Research on Cancer (IARC) is the world's authority on carcinogens

- IARC is part of the World Health Organization
- IARC classifies carcinogens based upon available scientific information
  - These classifications make up volumes called Monographs
- Experts without conflicts of interest from all over the world evaluate the scientific information
- Classification is based on the weight of scientific evidence
  - human evidence weighted most heavily

IARC Classifications Framework

Table 4. Integration of streams of evidence in reaching overall classifications (the evidence in *bold italic* represents the basis of the overall evaluation)

Stream of evidence			Classification based on strength of evidence
Evidence of cancer in humans[a]	**Evidence of cancer in experimental animals**	**Mechanistic evidence**	
Sufficient	Not necessary	Not necessary	**Carcinogenic to humans (Group 1)**
Limited or Inadequate	***Sufficient***	***Strong (b)(1) (exposed humans)***	
Limited	***Sufficient***	Strong (b)(2–3), Limited, or Inadequate	**Probably carcinogenic to humans (Group 2A)**
Inadequate	***Sufficient***	***Strong (b)(2) (human cells or tissues)***	
Limited	Less than Sufficient	***Strong (b)(1–3)***	
Limited or Inadequate	Not necessary	***Strong (a) (mechanistic class)***	
Limited	Less than Sufficient	Limited or Inadequate	**Possibly carcinogenic to humans (Group 2B)**
Inadequate	***Sufficient***	Strong (b)(3), Limited, or Inadequate	
Inadequate	Less than Sufficient	***Strong b(1–3)***	
Limited	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	
Inadequate	***Sufficient***	***Strong (c) (does not operate in humans)***[b]	**Not classifiable as to its carcinogenicity to humans (Group 3)**
All other situations not listed above			

Cancer – Can Any Thing Cause It?

Agents Classified by the IARC Monographs, Volumes 1–131

Group 1	Carcinogenic to humans	121 agents
Group 2A	Probably carcinogenic to humans	93 agents
Group 2B	Possibly carcinogenic to humans	320 agents
Group 3	Not classifiable as to its carcinogenicity to humans	501 agents

Some of the IARC Group 1 Human Carcinogens

Some of the 121 Chemicals Listed by IARC as Carcinogens to People

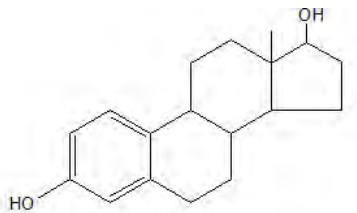
Note that in many cases data on cancer rates were collected under exposure conditions that no longer exist.

Some Occupational Exposures
- Boot and shoe manufacture (certain exposures)
- Furniture manufacture (wood dusts)
- Nickel refining
- Rubber industry (certain occupations)
- Underground hematite mining, when radon exposure exists.

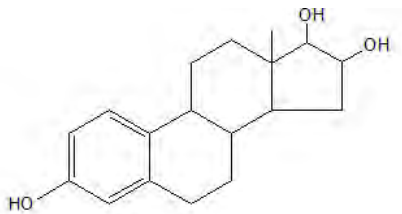
Some Chemicals
- Arsenic and arsenic compounds
- Asbestos (when inhaled)
- Chromium and certain chromium compounds (when inhaled)
- Benzene
- Diethylstilbestrol (DES)
- 2-Napthylamine, benzidine (starting materials for manufacture of certain dyes)
- Vinyl chloride (starting material for PVC plastic manufacture)
- Mustard gas

Some Chemical Mixtures
- Tobacco smoke
- Smokeless tobacco products
- Soots, tars, mineral oils*
- Analgesic mixtures containing phenacetin

* Mineral oils now in commercial production generally do not have the PAH content they had at the time the evidence of carcinogenicity was gathered.

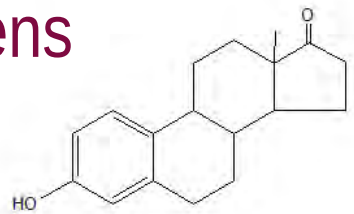


17β-Estradiol



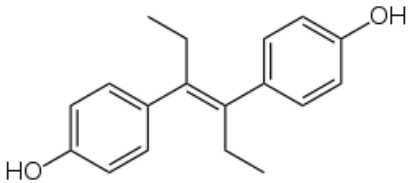
Estriol

Estrogens



Estrione

Diethylstilbesterol (DES)



Classifications by breast cancer site with sufficient or limited evidence in humans in IARC Monographs Volumes 1-133

Carcinogenic agents with *sufficient evidence* in humans	**Agents with *limited evidence* in humans**
Alcoholic beverages	Dieldrin, and aldrin metabolized to dieldrin
Diethylstilbestrol (DES)	Digoxin
Estrogen–progestogen oral contraceptives (combined)	Estrogen therapy, postmenopausal
Estrogen–progestogen menopausal therapy (combined)	Ethylene oxide
X- and Gamma-radiation	Night shift work
	Polychlorinated biphenyls
	Tobacco smoking

https://monographs.iarc.who.int/wp-content/uploads/2019/07/Classifications_by_cancer_site.pdf

Adapted from Table 4 in Cogliano *et al.* (2011); available from: http://jnci.oxfordjournals.org/content/early/2011/12/11/jnci.djr483.short?rss=1

All* the known risk factors for breast cancer are linked to total lifetime exposure to estrogen

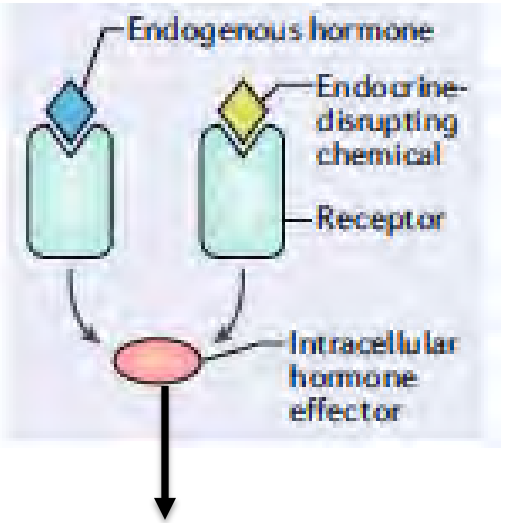
- Early puberty (periods before age 12)
- Late menopause (end of periods after age 55)
- Excess body fat (fat cells make estrogen)
- Alcohol (increases estrogen levels)
- DES (pretends to be estrogen)

*Radiation is a well- established environmental cause of breast cancer in men and women that has no clear link to estrogen

75% of human breast cancer is ER positive

ER positive means the
cancer cells have the estrogen receptor (ER)

Estrogen receptor (green) in the cancer cells
can be activated by
natural estrogen (blue diamond) or
endocrine disrupting chemicals
(EDCs, yellow diamond)



Activated ER
causes cancer growth

Diethylstilbestrol (DES)

- Synthetic form of estrogen
- Used to promote fetal growth and prevent miscarriage
- From 1938-1971 about 10 million women were exposed to DES when pregnant
- In 1971, the Food & Drug Administration (FDA) advised physicians to stop prescribing DES to pregnant women
  - because it was linked to a rare vaginal cancer in female offspring

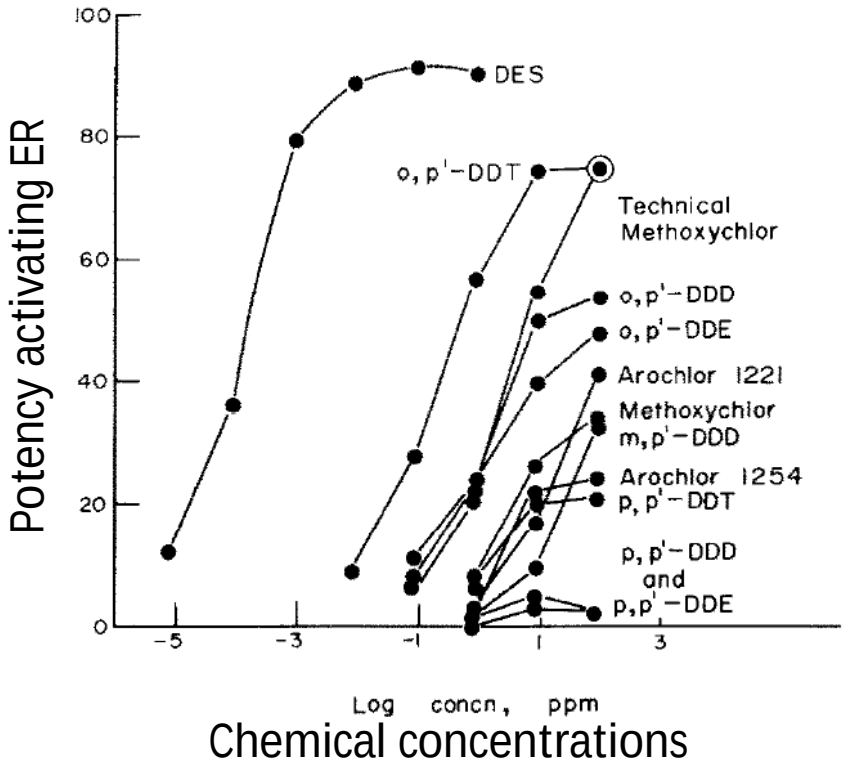
Reproductive risk associated with DES

- The women who took DES when pregnant
  - 30% increased risk of breast cancer
- Their daughters
  - Reproductive tract and breast cancers
  - Structural defects in reproductive tract
  - Preterm delivery
  - Infertility
- Their sons
  - Reproductive tract cysts
  - Structural defects in reproductive tract
  - Infertility
- Their grandkids
  - Structural defects in reproductive tract
  - Reproductive tract cancers in mice of this generation

DES is a synthetic estrogen.
Can other chemicals that mimic estrogens result in similar reproductive health adversities?

(A lot of toxicologists spend their careers investigating this)

Can we identify chemicals that increase risk of breast cancer by measuring if the chemicals activate ER?

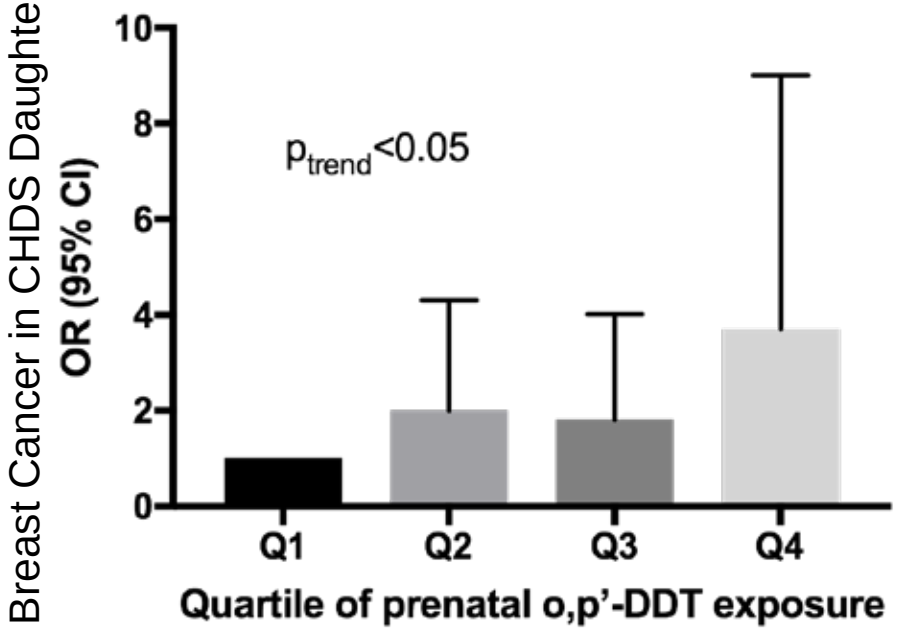


Chemical concentrations

- What does IARC say?
  - "no clear association was found between breast cancer and DDT or DDE…in adulthood"
  - the possible importance of early-life exposure to DDT remains unresolved

Child Health and Development Studies: breast cancer risk that is hard to study

- ~15,000 pregnant women in the Kaiser Permanente Health Plan joined the CHDS in 1960s.
- Early-life exposure to DDT in pregnant women associated with their increased risk of breast cancer
- Daughters had higher risk of breast cancer
- Granddaughters (in their 20s now) had earlier periods



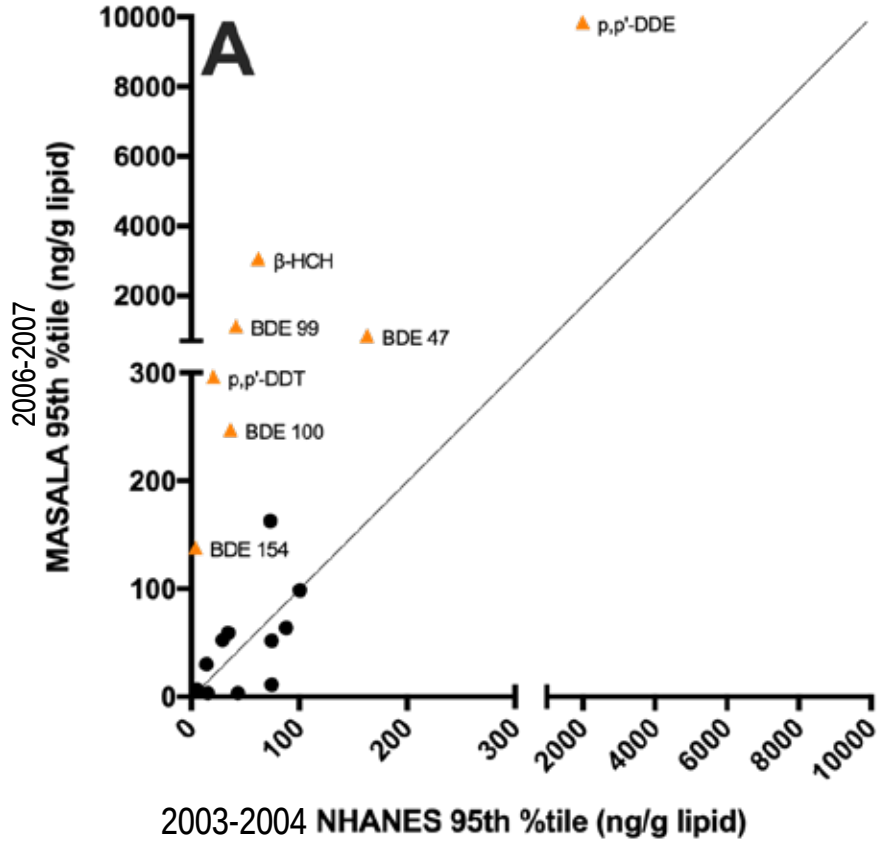
Cohn et al. 2019; Cohn, La Merrill et al. 2015; Cirillo, La Merrill et al. 2021

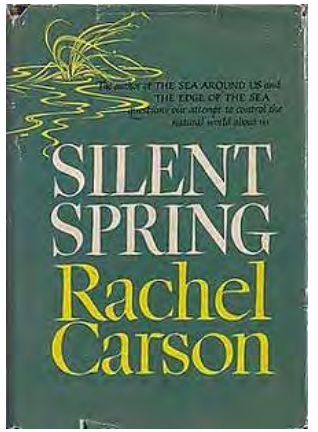
Cancer Hotspots

- Cancer Hotspot
  - A community has excess cases of cancer than expected by historical cancer data
- Breast cancer hotspots
  - Breast cancer mortality risk in hotspots linked to estrogenic exposures
    - Marin county, CA
      - Higher hormone replacement therapy (IARC sufficient evidence)
    - Long Island
      - Higher DDT

Ongoing DDT relevance in the USA:

Indian Asians living in USA have much higher levels of DDT and DDE than the "representative" US population



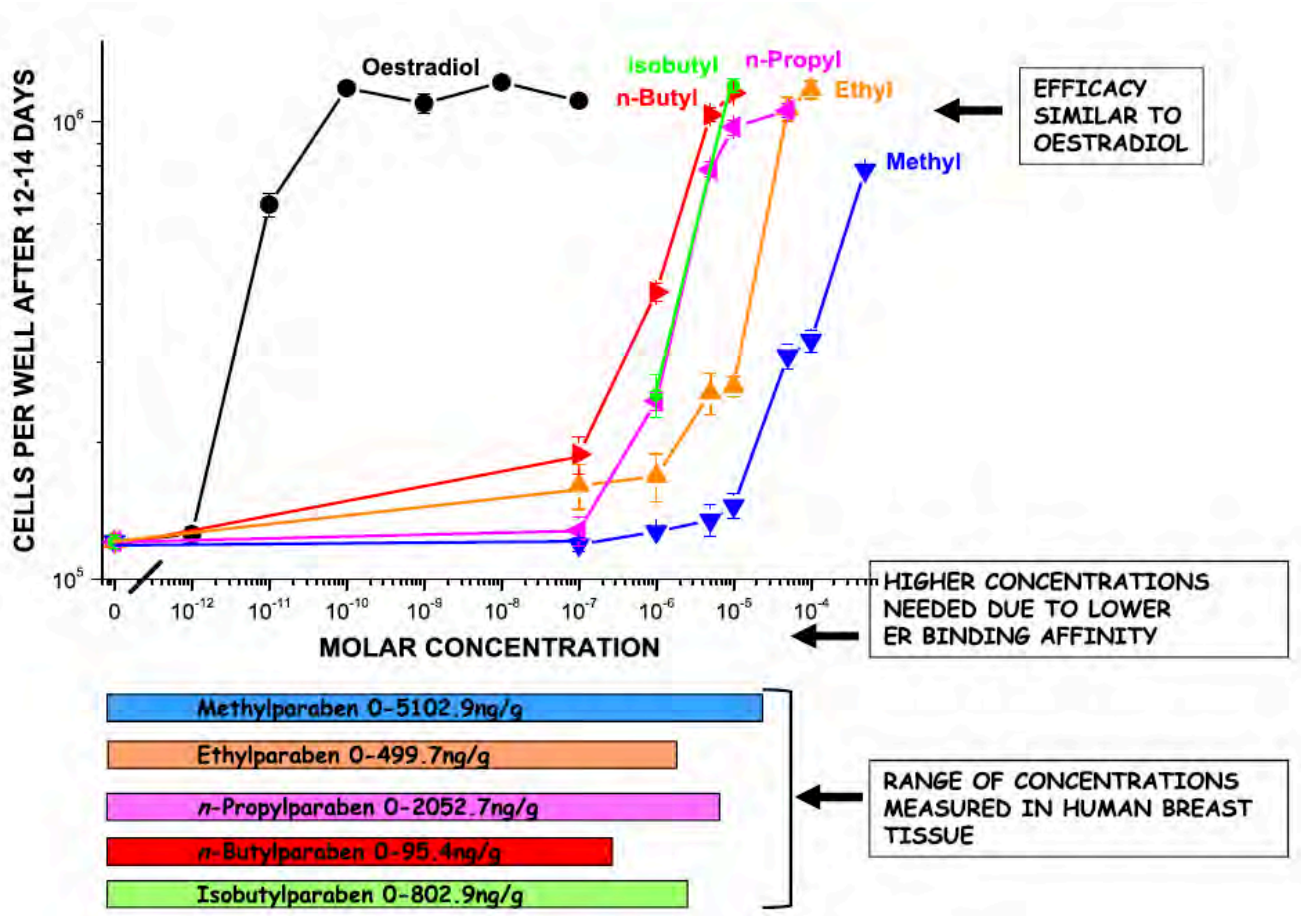


"We spray our elm trees and the following springs are silent of robin song… because the poison traveled"

La Merrill et al. 2019; Photographer: Alfred Eisenstaedt; National Portrait Gallery, Smithsonian Institution

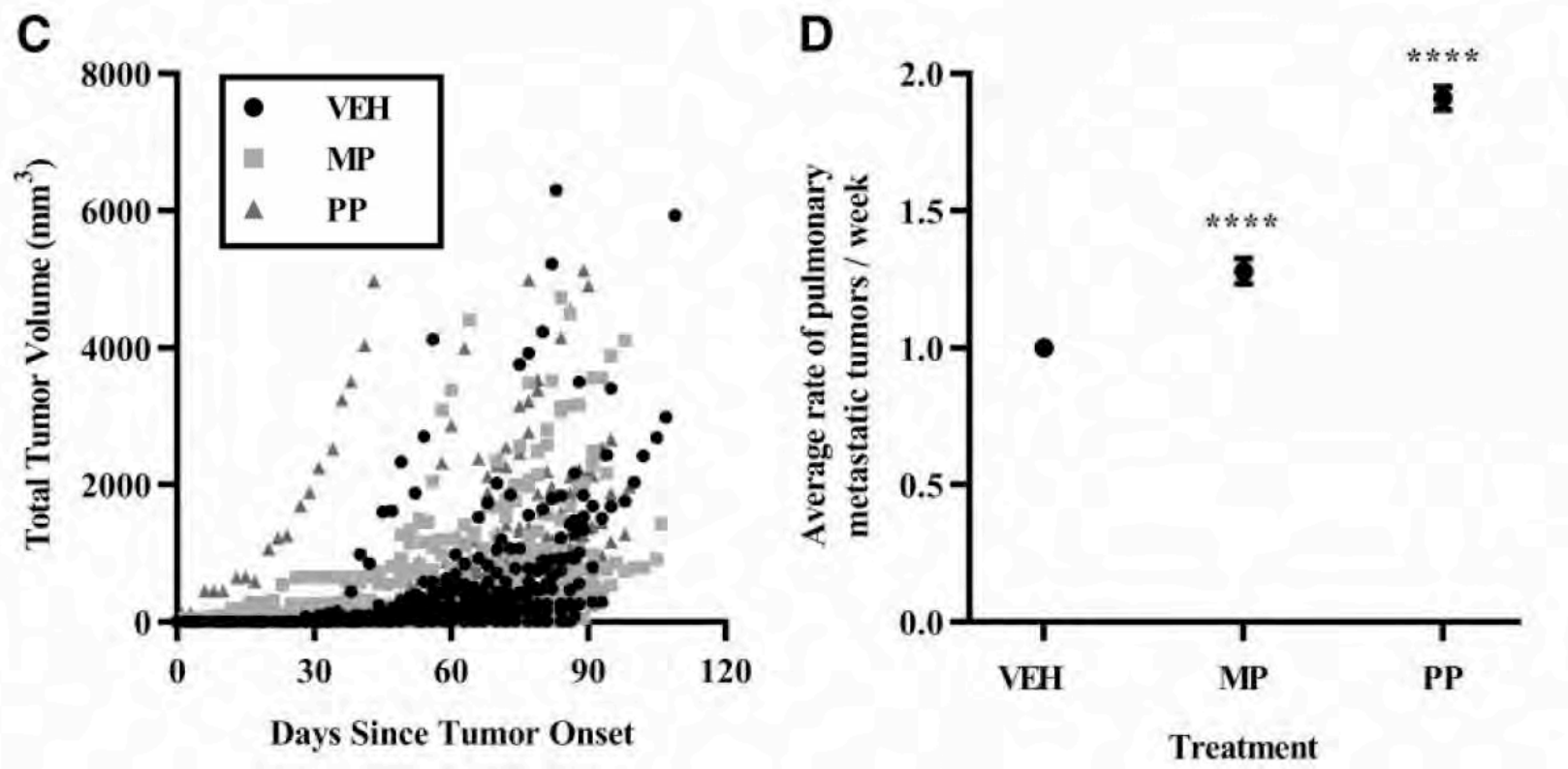
Can we identify chemicals that increase risk of breast cancer by measuring if the chemicals activate ER?

Parabens activate ER-dependent growth of human breast cancer cells



Khanna and Darbre 2013; **Darbre and Harvey 2014 (MCF-7);** Okubo et al. 2001 (MCF-7, all parabens inhibited by estrogen blocking ICI182,780)

Methylparaben and propylparaben increased the rate of breast tumor growth and its metastasis in mice



Tong et al. 2022

What can we do to reduce risk of breast cancer?

- IARC Carcinogenic agents with sufficient or limited evidence for their associations with human breast cancer
  - Drink less alcohol
  - Smoke less tobacco
  - Fly less to minimize radiation exposure
  - Consider contraceptives and menopause therapies that don't contain estrogen
  - Eat lower on the food chain since levels of 'limited evidence' agents magnify in animal fats up the food chains
    - Covers potential risk from DDT too
    - Also better for reducing your foot print on climate change
- Experimental studies in rodents and in human cells indicate that environmental chemicals that activate ER increase risk of breast cancer
  - Need human studies to confirm
  - Human studies can be very difficult due to long time for cancer to arise and other difficulties with measuring chemical exposures
- Precautionary principle can be implemented while we wait for the science to fill in the research gaps
  - Read the label with care
  - Look for phrase "paraben-free"
  - Avoid ingredients: paraben, estrogen, estrone, estriol
  - Avoid products that contain placental extracts
    - Estrogen and other hormones are present in animal placenta

Lindsey Treviño, Ph.D.

Assistant Professor, Division of Health Equities and
Department of Population Sciences

City of Hope

Break

We will resume at 3:10 PM

Maternal-Child Health Risks and Risk Reduction

Carmen Messerlian, Ph.D., Rita Strakovsky, Ph.D., R.D., Christine Langton, Ph.D.

Moderated by Deb Kilday

Maternal-Child Health Risks and Risk Reduction

Carmen Messerlian, Ph.D.

Assistant Professor

Environmental Reproductive, Perinatal, and Pediatric Epidemiology

Beyond Pregnancy – How the Environment Shapes Women's Health

Carmen Messerlian, PhD

Harvard Chan School of Public Health

Endocrine Disrupting Chemicals and Women's Health Symposium

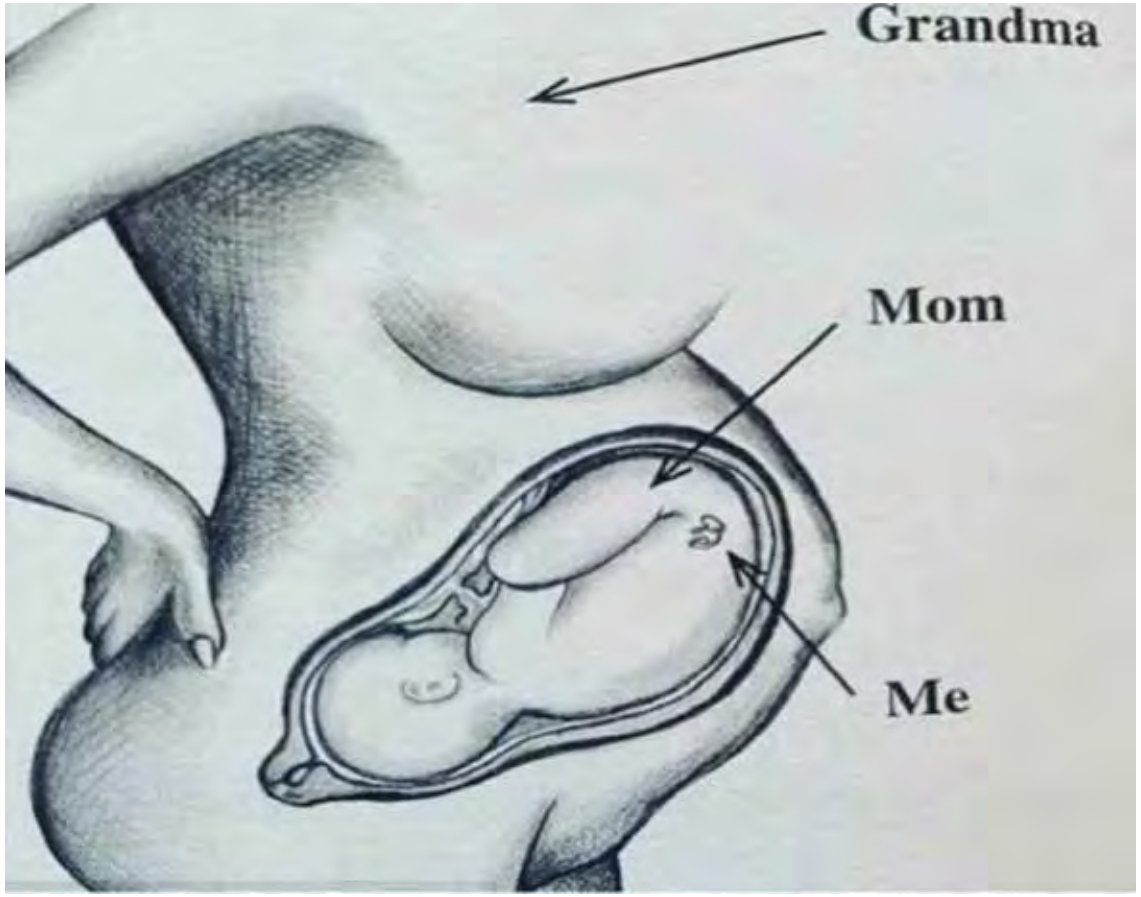
July 18, 2023

SEED PROGRAM
SCIENTIFIC EARLY LIFE ENVIRONMENTAL HEALTH & DEVELOPMENT PROGRAM

HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

MGH 1811

The Origins of Reproductive Health



F0

F1

F2

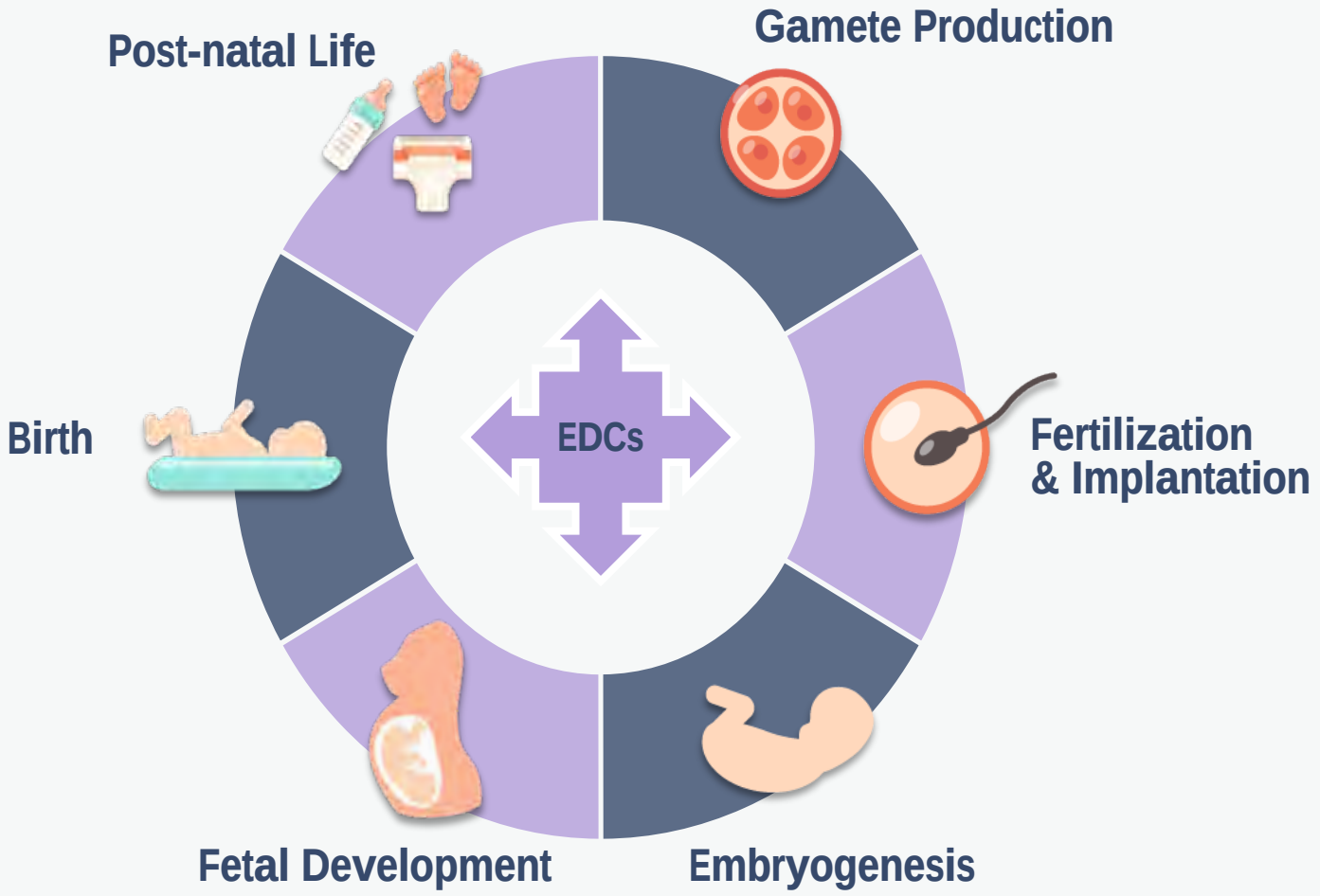
F3+

Multi-generational inheritance

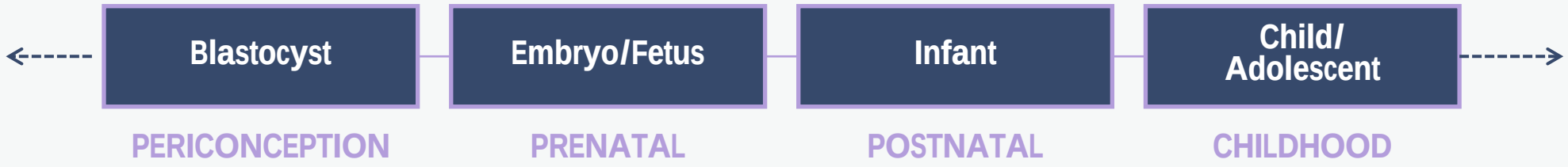
Inter-generational inheritance

The Reproductive Cycle

- Complex hormonal, molecular processes work in synchrony
- Control and interplay – organs, tissues, cells
- Timing of events - disruption
- Opportunity for adaptation or aberration



Timing of Exposure



Adapted from: TJ Woodruff, SJ Janssen, LJ Guilllette Jr, L Giudice. (2010). Environmental Impacts on Reproductive Health and Fertility. Cambridge University Press.

Preconception Origins of Health and Disease

Most studies focus on *in utero* exposure

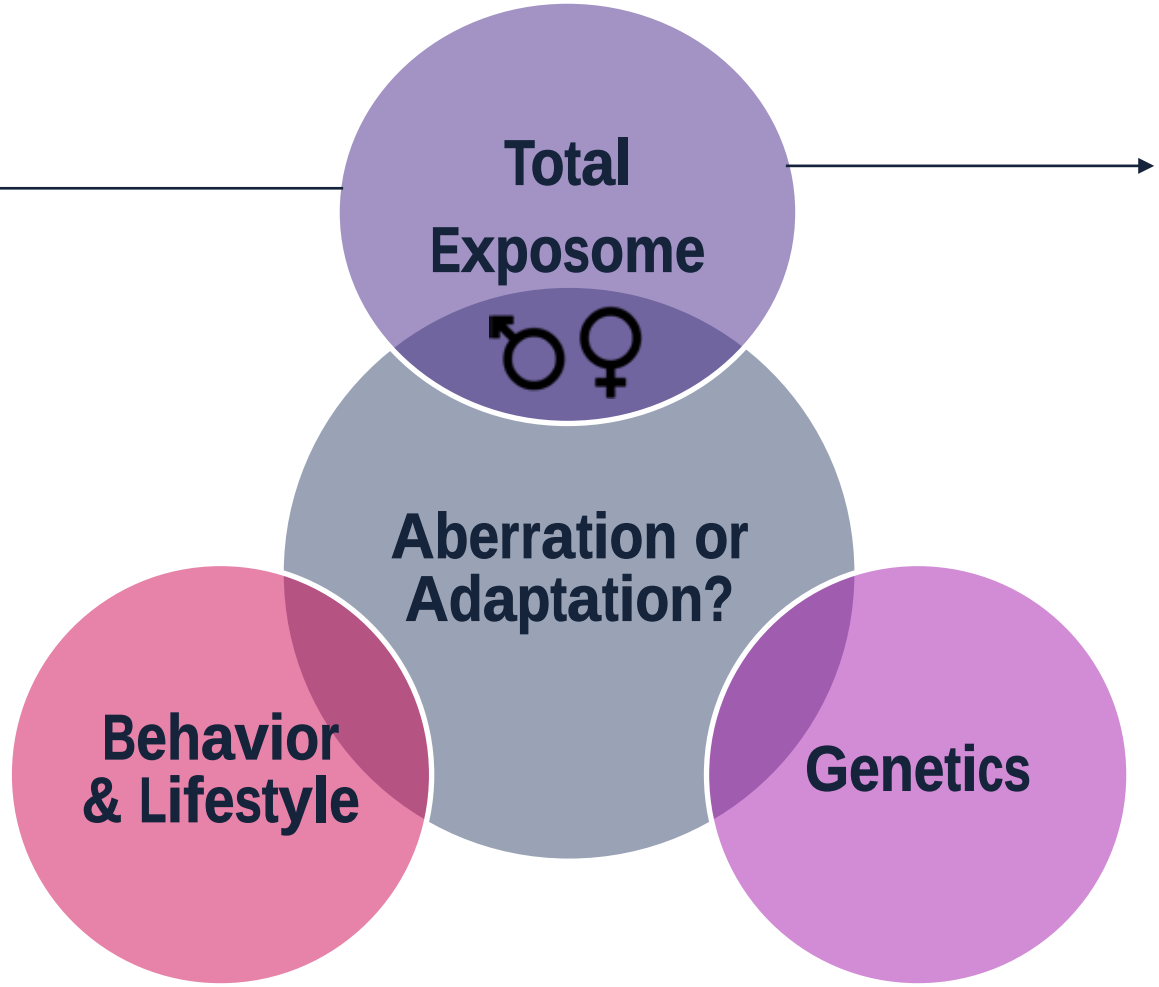
Paternal, maternal preconception environments impact fertility, pregnancy, child health

Few studies able to examine this critical period of vulnerability such as pre and periconception

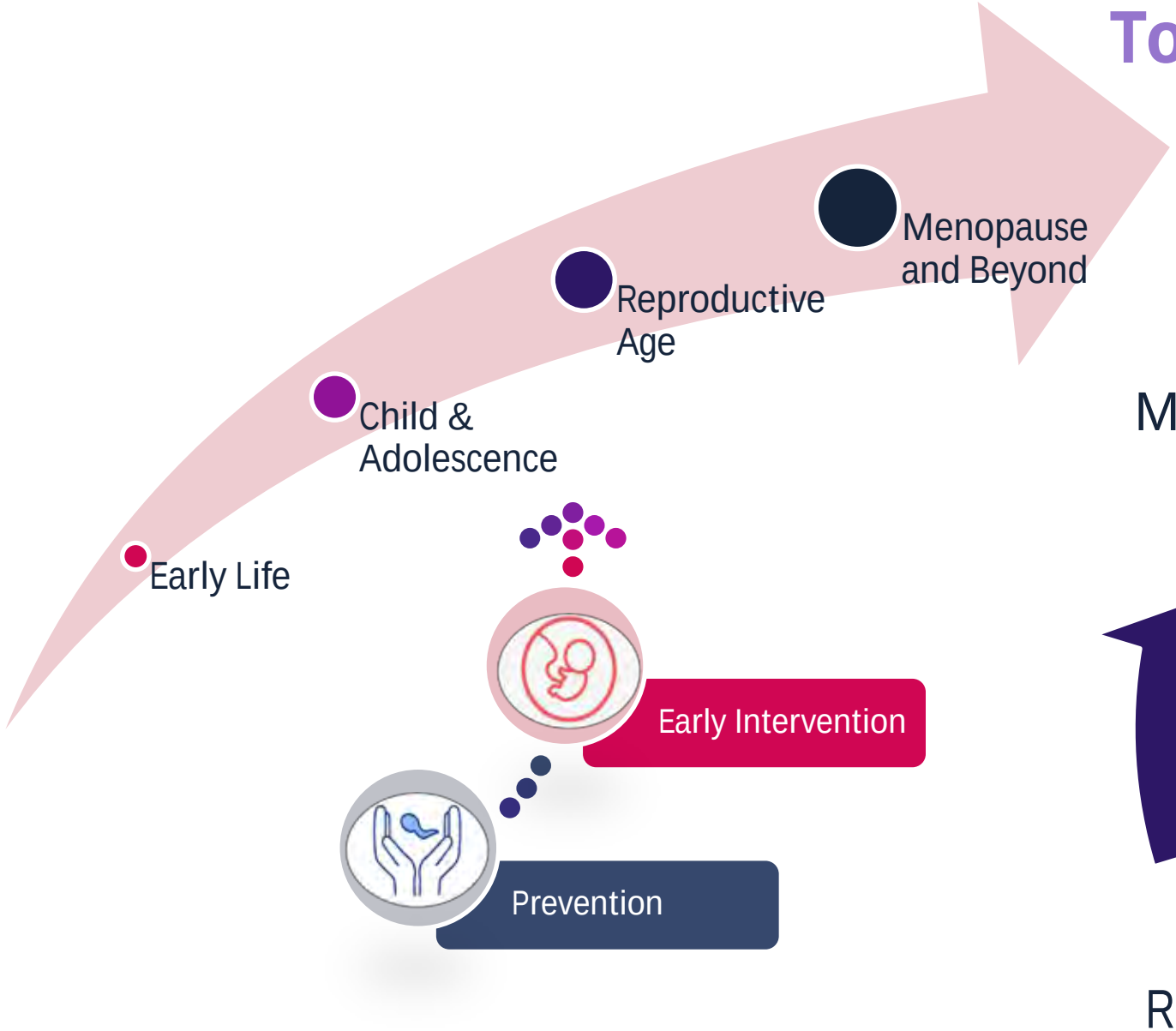
Reproductive Success?

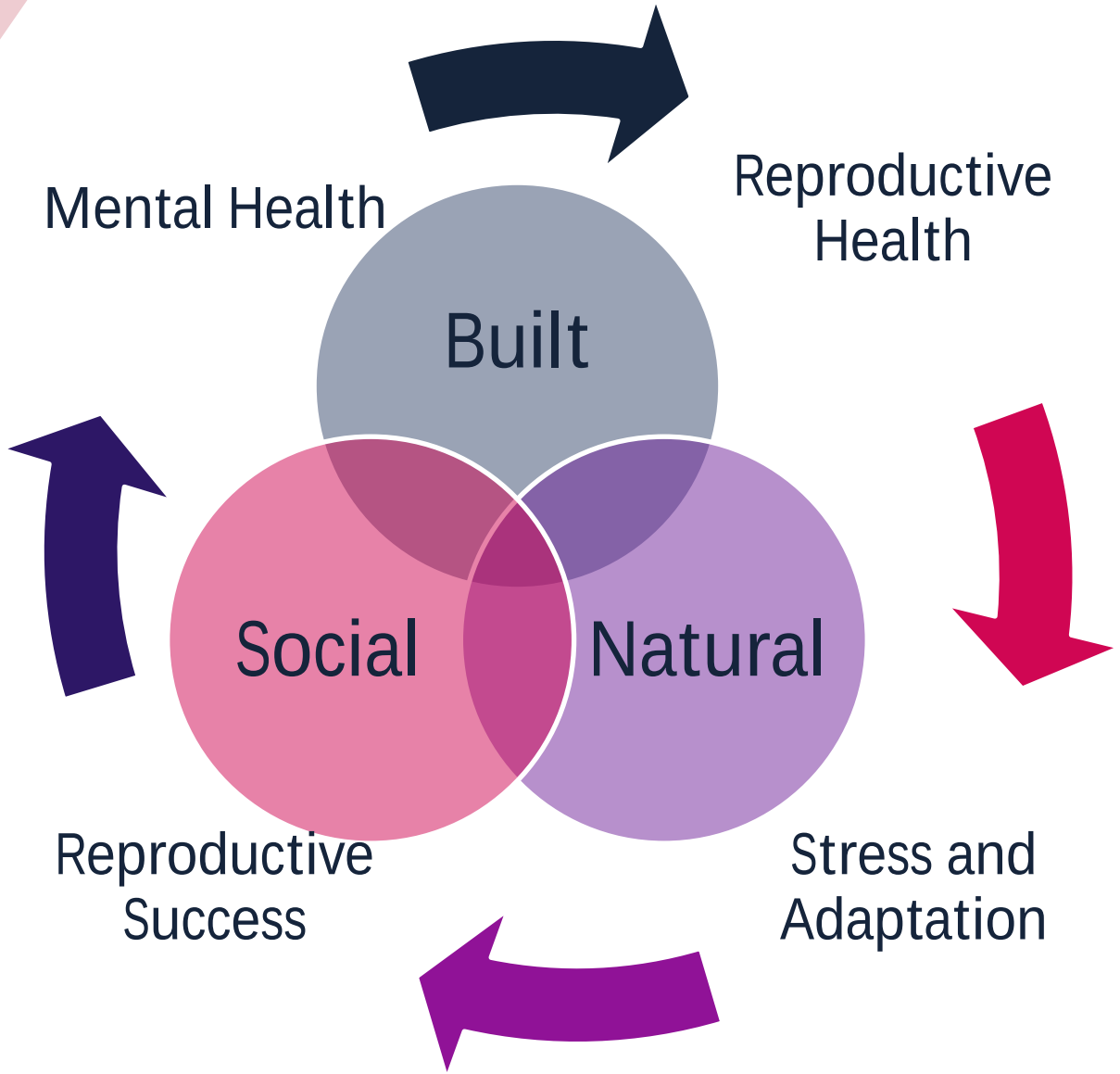
Social Environment

Natural and Built Environment

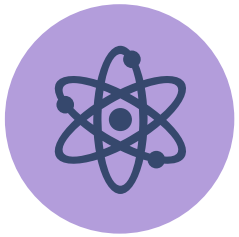


Towards an Integrated Model





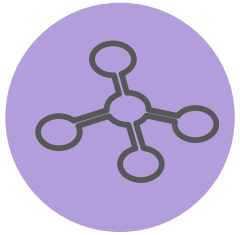
The Built Environment



EDCs

Endocrine Disrupting Chemicals (EDCs)

Exogenous chemicals that interfere with any aspect of endocrine system or hormonal action



PHTHALATES

High Molecular Weight
Medical devices, toys

Low Molecular Weight
Paints, adhesives
Personal care products

BPA

Plastic bottles
Food packaging
Toys

PFAS

Water contamination
Meat/dairy
Food contact material

MIXTURES

Beyond a single-chemical problem, real word exposure scenarios are much more complex

Environment International
Volume 151, June 2021, 106440

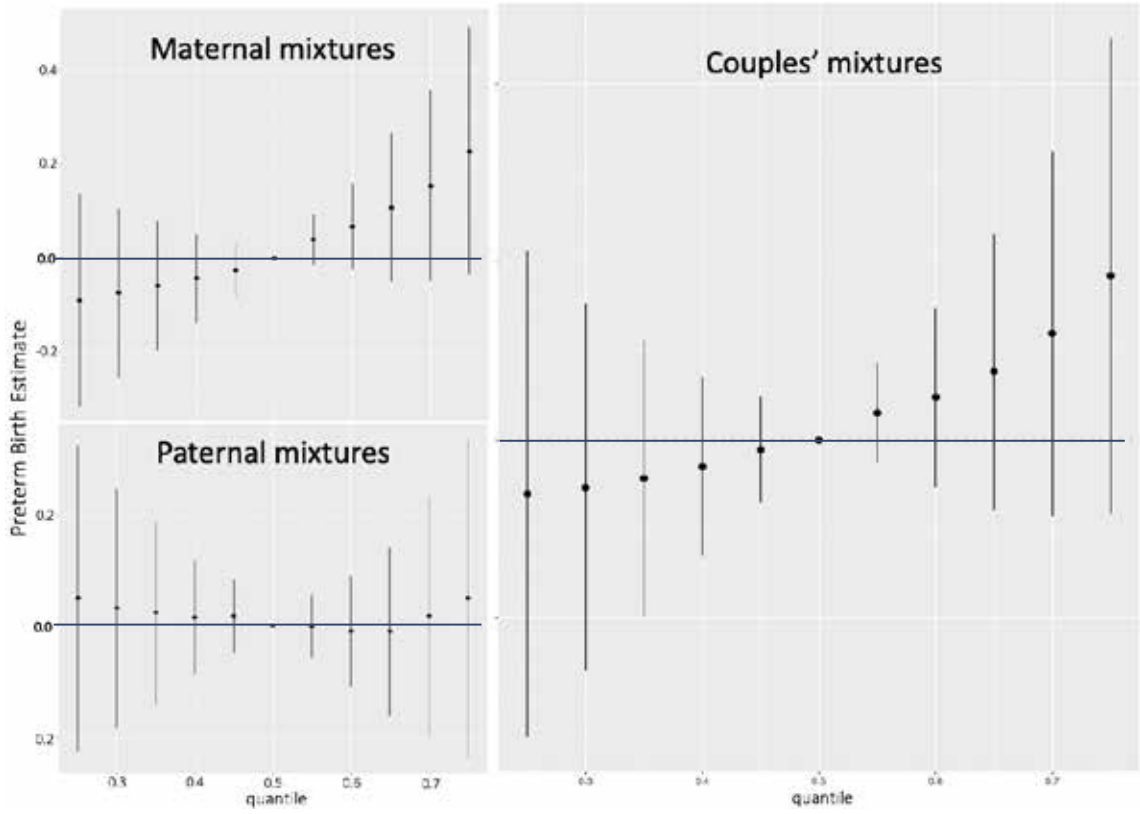
Parental preconception exposure to phenol and phthalate mixtures and the risk of preterm birth

Yu Zhang [a c], Vicente Mustieles [b c d], Paige L. Williams [c f], Blair J. Wylie [g], Irene Souter [h i], Antonia M. Calafat [j], Melina Demokritou [a], Alexandria Lee [e], Stylianos Vagios [h i], Russ Hauser [a e], Carmen Messerlian [a e h i]

- Paternal preconception DEHP and maternal BPA à higher preterm birth, holding all other biomarker at median concentration

- Higher preterm birth across quantiles of maternal and couples' total mixture concentrations

CUMULATIVE EFFECT OF TOTAL MIXTURE

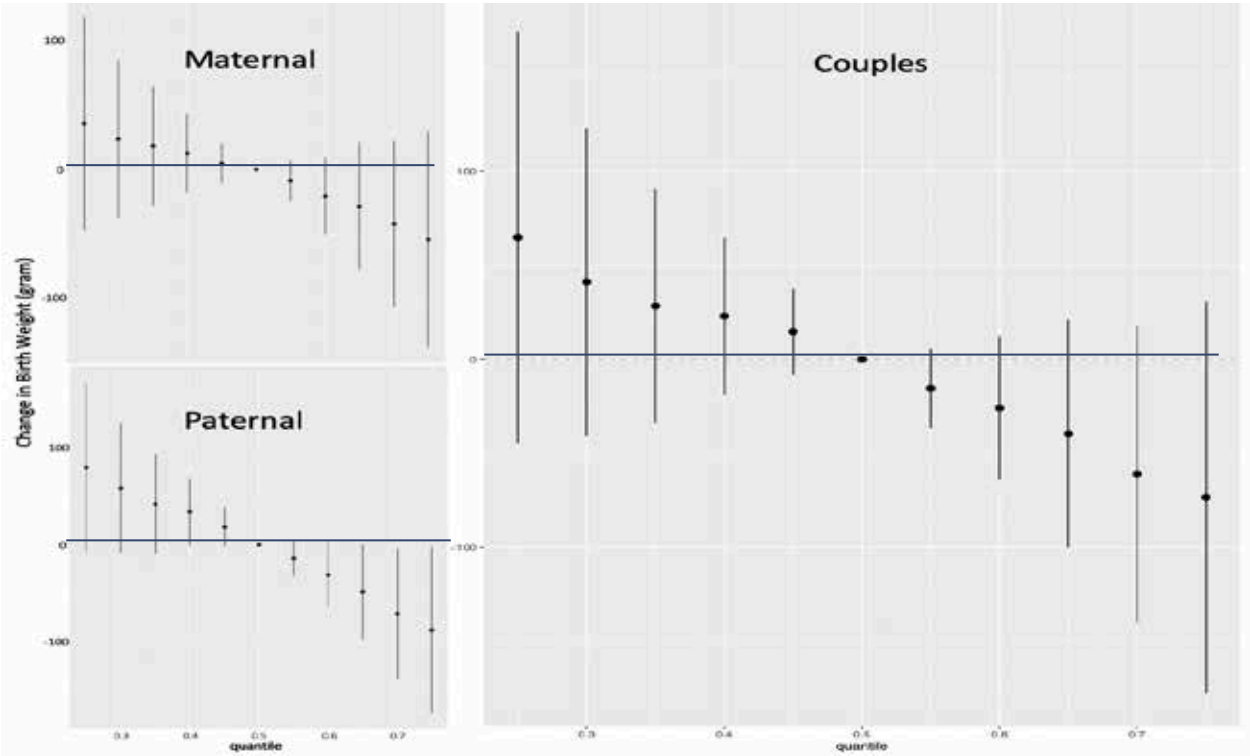


Increasing trend of **preterm birth** across quantiles of **maternal** and **couples**' total preconception mixture concentrations

Couples' Mixtures & Birthweight

- Paternal MBP and maternal preconception BPA à decreased birthweight, holding all other biomarkers at median concentration
- Lower birthweight across quantiles of maternal, paternal and couples' total mixture concentrations

CUMULATIVE EFFECT OF TOTAL MIXTURE



Decreasing trend of **birthweight** across quantiles of **maternal**, **paternal** and **couples**' total preconception mixture concentrations

PFAS and Human Health

- PFAS - found in the water we drink, the food we eat, the air we breath
- High production volume synthetic water and stain resistant chemicals
- Countless diverse commercial and consumer applications (non-stick pans, textiles, food packaging, water)
- Universally detected in general population worldwide
- Resist environmental degradation and then bioaccumulates in food-chain





Preconception PFAS and Reproduction (PREPARE) Study

Funded by:

NIEHS R01ES031657, 2020-2025

SEED PROGRAM
SCIENTIFIC EARLY LIFE ENVIRONMENTAL HEALTH & DEVELOPMENT PROGRAM

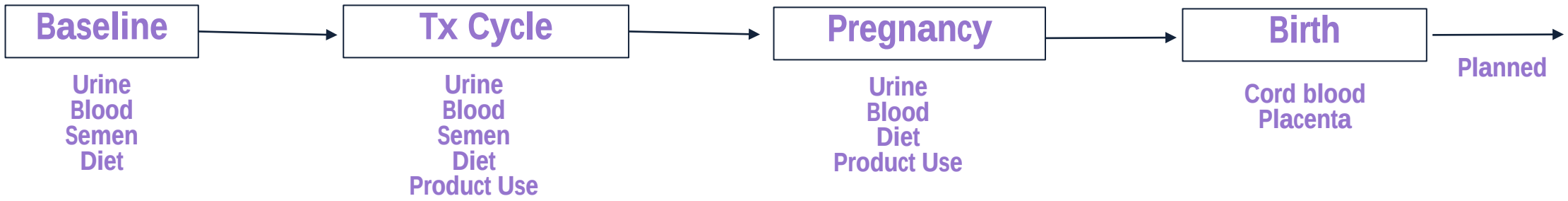
HARVARD T.H. CHAN SCHOOL OF PUBLIC HEALTH

MGH 1811

NIH NIEHS

The PREPARE Study Aims

Measure preconception serum PFAS concentrations among female and their male partners recruited from 2003 to 2019 in the EARTH Study and then the Prepare Study 2022-2025 at the Massachusetts General Hospital Fertility Center

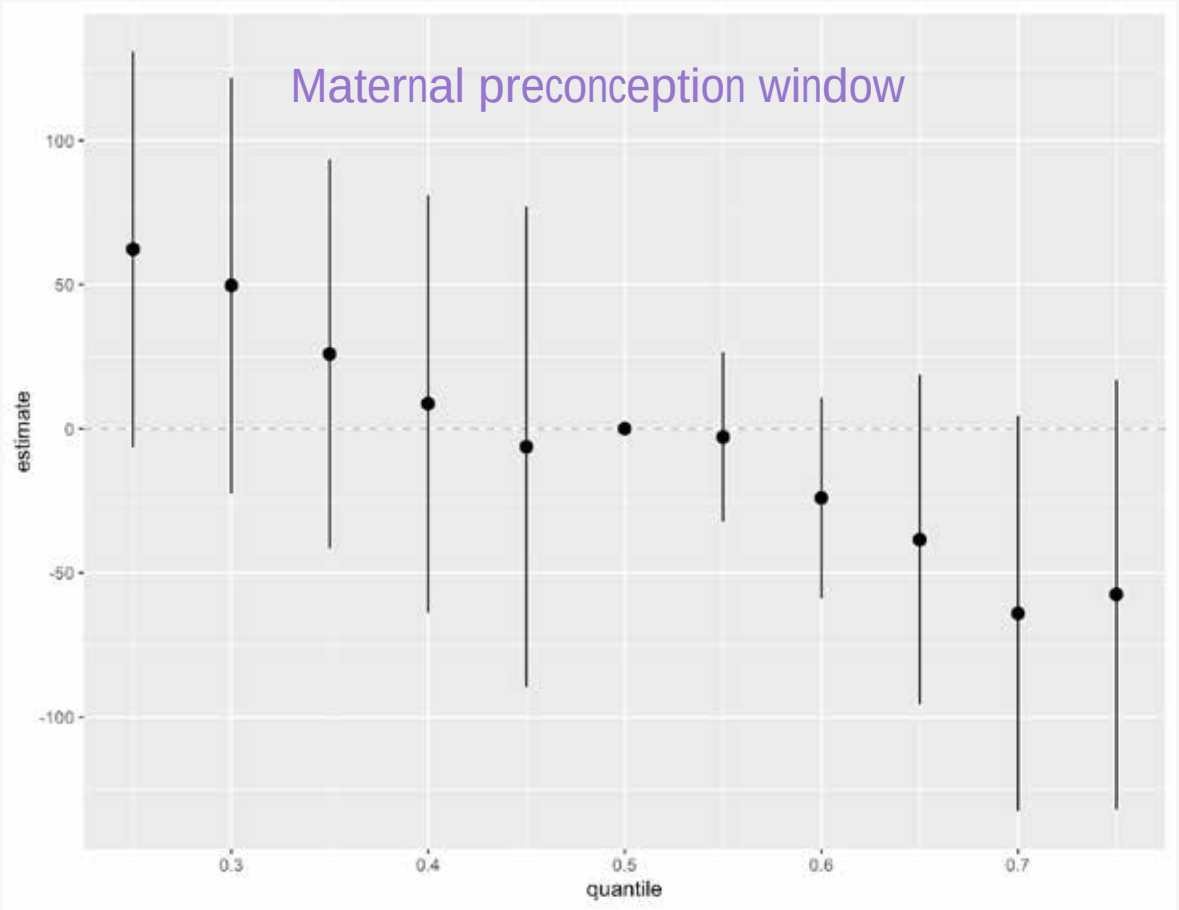


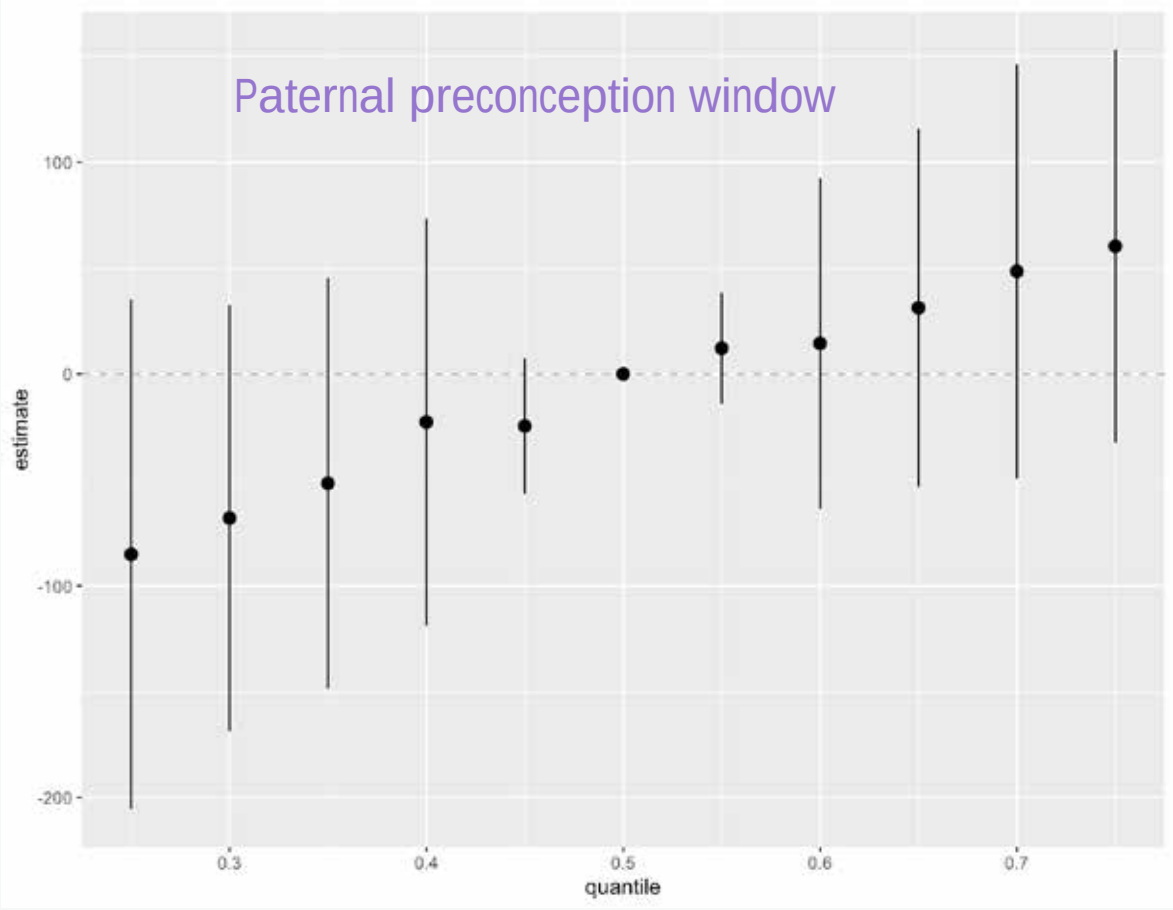
PFAS and Birth Weight

Maternal and paternal preconception PFAS concentrations and birthweight (g) among 312 singletons

PFAS	Maternal Beta (95% CI)	Paternal Beta (95% CI)
PFOA	-41.32 (-163.24, 80.61)	124.86 (-64.35, 314.06)
PFOS	**-161.44 (-268.31, -54.58)**	**147.81 (-7.9, 303.52)**
PFNA	-40.7 (-140.79, 59.38)	105.73 (-64.91, 276.36)
PFHxS	**-94.26 (-180.4, -8.11)**	**127.13 (-2.75, 257)**
PFDA	-46.93 (-161.62, 67.77)	41.65 (-136.92, 220.22)
PFUnDA	-55.44 (-140.01, 29.14)	10.7 (-116.01, 137.41)
Mixture	**-96.90 (-195.75, 1.96)**	80.31 (-66.42, 227.04)

Joint Effect of PFAS Mixture on Birth Weight





PFAS Concentrations and Thyroid Function in 287 Females

		Percent Change (95% CI)
Total Triiodothyronine (TT3)		
	PFOA	**-4.22 (-7.9, -0.39)**
	PFOS	-2.96 (-6.2, 0.39)
	PFHxS	-2.58 (-5.31, 0.23)
	PFNA	**-3.03 (-5.95, -0.01)**
	PFUnDA	**-4.04 (-6.51, -1.5)**
	PFDA	**-5.56 (-8.82, -2.17)**
	Mixture	**-4.45 (-7.15, -1.67)**
FT4/FT3		
	PFOA	1.22 (-1.36, 3.87)
	PFOS	0.58 (-1.65, 2.86)
	PFHxS	**1.16 (-0.71, 3.06)**
	PFNA	**2.22 (0.18, 4.29)**
	PFUnDA	**2.45 (0.71, 4.23)**
	PFDA	**3.2 (0.83, 5.62)**
	Mixture	**1.78 (-0.13, 3.73)**

PFAS & Nutrient Modifiers

Observational Study > Lancet Planet Health. 2023 Jun;7(6):e449-e458.
doi: 10.1016/S2542-5196(23)00088-8.

Folate concentrations and serum perfluoroalkyl and polyfluoroalkyl substance concentrations in adolescents and adults in the USA (National Health and Nutrition Examination Study 2003–16): an observational study

Yu Zhang [1], Vicente Mustieles [2], Yi-Xin Wang [3], Yang Sun [4], Juliana Agudelo [5], Zainab Bibi [6], Nicole Torres [6], Youssef Oulhote [7], Angela Slitt [5], Carmen Messerlian [8]

NHANES
2003 - 2016 cycles
2,802 Adolescents
9,159 Adults

Adults: Folate in Red Blood Cells (RBC) à PFAS

Adjusted % Change in Serum PFAS Concentration Per 2.7-fold Increase in RBC Folate

Biomarkers	Adjusted Percent Change (95%CI)	Adjusted (+diet) Percent Change (95%CI)
PFOA	-12.45% (-17.28%, -7.35%)	-11.18% (-16.36%, -5.68%)
PFOS	-25.30% (-29.67%, -20.65%)	-23.54% (-28.27%, -18.49%)
PFHxS	-21.65% (-26.19%, -16.82%)	-20.47% (-25.35%, -15.27%)
PFNA	-11.70% (-17.32%, -5.70%)	-9.50% (-15.54%, -3.02%)

Adolescents: Folate in Red Blood Cells (RBC) à PFAS

Adjusted % Change Serum PFAS Concentration Per 2.7-fold Increase in RBC Folate

Biomarkers	Adjusted Percent Change (95%CI)	Adjusted (+diet) Percent Change (95%CI)
PFOA	-7.34% (-16.57%, 2.91%)	-6.51% (-16.49%, 4.67%)
PFOS	-24.36% (-33.21%, -14.34%)	-25.14% (-34.18%, -14.86%)
PFHxS	-13.00% (-21.87%, -3.12%)	-14.68% (-23.51%, -4.84%)
PFNA	-12.29% (-26.12%, 4.12%)	-11.14% (-26.01%, 6.72%)

Environment International
Volume 164, June 2022, 107239

Full length article

Association between serum per- and polyfluoroalkyl substances concentrations and common cold among children and adolescents in the United States

Yu Zhang [a], Vicente Mustieles [b, c, d], Yang Sun [a, e], Youssef Oulhote [f], Yi-Xin Wang [g], Carmen Messerlian [a, c, h]

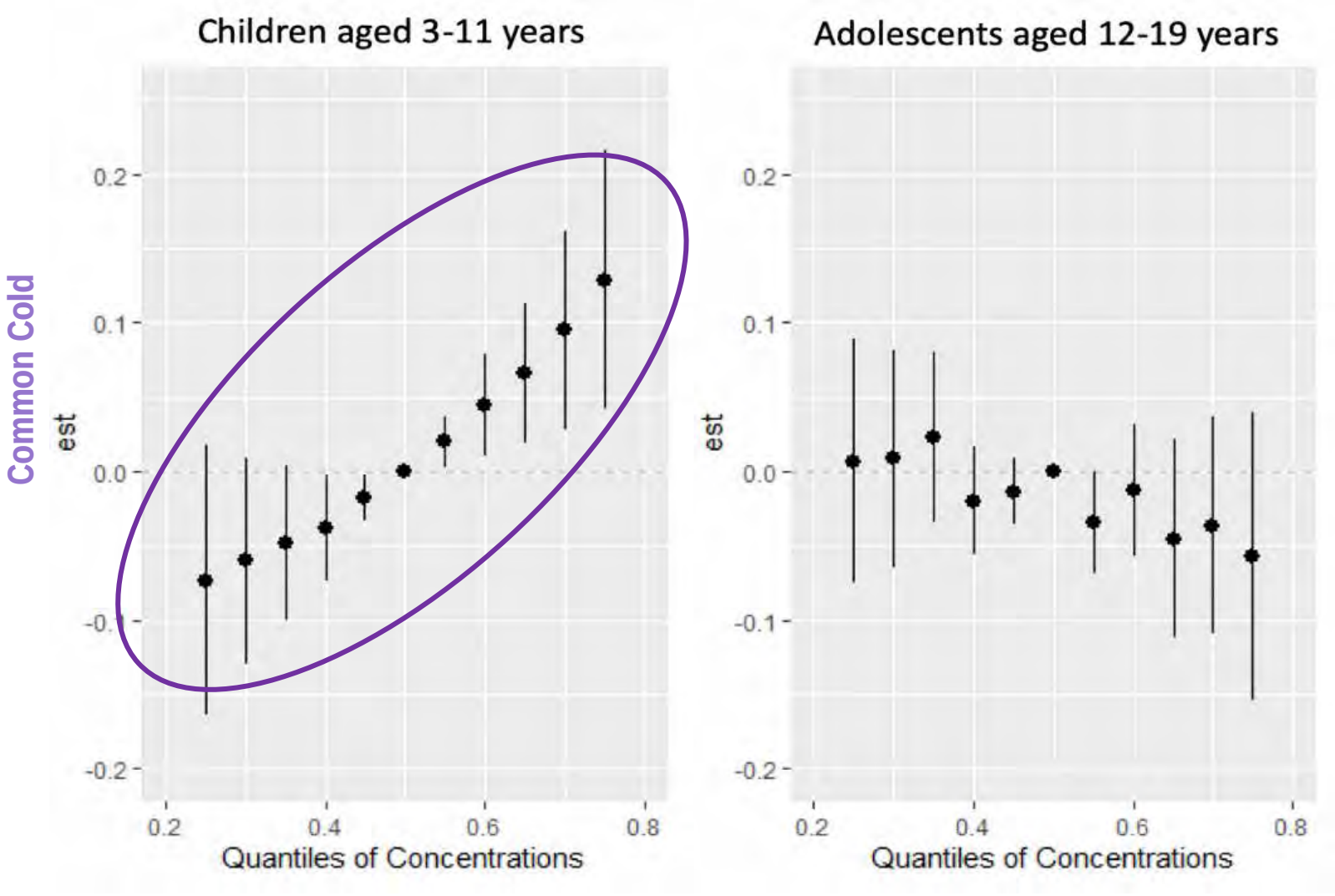
PFAS and Children's Health

NHANES

517 children 3–11 years, 2013–2014 cycles

2732 adolescents 12–19 years, 2003–2016 cycles

Change in common cold estimate per 5th percentile increase or decrease in the total PFAS mixture concentrations compared with the median total mixture concentration



PFAS and Folate in Project Viva

Early Pregnancy Plasma PFAS Concentrations and Birthweight, Stratifying By Folate Groups

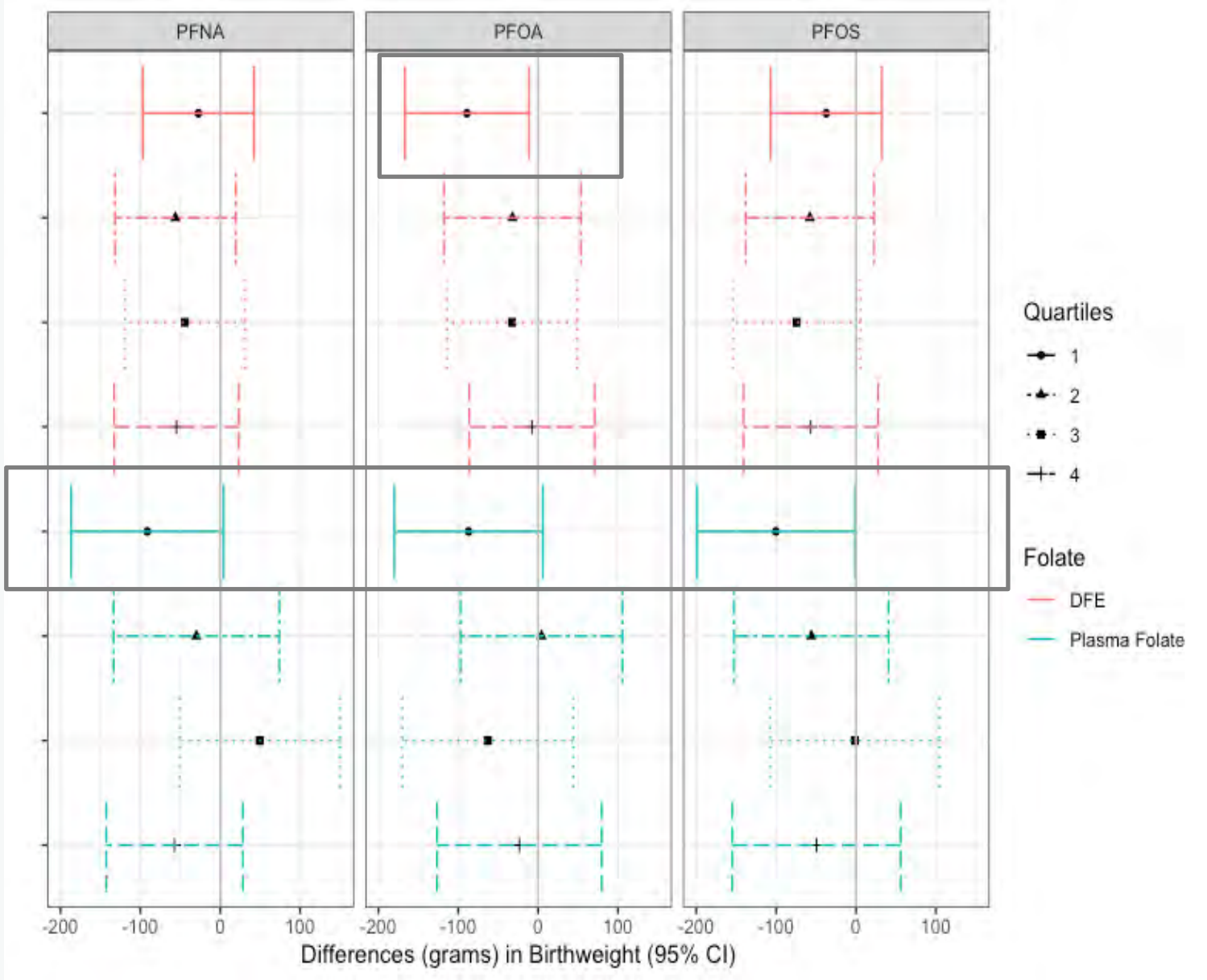
JAMA Netw Open. 2023 May 1;6(5):e2314934. doi: 10.1001/jamanetworkopen.2023.14934.

Association of Early Pregnancy Perfluoroalkyl and Polyfluoroalkyl Substance Exposure With Birth Outcomes

Yu Zhang [1], Vicente Mustieles [2], Qi Sun [3 4 5 6], Brent Coull [1 7], Thomas McElrath [5 6 8], Sheryl L Rifas-Shiman [9], Leah Martin [1], Yang Sun [1 6], Yi-Xin Wang [1 3], Emily Oken [3 9], Andres Cardenas [10], Carmen Messerlian [1 6 11]

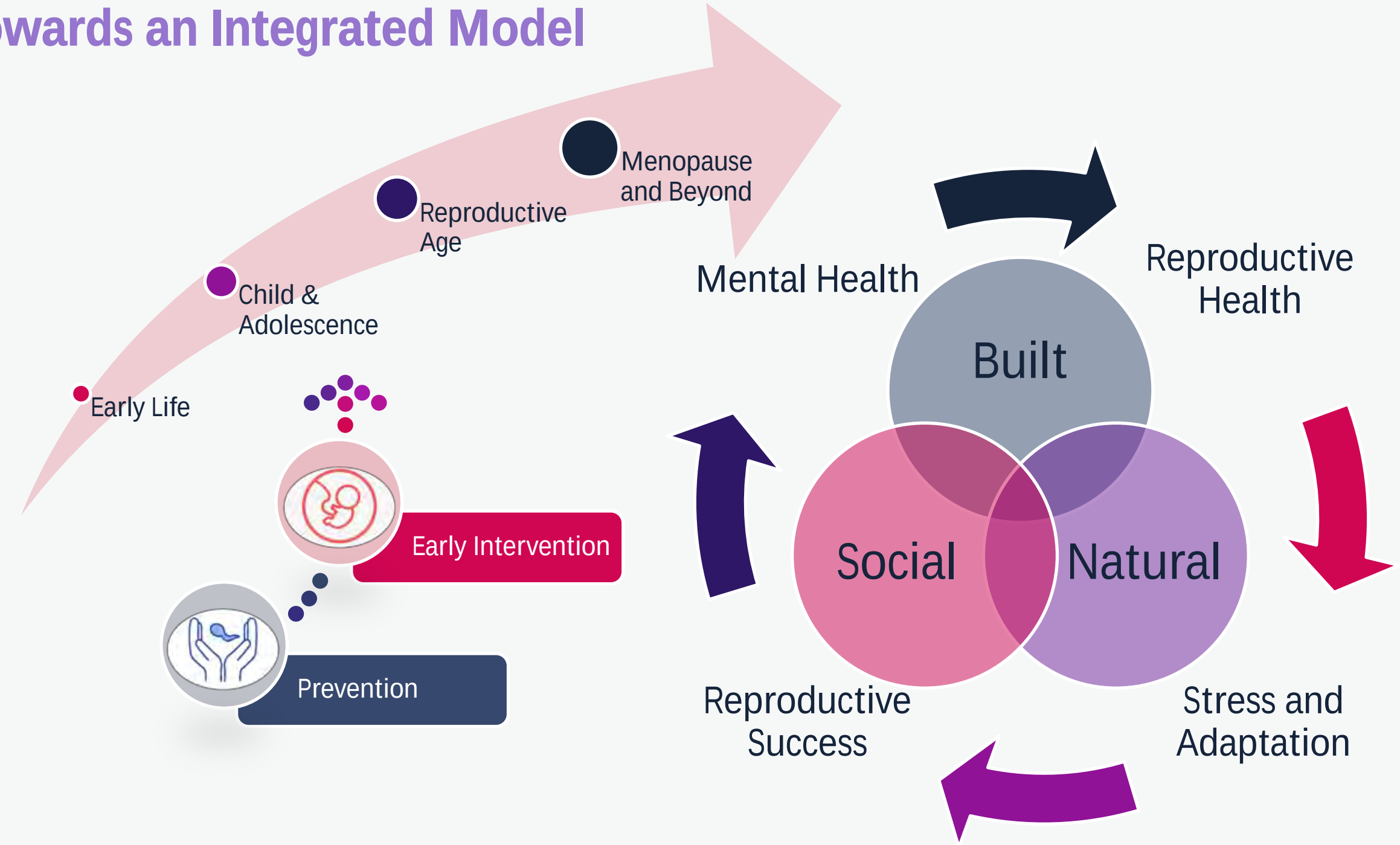
Affiliations + expand

PMID: 37256622 PMCID: PMC10233420 DOI: 10.1001/jamanetworkopen.2023.14934



- **PFOA - lower birthweight only in the lowest quartile group of dietary folate equivalent (DFE) intake**
- **PFNA, PFOA, PFOS - lower birthweight only in the lowest quartile group of plasma folate concentration**

Towards an Integrated Model



The Natural Environment

WATER

Disinfection by Products (DBP)

Municipal water

Swimming pools

Inhalation, dermal, ingestion

AIR

Ambient Air Pollutants

Nitrous Oxide (N02)

PM2.5

Ozone (O3)

DBPs and Human Health

NHANES

Xiaogan DBP Study

All Cause Mortality (published)

Oxidative Stress (published)

Birth Outcomes (published)

Neurodevelopment (published)

Fetal Growth (published)

Asthma, Children/Adolescents (published)

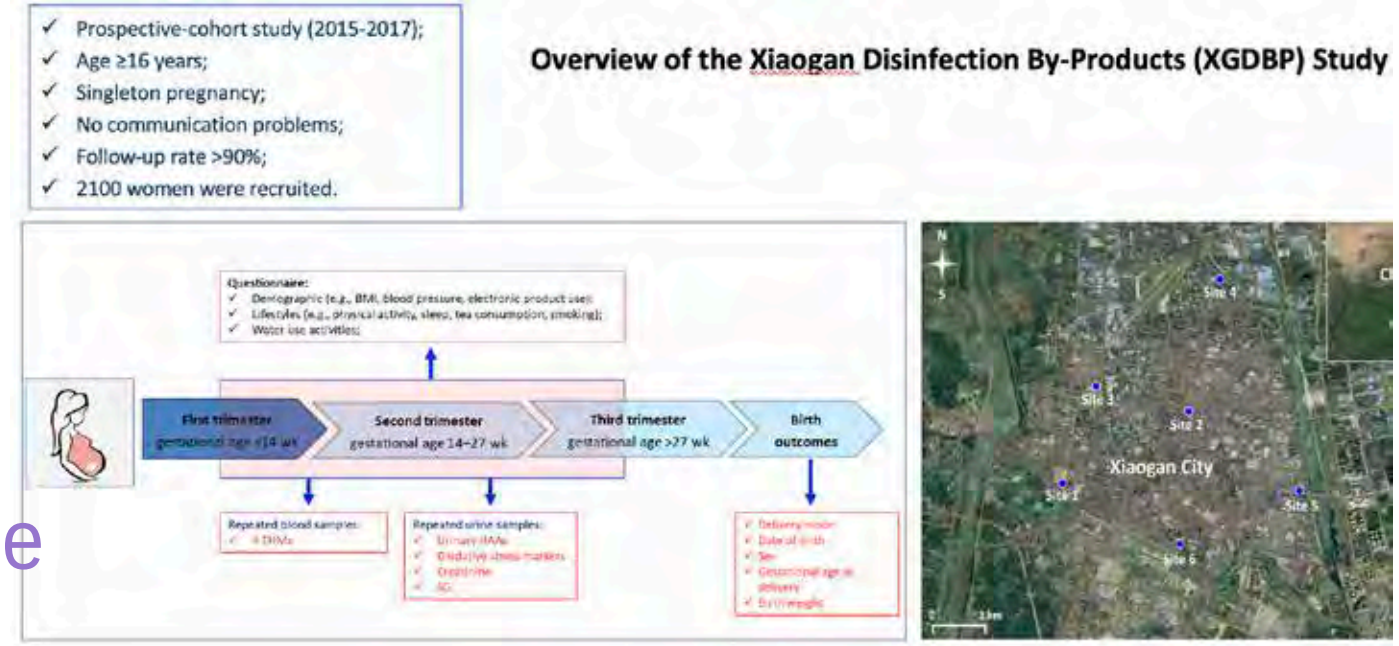
Thyroid Function (published)

Lung Function (published)

Allergic Sensitization (published)

Disinfection By Products (DBP) and Birth Outcomes

- Oxidative Stress
- Birthweight
- Fetal Weight
- Small for Gestational Age (SGA)



Xiaogan DBP Study

Windows of Vulnerability

ehp Environmental Health Perspectives

HOME ISSUE IN PROGRESS ARCHIVES COLLECTIONS AUTHORS REVIEWERS ABOUT

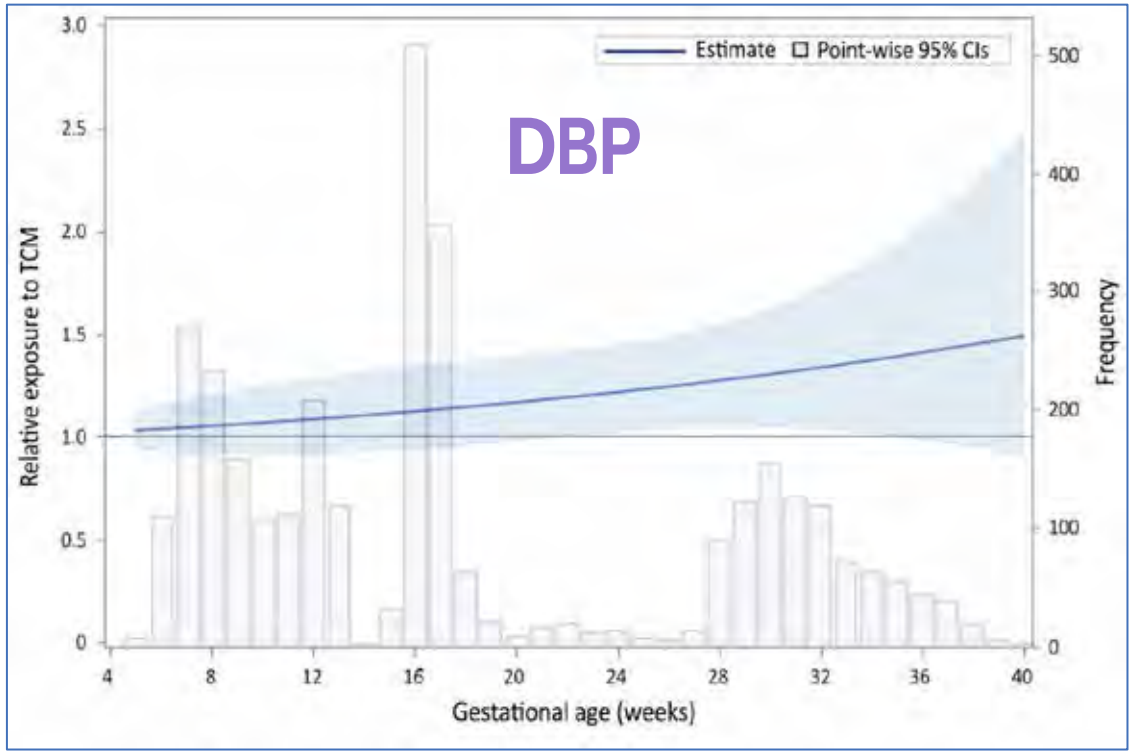
Open Access

Vol. 128, No. 10 | Research

Trimester-Specific Blood Trihalomethane and Urinary Haloacetic Acid Concentrations and Adverse Birth Outcomes: Identifying Windows of Vulnerability during Pregnancy

Yang Sun, Yi-Xin Wang, Chong Liu, Ying-Jun Chen, Wen-Qing Lu, and Carmen Messerlian

Published: 7 October 2020 CID: 107001 https://doi.org/10.1289/EHP7195



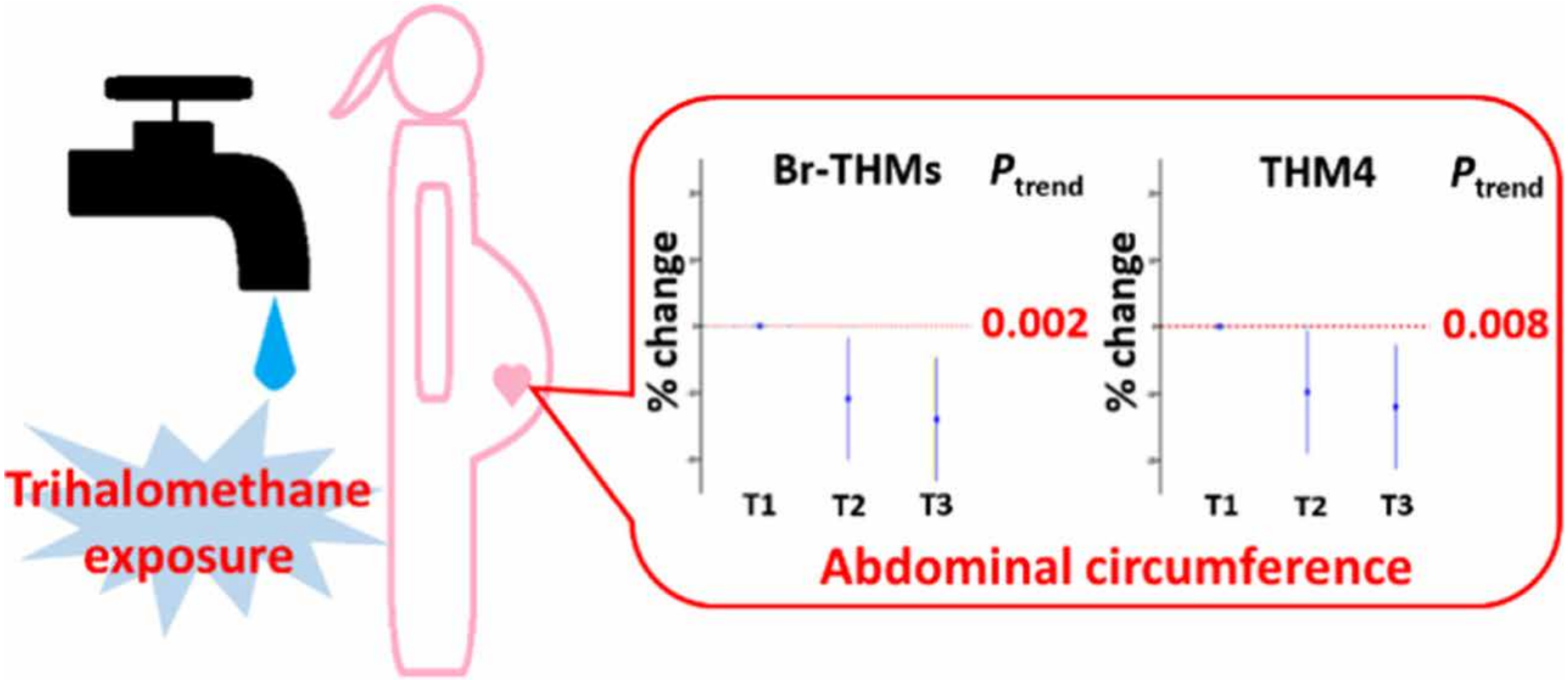
Relative exposure to TCM concentrations across gestational weeks, comparing SGA with non-SGA births

DBP and Fetal Growth

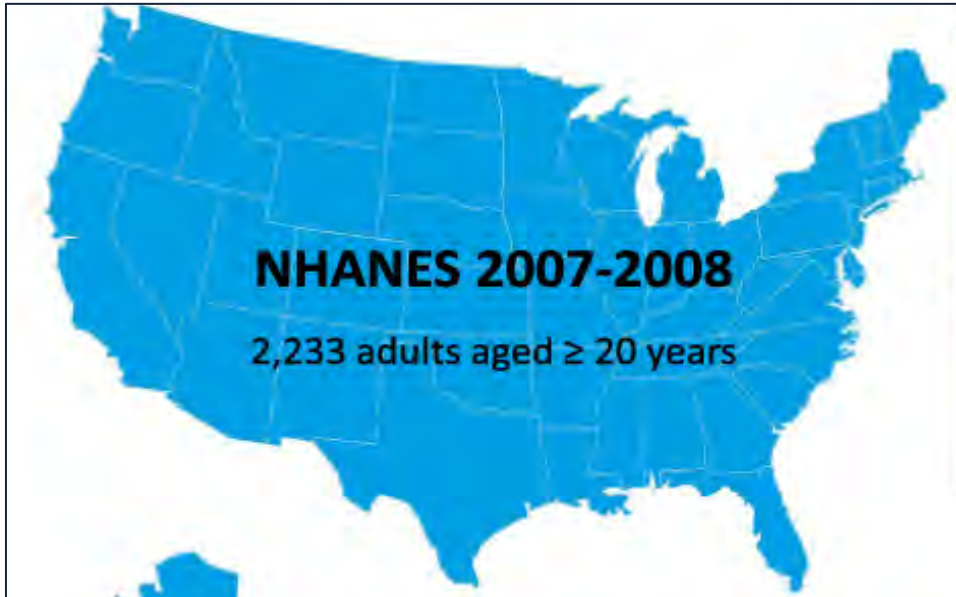
> Environ Sci Technol. 2021 Dec 7;55(23):16011-16022. doi: 10.1021/acs.est.1c04926. Epub 2021 Nov 23.

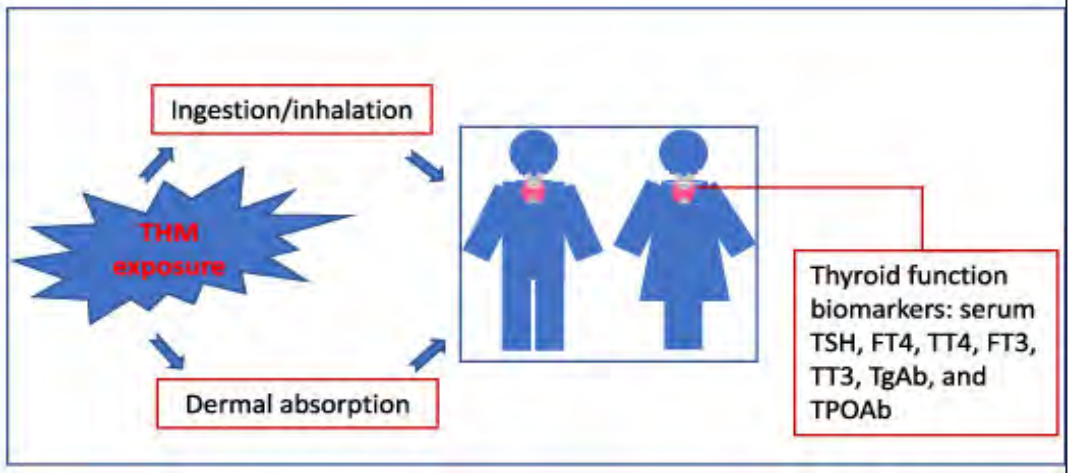
Prenatal Exposure to Disinfection Byproducts and Intrauterine Growth in a Chinese Cohort

Chong Liu [1 2], Yang Sun [3 4], Vicente Mustieles [5 6], Ying-Jun Chen [7], Li-Li Huang [8], Yan-Ling Deng [1 2], Yi-Xin Wang [9], Wen-Qing Lu [1 2], Carmen Messerlian [3 4]



DBP and Thyroid Function





Adjusted percent difference in serum thyroid function measures in relation to blood THM concentrations

Blood THMs (pg/mL)	FT4	TT3	FT4/FT3	TT4/TT3	FT3/TT3
TCM	**3.24 (1.29, 5.19)***	0.72 (-2.45, 3.93)	**3.05 (0.45, 5.68)***	1.16 (-2.87,5.25)	-0.54 (-3.44, 2.39)
BDCM	**2.65 (0.32, 5.01)***	-1.92 (-5.46, 1.67)	2.65 (-1.03, 6.38)	**4.26 (0.21, 8.39)***	1.91 (-1.39, 5.26)
DBCM	0.92 (-2.96, 4.86)	**-4.31 (-7.42, -1.16)***	1.21 (-3.47, 5.99)	**4.17 (0.83, 7.57)***	**4.04 (1.27, 6.85)***
Br-THMs	1.25 (-2.23, 4.78)	**-4.80 (-7.71, -1.85)***	2.08 (-2.36 ,6.60)	**5.44 (1.83, 9.11)***	**4.00 (0.91, 7.14)***
TTHMs	**3.16 (0.58, 5.77)***	-1.66 (-4.49, 1.62)	3.28 (-0.17, 6.78)	3.20 (-1.17, 7.65)	1.52 (-1.49, 4.57)

*P < 0.05.

Environmental Exposures & Cerebral Palsy (CP)

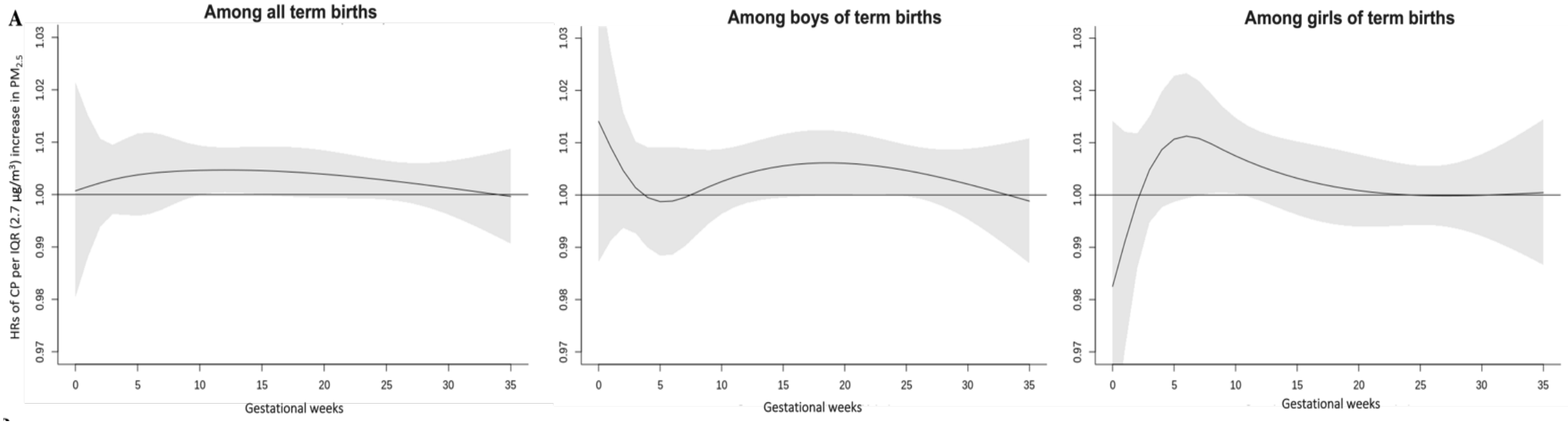
Y Hu , Y Zhang , R Talarico , X Qiu , J Schwartz, DB Fell, M Oskoui, E Lavigne, C Messerlian

Prenatal Exposure to Ambient Air pollution and Cerebral Palsy (CP) in Ontario, Canada Submitted, JAMA

IC/ES Data Discovery Better Health

Prenatal Exposure to Ambient Air Pollution & CP

Overall and sex-specific HRs of CP among term births



- >1.6 million singleton births in Ontario
- Multipollutant Cox proportional hazards model with distributed non-linear lag – weekly AP
- $PM_{2.5}$ exposure during pregnancy increased CP risk by ~10%

Prenatal Exposure to Ambient Air Pollution & Cerebral Palsy

Overall and sex-specific HRs of CPs among term births

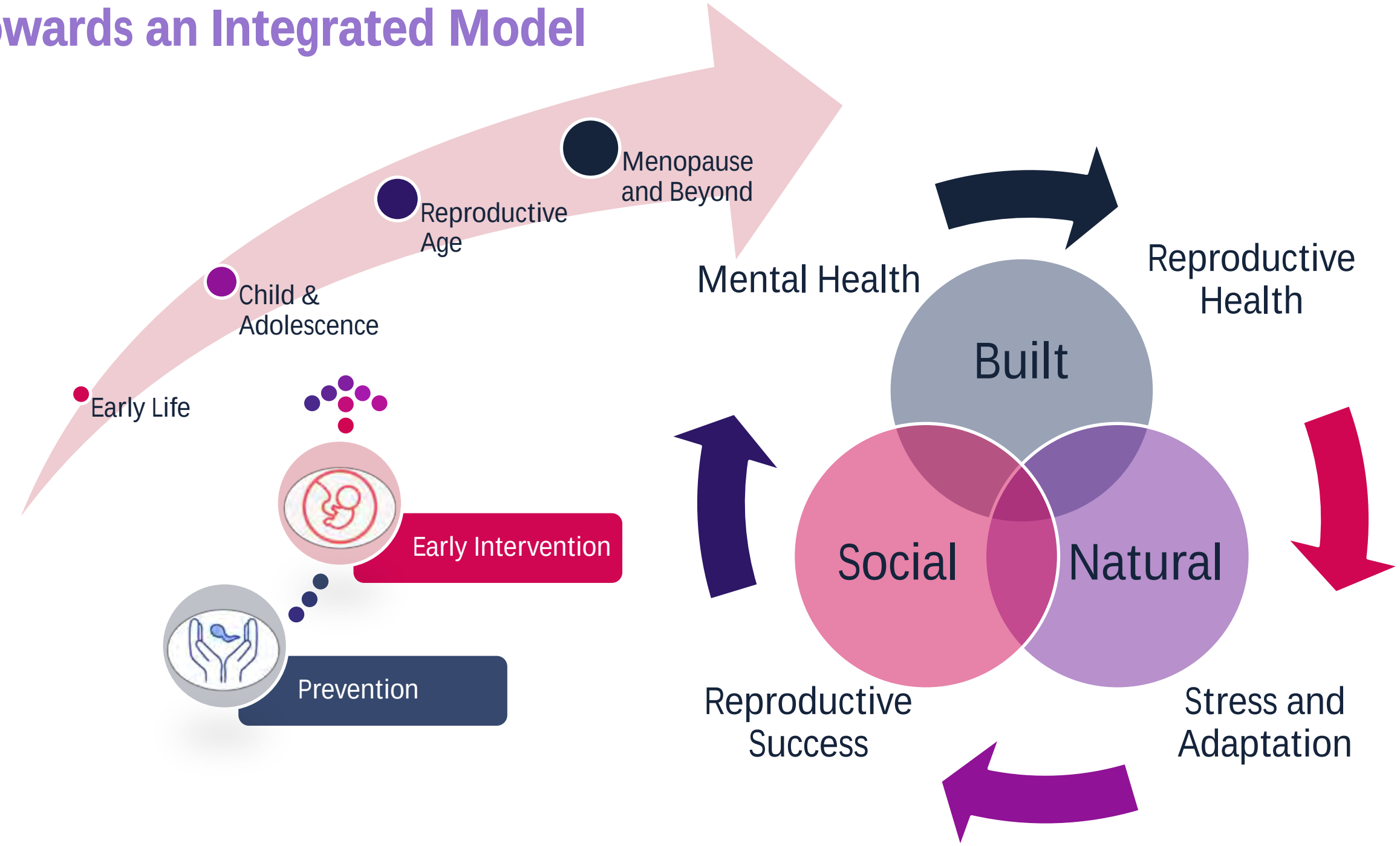
Pollutant	HRs (95% CI)[a]			p-value[b]
	All	Males	Females	
$PM_{2.5}$ (per 2.7ug/m^3)	1.11 (1.03, 1.20)	1.14 (1.02, 1.26)	1.06 (0.96, 1.22)	0.8463
NO_2 (per 10ppb)	0.93 (0.84, 1.02)	0.95 (0.83, 1.08)	0.90 (0.77, 1.04)	0.2207
O_3 (per 7ppb)	0.97 (0.90, 1.04)	0.93 (0.85, 1.02)	1.02 (0.92, 1.15)	0.7155

[a] The lengths of the exposure period during pregnancy for calculation of cumulative HRs are week 0-week 36 for the term births

[b] The p-values of effect modification by sex was derived by adding an interaction term between child's sex and each air pollutant and conducting Likelihood Ratio test with the primary models separately

We found positive associations between exposures to $PM_{2.5}$ over gestational weeks 0 to 33 and increased CP risk among term births with the statistically higher risk between weeks 11 and 14

Towards an Integrated Model



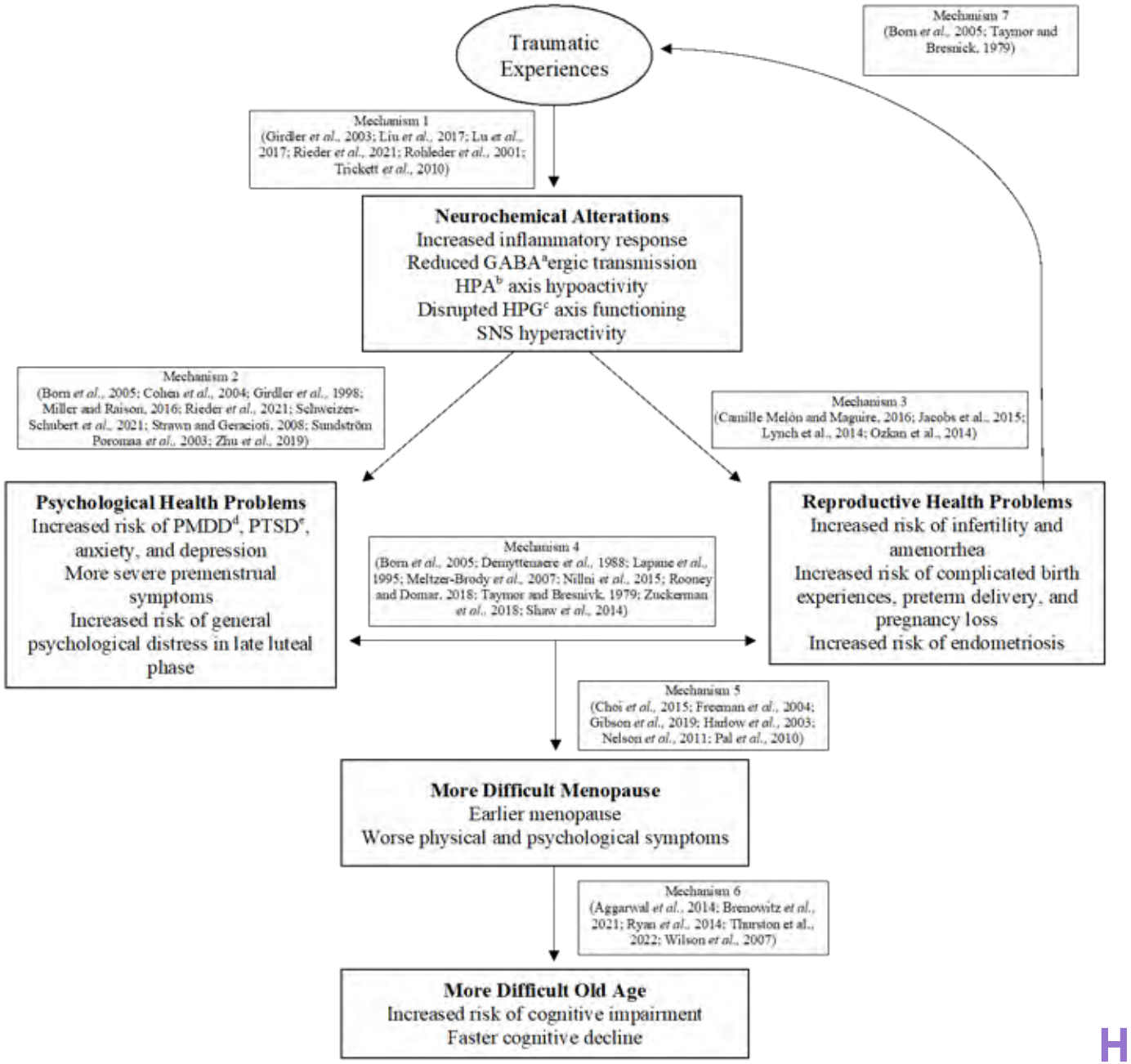
The Social Environment

Early Life Stress

Trauma
Stressful Life Experience
Child Maltreatment
Familial Dysfunction

Sexual and Reproductive Exposure

Age at Menarche
Sexual Experience
Fertility
Pregnancy
Post Partum
Menopause



Trauma and Reproductive Health Across Life

Hillcoat & Messerlian, Human Reproduction

The Preconception Intervention Program for Healthy Reproduction (PIPER) Project

Funded by:

Harvard Scientific Advancement Award (PI: Messerlian)

R01 NIEHS, to be resubmitted (PI: Messerlian)

SEED PROGRAM
SCIENTIFIC EARLY LIFE ENVIRONMENTAL HEALTH & DEVELOPMENT PROGRAM

MGH 1811

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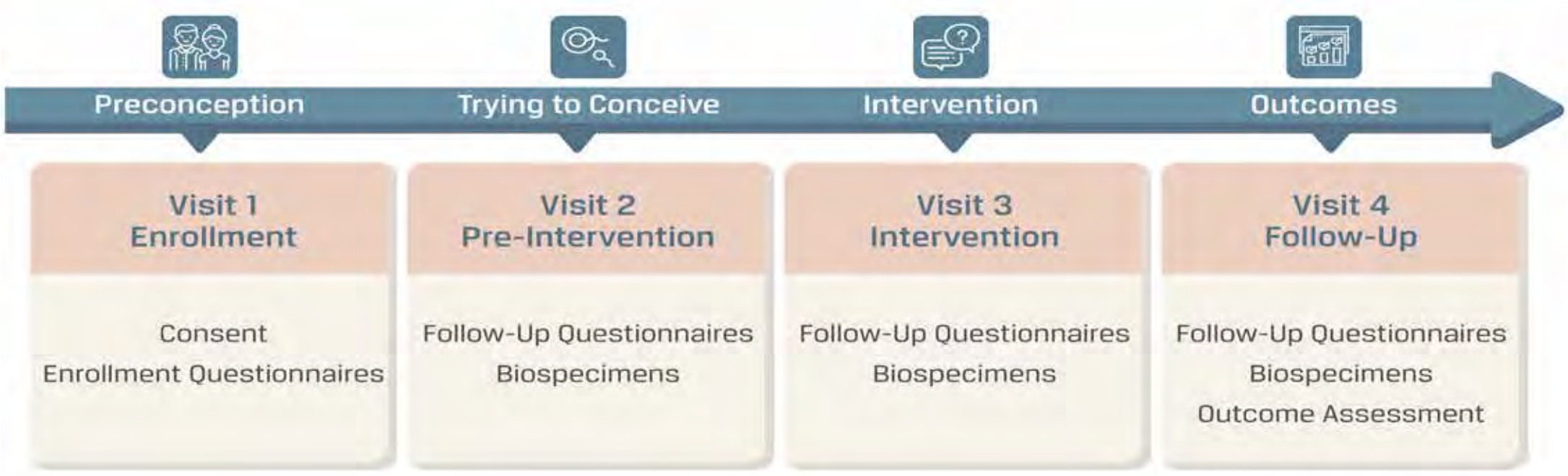
NIH NIEHS

The PIPER Project



Implement a randomized controlled trial design to examine the impact of our intervention on fertilization, implantation, and pregnancy loss

The PIPER Intervention

Food & Drinks

Chemicals may come out of plastic containers into your food and drinks

Use glass or stainless steel containers for food and drinks

Avoid microwaving food in plastic containers, instead use glass or ceramic containers

Brew coffee in a glass or stainless steel French press instead of a plastic coffee maker

Choose organic produce, meat, and dairy when possible

- Choose lower % fat, higher % lean meats
- Look for the USDA organic label
- Look for 5 digit PLU labels that start with the number 9—these indicate the produce is organic

Cook more meals at home with fresh ingredients

Studies have shown that people who eat more meals prepared outside the home have higher levels of BPA

Choose safer plastics:

Plastics to avoid:

Choose fresh or frozen instead of canned food or drinks

BPA and phthalates can migrate from the linings of cans and plastic packaging into food and drinks

Replace plastic cooking utensils with wood or stainless steel

Tips to Reduce Your Exposure

Personal Care Products & Cosmetics

Seek products that say "fragrance-free" on the label

Avoid buying personal care products with the words "fragrance" or "parfum" on the label

Avoid toothpaste, deodorant, & other products containing triclosan or triclocarban

Avoid products that say "antibacterial"

Avoid chemical UV filters in daily moisturizers and sunscreens

Choose shade, hats, and tightly woven clothing instead of sunscreen when you can

Choose safer period products

Tampons, pads, liners - Look for:

- 100% organic cotton or natural materials
- unscented and fragrance-free
- plastic-free packaging
- plastic-free applicator or tampon without applicator

Menstrual cups - Look for:

- 100% medical-grade silicone
- non-toxic cleaning methods

Look for plant-based products

The USDA Organic label or "Made with organic ingredients" indicates products mostly made of plants

Avoid nail polish & nail polish remover

Choose mineral instead of chemical sunscreens

- ✗ Benzophenone
- ✗ Oxybenzone
- ✗ Octinoxate
- ✗ Octyl methoxycinnamate
- ✓ Zinc oxide
- ✓ Titanium dioxide

Avoid cosmetics and personal care products with parabens listed on the label

Common names for these chemicals include butyl paraben, ethyl paraben, methyl paraben, and propyl paraben

Home & Cleaning

Chemicals in plastic end up in your dust, Keep dust levels low

Wipe surfaces with a damp cloth and use a vacuum with a HEPA (high-efficiency particulate air) filter

Clean with plain water, baking soda, vinegar, or castile soap when possible

Open windows or use vents when using products that have strong smells

Don't use fabric softeners or dryer sheets

try baking soda, vinegar, or dryer balls to soften clothes if needed

Get rid of mothballs

Avoid products with fragrances

Air fresheners
Cleaning products
Soaps
Dryer sheets
Laundry detergents & fabric softeners
Candles

Use nontoxic alternatives to toilet bowl deodorizers,

such as lemon juice, borax, baking soda, or white vinegar

Avoid tracking pollutants into your home

Place rugs in doorways and take off your outdoor shoes at the door

Use the Label to Find Safer Products: Cleaners & Laundry Detergents

Look for	Avoid
• Fragrance-free • Plant-based, "Made with organic ingredients" • Safer Choice logo	• "Poison", "danger", "caution", or "warning" on the label • Products advertised as **antibacterial, antimicrobial,** or **stain-protecting**

Educational Resources

EDCs & Reproductive Health

Understanding everyday toxicants and how you can minimize your exposure

Additional Resources

Learn more about the chemicals in your everyday products and environment:

The Environmental Working Group: www.ewg.org

Explore our website: www.seed-program.org

Contact & Follow us: SEED@hsph.harvard.edu (Twitter and Instagram) @drmesserlian

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How can I minimize my EDC exposure?

CHEMICAL FREE

- Wash your hands frequently, especially before meals
- Rinse produce thoroughly and buy organic when possible
- Avoid foods and beverages stored in plastic or canned containers
- Filter your water and use glass or metal storage containers
- Replace personal care and household products that have fragrance with safer products
- Look for phthalate-, paraben-, and chemical-free products
- Look for these labels:

100% FRAGRANCE FREE

EDCs

Endocrine-disrupting chemicals (EDCs) interrupt normal hormonal activity by mimicking, blocking, or altering hormones and changing the way that they function in the body.

EDCs include phthalates, phenols, per- and polyfluoroalkyl substances (PFAS), and other toxicants. Some EDCs are rapidly removed from the body, while others can remain in the body for a long time. Most people are exposed to multiple EDCs daily, allowing these chemicals to remain at harmful concentrations.

These chemicals damage your health

Current research suggests that EDCs lead to adverse health effects. These chemicals can:

- ✕ Alter reproductive health
- ✕ Reduce immune function
- ✕ Increase cancer risk
- ✕ Change metabolic function
- ✕ Decrease brain function

How do EDCs enter the body?

- Ingestion
- Inhalation
- Absorption

Where can EDCs be found?

- Personal care products, nail polish, and cosmetics
- Takeout containers, plastic packaging, canned foods and beverages, and plastic kitchen and storage items
- Unfiltered water sources and processed drinks
- Furniture, electronics, cleaning products, candles, fresheners, and household dust
- Cigarette smoke, e-cigarettes, and other smoking devices

Advancing the Field

- Focus on Prevention and Early Intervention
- Integration of Built, Natural, and Social Environments
- Holistic Reproductive Health across the Lifecourse, including men in the process
- Moving to a multidimensional integrated framework of health and wellbeing through multimodal AI

ACKNOWLEDGEMENTS

Preconception PFAS Exposure and Reproduction (PREPARE) Study

R01ES031657 (PI: Messerlian)

NIEHS Program Officers

Antonia Calafat
Centers for Disease Control and Prevention

All SEED Program Team Members
Collaborators
Participants

Vincent Center for Reproductive Biology

Massachusetts General Hospital

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CDC

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Conclusions

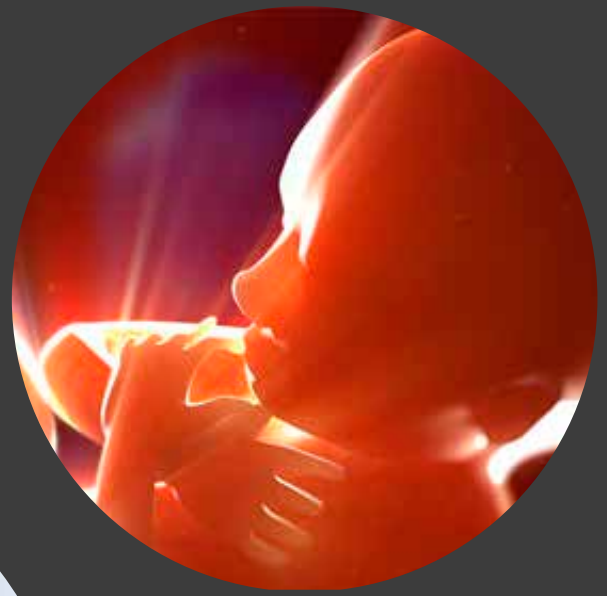
- With increasing exposure to complex environments, we need more novel and multifaceted preventive strategies
- A holistic lens that considers built, natural, and social environments across the lifecourse is needed to solve for real world reproductive health issues focused on prevention
- Multimodal AI, interventions, and education are just the beginning translating evidence into prevention, personalized care, and improved outcomes

Maternal-Child Health Risks and Risk Reduction

Rita Strakovsky, Ph.D., R.D.

Associate Professor

Michigan State University



Exposure to non-persistent endocrine disrupting chemicals, maternal metabolic health, and roles of diet quality

Rita Strakovsky, Ph.D., R.D.
Associate Professor

Michigan State University
East Lansing, MI
strakovs@msu.edu

Office on Women's Health Endocrine Disrupting Chemicals and Women's Health Symposium. July 18 & 19, 2023.

OASH | Office on Women's Health

Maternal-Child Health Risks and Risk Reduction

Christine Langton, Ph.D.

Postdoctoral Fellow, Women's Health Group, Epidemiology Branch

National Institute of Environmental Health Sciences

PRE-RECORDING

Endocrine Disrupting Chemicals and Women's Health Symposium

Menopause and Uterine Fibroid Research

Christine R. Langton, Postdoctoral Fellow
Women's Health Group, Epidemiology Branch

National Institute of Environmental Health Sciences

Objectives of Presentation

- Early life exposures and later in life health
  - Menopause research
  - Uterine fibroid research

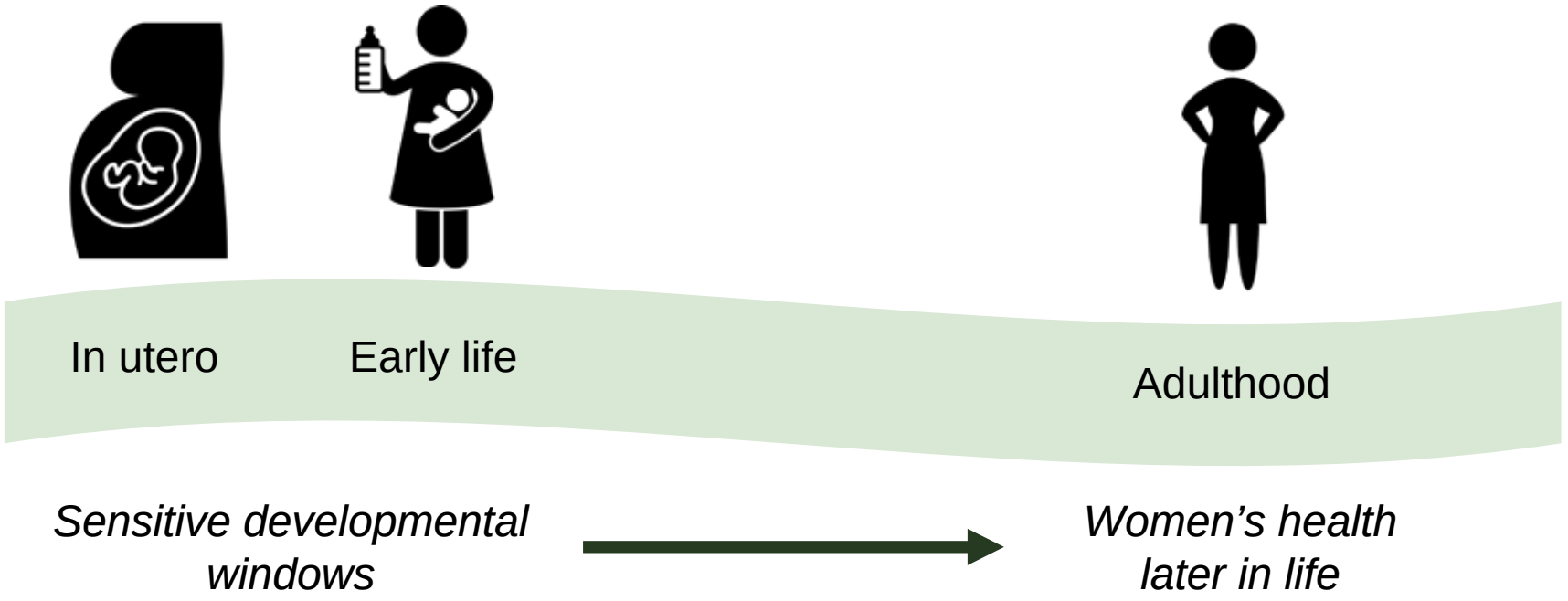
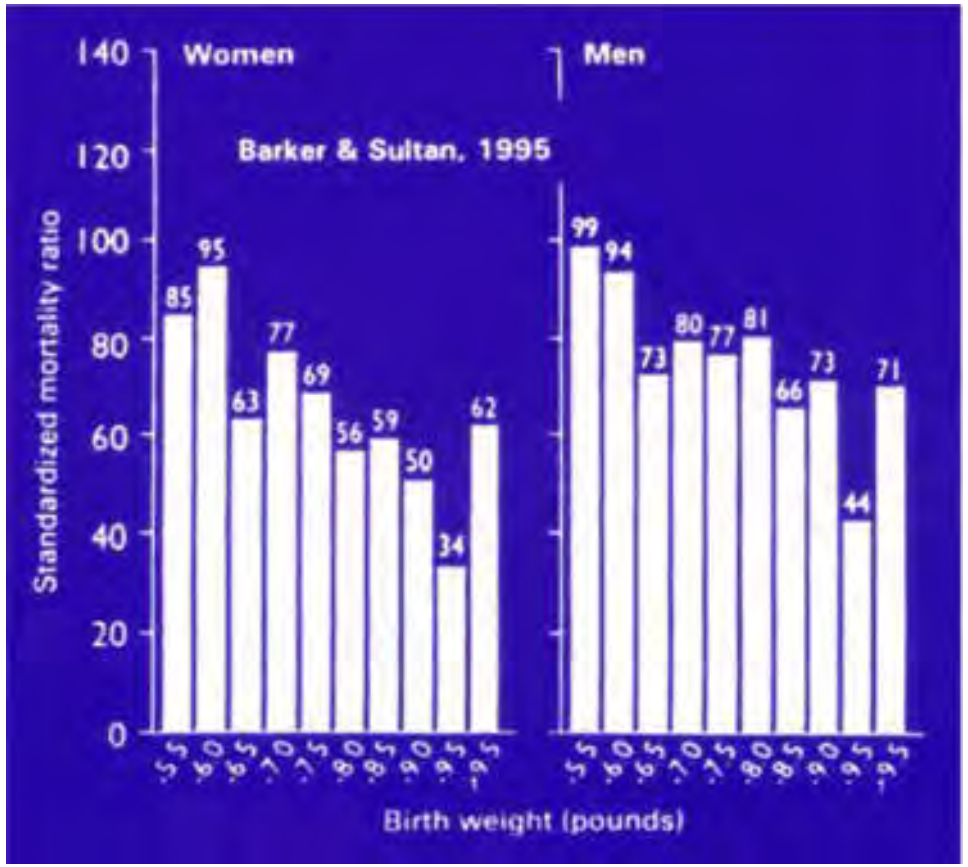


Image sources from Noun Project: Fetus, Lars Meiertoberens; Midwife, Gan Khoon Lay; Female aging, Marie Van den Broeck.

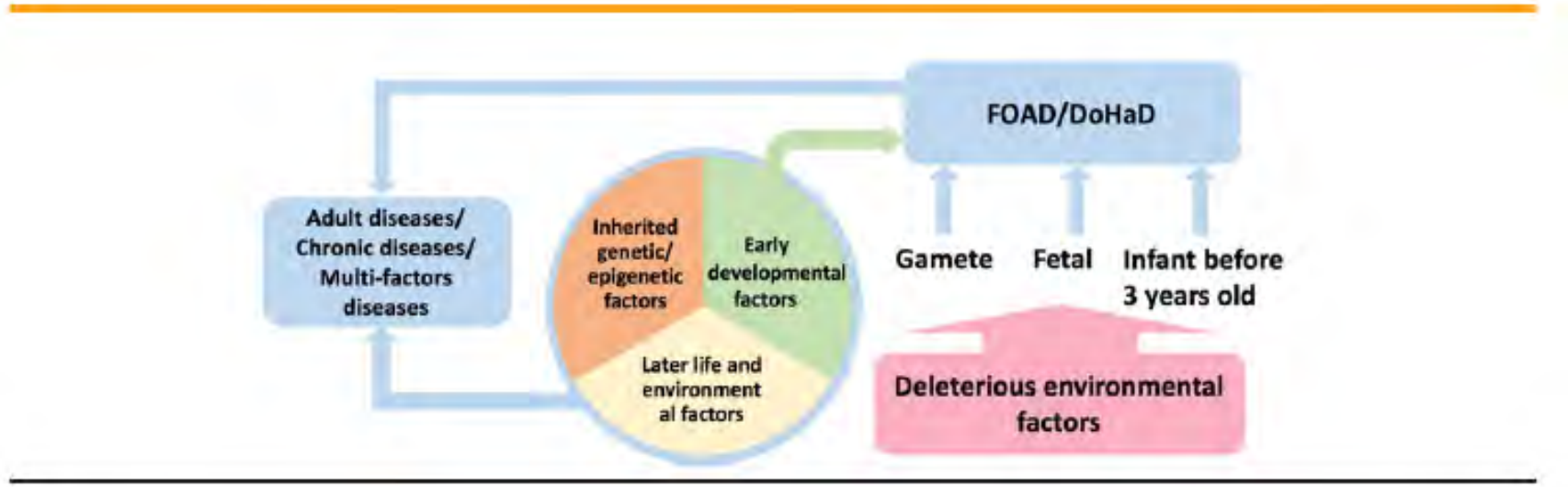
Fetal Origins of Adult Disease (FOAD)



- Developmental plasticity
- Beneficial in short-term to promote survival and reproduction
- Detrimental in long-term

Source: Calkins K, Devaskar SU. Fetal Origins of Adult Disease. *Curr Probl Pediatr Adolesc Health Care.* 2011;41(6):158-76.

Developmental Origins of Health and Disease (DoHaD)



- DoHaD linked to adult diseases
  - Type II diabetes, hypertension, CVD, cancer

Source: Li X et al. “Three Hits” Hypothesis for Developmental Origins of Health and Disease in View of Cardiovascular Abnormalities. *Birth Defects Res.* 2017;109:744-757.

In Utero Exposures and Menopause

American Journal of Epidemiology

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January 6, 2022

Original Contribution

Association of In Utero Exposures With Risk of Early Natural Menopause

Christine R. Langton*, Brian W. Whitcomb, Alexandra C. Purdue-Smithe, Lynnette L. Sievert, Susan E. Hankinson, JoAnn E. Manson, Bernard A. Rosner, and Elizabeth R. Bertone-Johnson

* Correspondence to Dr. Christine Langton, Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, 715 North Pleasant Street, Amherst, MA 01003-9304 (e-mail: clangton@umass.edu).

Initially submitted May 7, 2021; accepted for publication December 30, 2021.

Menopause

"permanent cessation of menstruation recognized after 12 consecutive months of amenorrhea with no other obvious pathological or physiological cause"

- Determinants of age at menopause
  - Number of oocytes at birth
  - Degeneration of oocytes due to atresia
  - Threshold oocytes needed to produce sufficient hormones to maintain menstrual cyclicity
- Average age 51

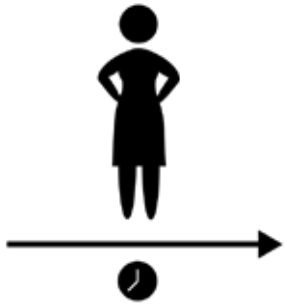


Image source adapted from Noun Project: Marie Van den Broeck

Early Natural Menopause

- Cessation of ovarian function before age 45
- Affects ~10% of women in Western populations
- Increased risk:
  - cardiovascular disease
  - osteoporosis
  - cognitive decline
  - premature mortality
- Interferes with family planning

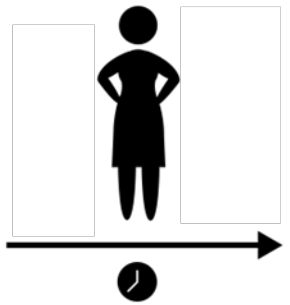


Image adapted from Noun Project: Marie Van den Broeck

Diethylstilbestrol (DES)

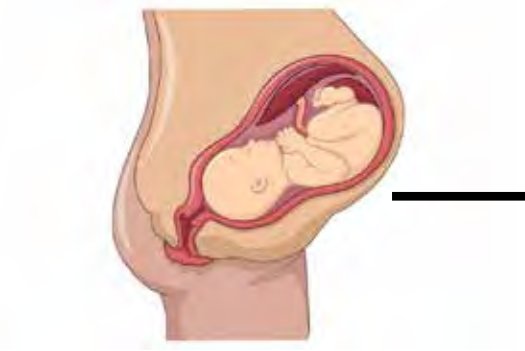
Increased Risk in DES daughters

- Clear cell adenocarcinoma of vagina and cervix
- Breast cancer
- Adverse reproductive outcomes
  - infertility
  - spontaneous abortion
  - ectopic pregnancy
  - preeclampsia
  - preterm delivery
  - stillbirth
  - neonatal death

Source: https://www.cancer.gov/about-cancer/causes-prevention/risk/hormones/des-fact-sheet#what-is-des

Ovarian Aging

Peak Reserve
4th month of fetal development



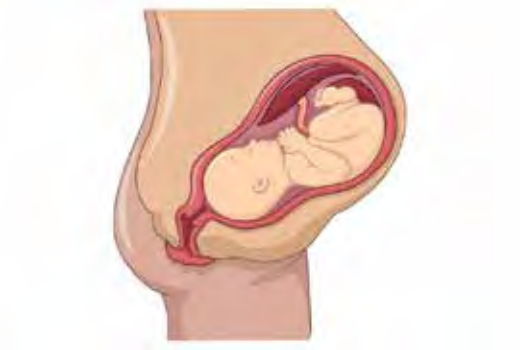
Birth | Menarche | Menstrual cycles | Menopause

~6-7m oocytes | ~1-2m oocytes | Recruitment & Atresia | ~1,000 oocytes

Oocyte Quantity

DES and Ovarian Aging

Peak Reserve
4th month of fetal development



~6-7m oocytes

- DES passes across placenta
- Animal studies
  - Absence of corpus luteum
  - Polyovular follicles
  - Ovarian cysts
- DES *may* affect:
  - Initial cohort of follicles
  - Rate of fetal oocyte atresia

Source: Reed CE, Fenton SE. Exposure to Diethylstilbestrol during Sensitive Life Stages: A legacy of heritable health effects. *Birth Defects Res C Embryo Today.* 2013;99(2):134-46.

Nurses' Health Study II

- Prospective cohort study
- 116,429 female registered nurses from 14 states
- Aged 25-42 years in 1989
- Questionnaires every 2 years, on-going
- Assess medical history and lifestyle behaviors
- Follow-up >89% for each cycle

Menopause Assessment

- Baseline and biennial questionnaires
  - Menstrual periods ceased permanently
  - Age at cessation
  - Natural cessation or due to surgery, radiation, chemotherapy
  - Use of hormone therapy
- Cases: natural menopause before age 45

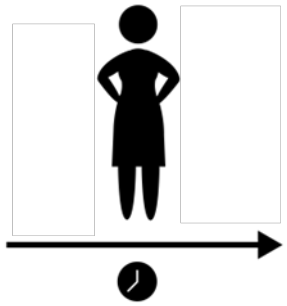
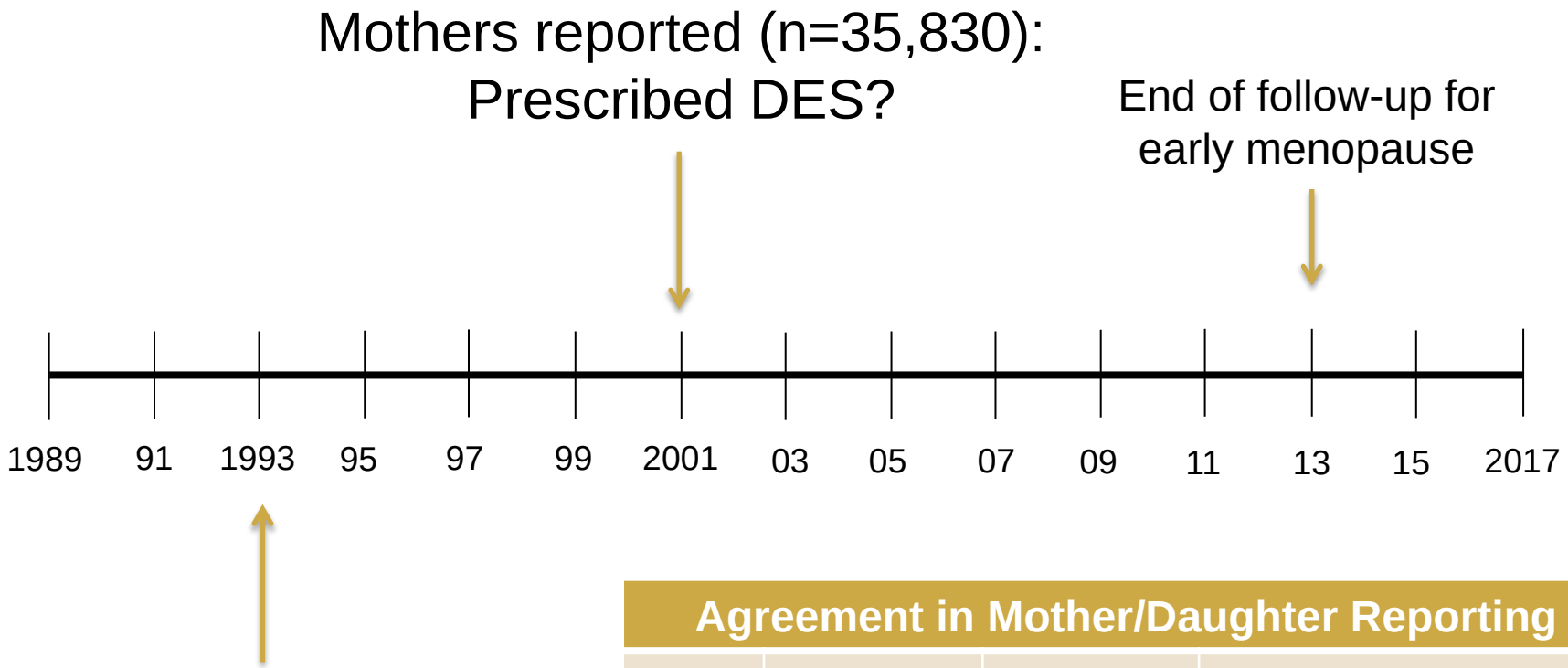


Image adapted from Noun Project: Marie Van den Broeck

DES Assessment, Participants and Mothers



Agreement in Mother/Daughter Reporting			
Yes	**No**	**Total**	**Kappa**
567	26,248	27,180	K=0.75 (95% CI: 0.72-0.77)

Statistical Models

- Cox proportional-hazards models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs)

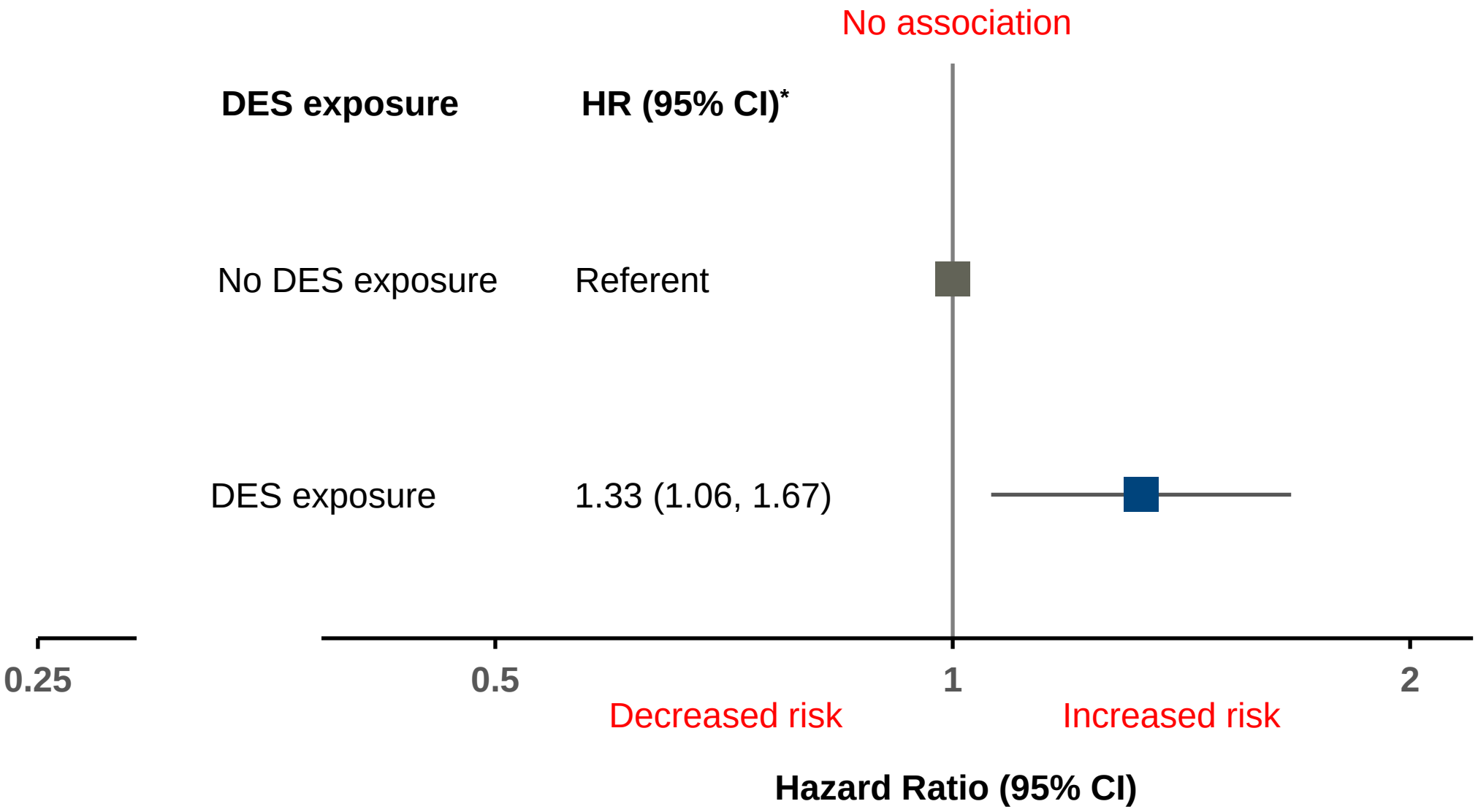
Model 1	Model 2	Model 3
Unadjusted	**Time-varying Participant Factors**	**In utero Factors**
§ Age as time scale	§ Age at menarche* § Smoking § Alcohol § BMI § Vitamin D § Menstrual cycle length § Infertility § Parity § Breastfeeding § Oral contraceptives § Tubal ligation	§ Part of multiple birth § Cigarette exposure § Prematurity § Birth weight

*Not time-varying

Demographics at Baseline

Characteristic	All Participants (n=106,633)	In utero DES exposure (n=2,401)
Age, mean	34.1	34.5
Non-Hispanic White	94%	97%
Cigarette smoking, pack-years, mean	17.9	13.0
Infertility due to ovulatory disorder	4.7%	7.8%

Risk of Early Menopause by In Utero DES Exposure



*Adjusted for age and questionnaire cycle (as time scale), age at menarche, time-varying smoking, alcohol, BMI, vitamin D, parity, breastfeeding, infertility, menstrual cycle length, oral contraceptives, tubal ligation, and other in utero exposures: cigarette smoke exposure, part of multiple birth, prematurity, birth weight.

Conclusions

In utero DES exposure and menopause

- **Increased risk** of early menopause (<45 years)
- Consistent with prior epidemiological studies
- Facilitate early identification for CVD risk reduction strategies
- Potential multigenerational effects

https://www.cancer.gov/about-cancer/causes-prevention/risk/hormones/des-fact-sheet#what-health-issues-might-des-grandchildren-have

Soy-based Infant Formula and Uterine Fibroids

ehp Environmental Health Perspectives

HOME ISSUE IN PROGRESS ARCHIVES COLLECTIONS AUTHORS REVIEWERS ABOUT INTRODUCING *JHP*

Open Access

Vol. 131, No. 1 | Research

Soy-Based Infant Formula Feeding and Uterine Fibroid Development in a Prospective Ultrasound Study of Black/African-American Women

Christine R. Langton, Quaker E. Harmon, Kristen Upson, and Donna D. Baird

Published: 25 January 2023 | CID: 017006 | https://doi.org/10.1289/EHP11089

Uterine Fibroids

- Non-cancerous tumors of myometrium
- >70% of reproductive age women
- Symptomatic fibroids can lead to severe morbidity and hysterectomy
- Black/African American women disproportionally burdened

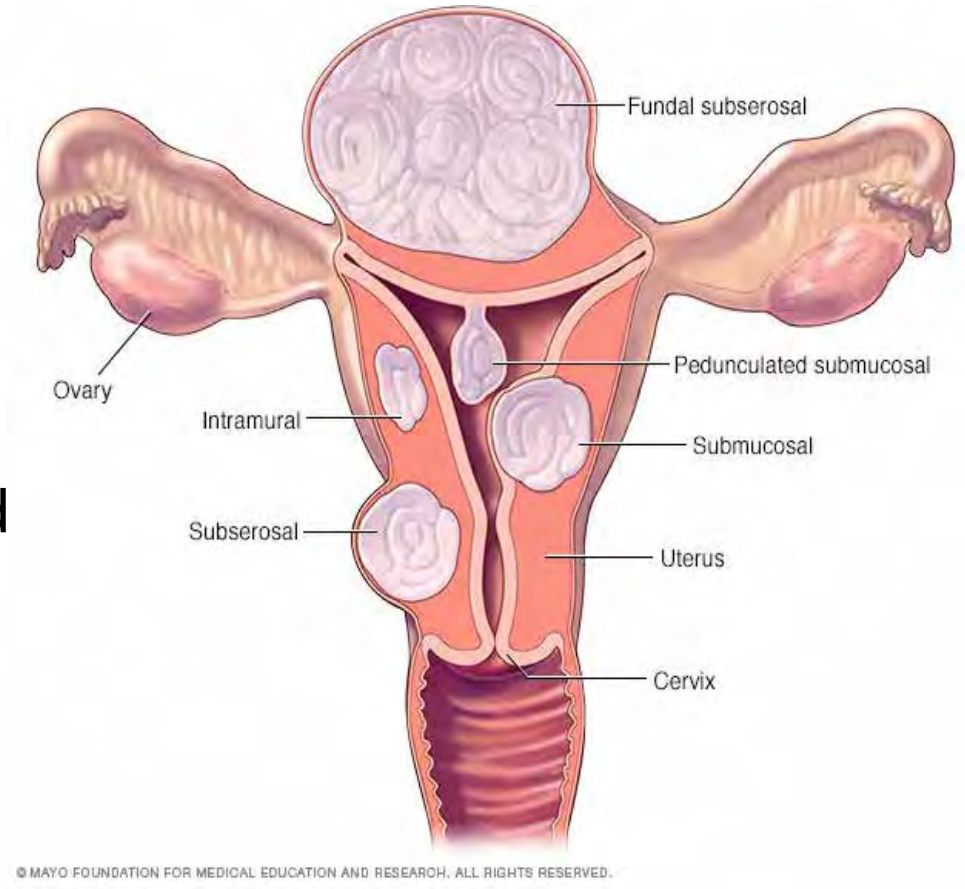
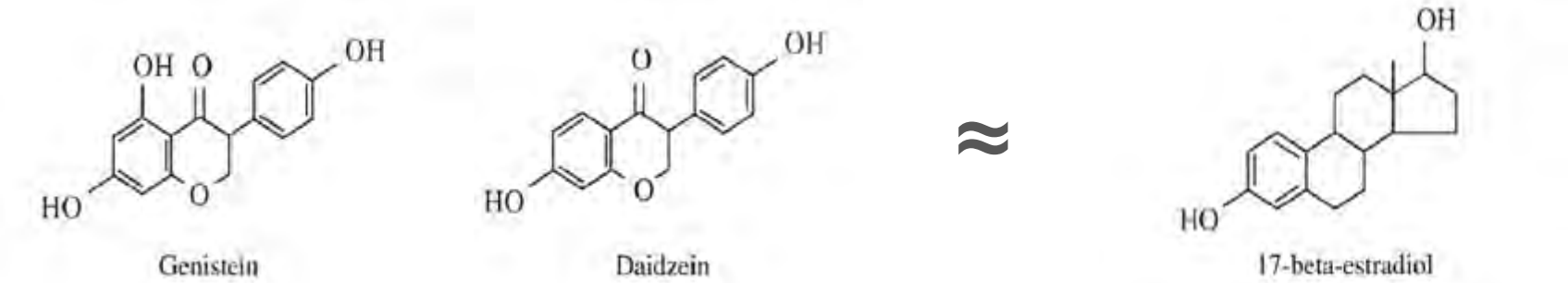


Image source: https://www.mayoclinic.org/diseases-conditions/uterine-fibroids/symptoms-causes/syc-20354288

Soy has Phytoestrogens



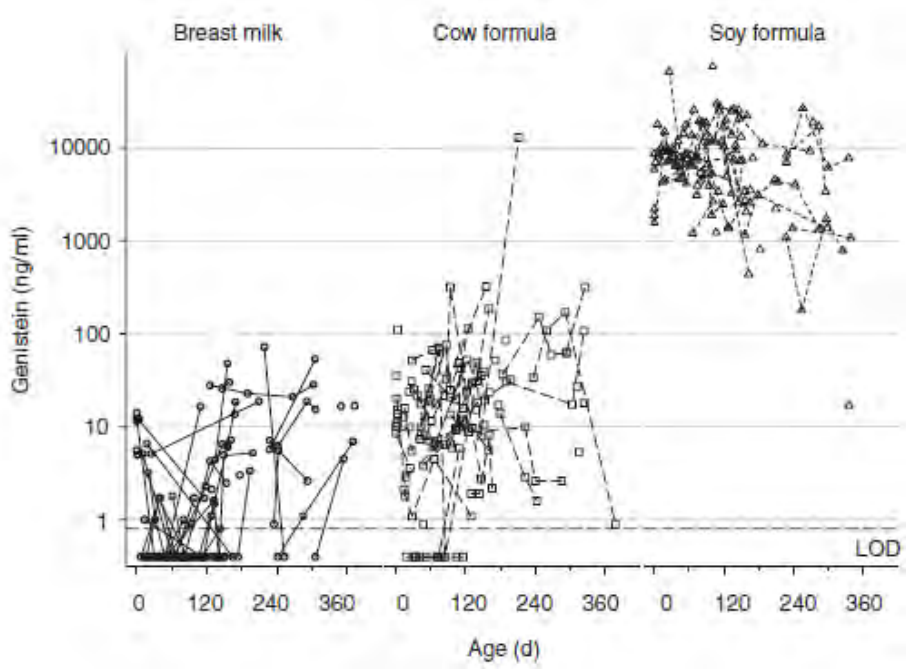
- Isoflavones act as endocrine disruptor

- Postnatal treatment to lab animals
  - Alters rodent reproductive tract including uterus (Suen et al. 2021)
  - Increased fibroid development in Eker rats (Greathouse et al. 2012)
- Exposure during sensitive developmental windows detrimental effects on reproductive systems

Image adapted: Pilšáková L, Riečansky I, Jagla F. The physiological actions of isoflavone phytoestrogens. *Physiol Res.* 2010;59(5):651-664.

Soy-based Infant Formula

- Linked to reproductive conditions
  - early/late menarche, menstrual irregularities, endometriosis
- Proliferative vaginal tissue and slower rate of uterine involution in soy-fed infants[1]
- Contains high levels of phytoestrogens
- Consumed by 12% US infants



[1]Adgent et al. A longitudinal study of estrogen-responsive tissues and hormone concentrations in infants fed soy formula. *J Clin Endocrinol Metab*. May 1 2018;103(5):1899-1909; Figure adapted: Cao et al. Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula. *J Expo Sci Environ Epidemiol.*. 2009 Feb;19(2):223-224.

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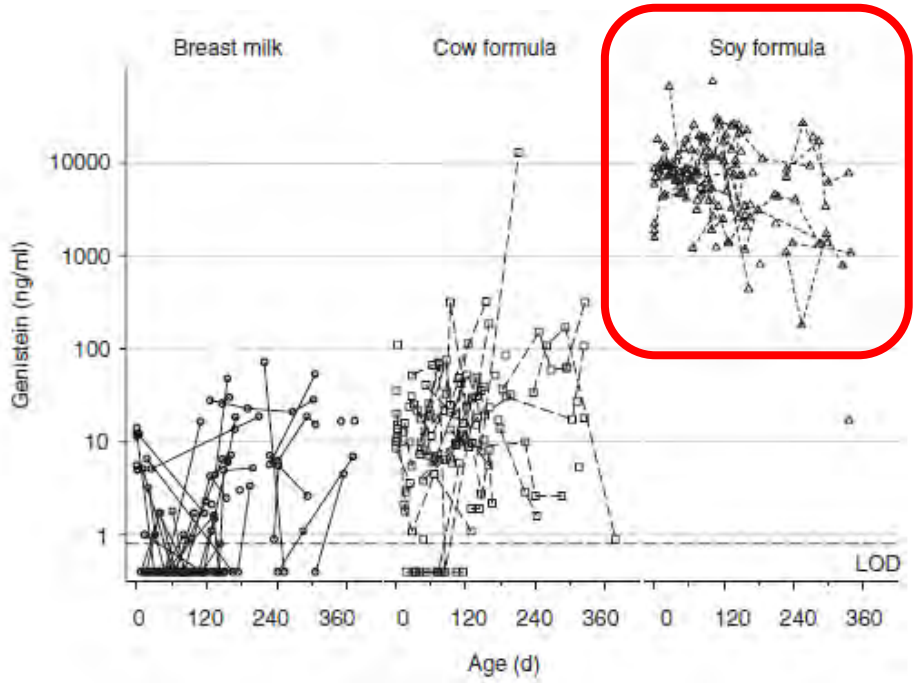

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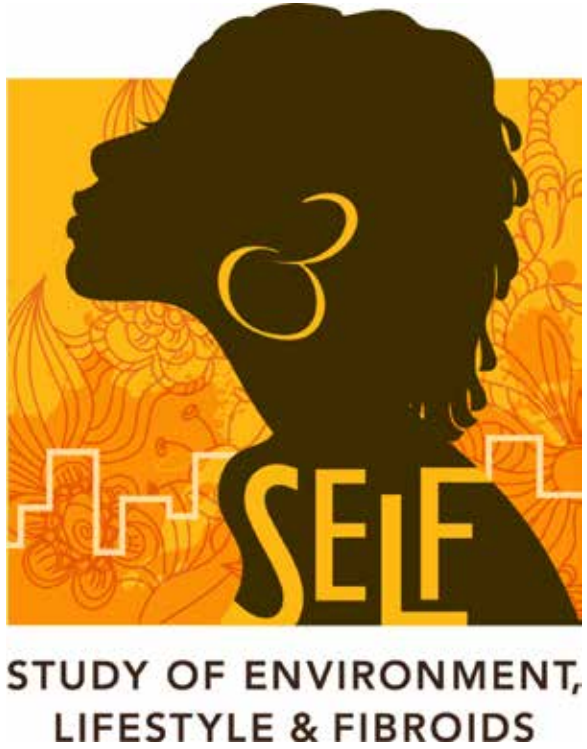
Study of Environment, Lifestyle & Fibroids (SELF)



- Prospective cohort with community-recruited participants (n=1,693)
- Detroit, Michigan area
- Self-identified Black or African American women
  - Ages 23-35
  - Premenopausal
  - No prior clinical diagnosis of fibroids

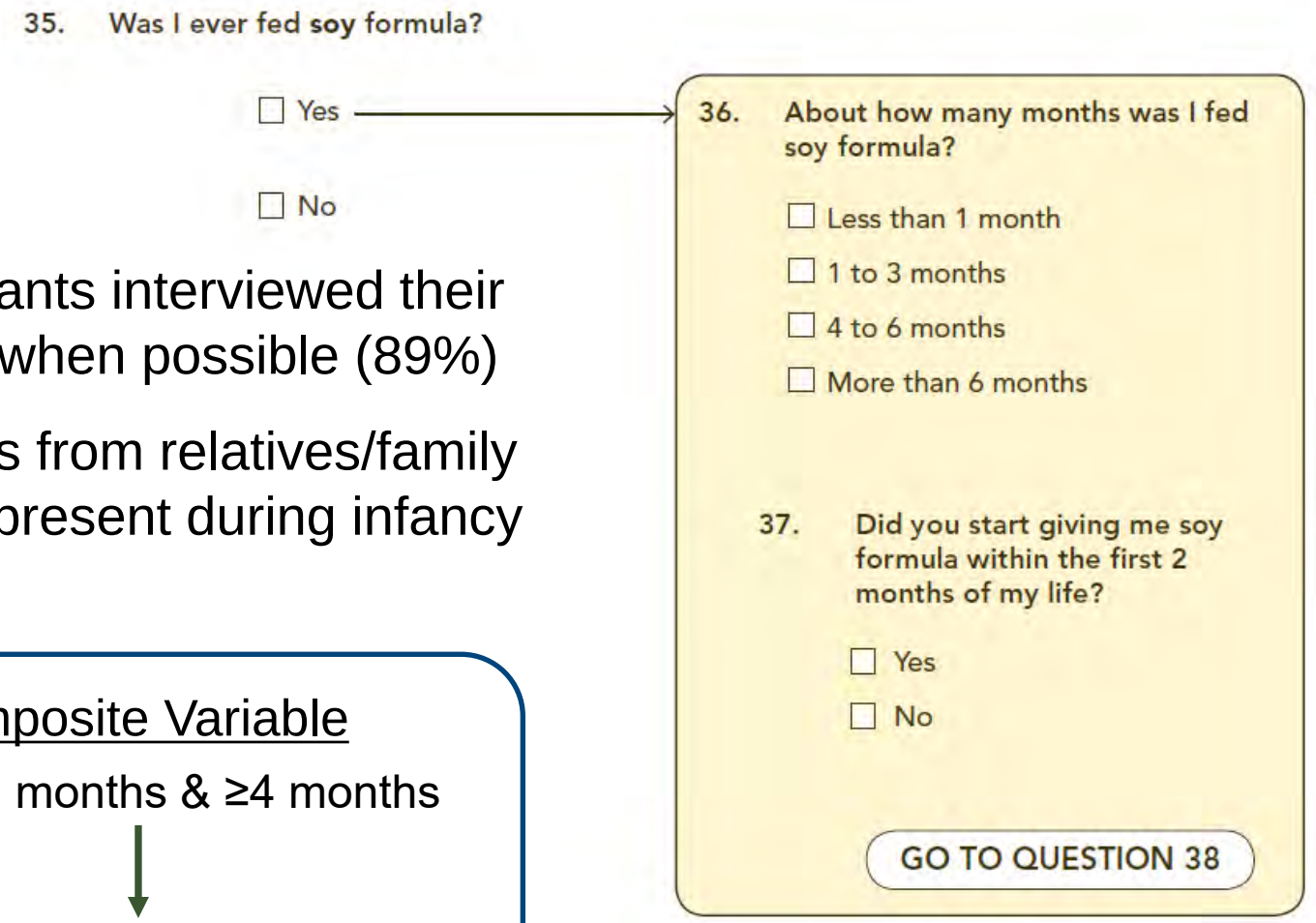
SELF – Study Design

Visit 1	Visit 2	Visit 3	Visit 4
n=1,693	88% response	86% response	91% response
2010–2012	2012–2015	2014–2016	2016–2018
	~18-20 mos.	~18-20 mos.	~18-20 mos.

Every visit

Ultrasound
Questionnaires
Clinical Measurements
Biospecimen

Soy Formula Assessment, Mothers Interviewed



35. Was I ever fed soy formula?

☐ Yes → 36. About how many months was I fed soy formula?

☐ Less than 1 month
☐ 1 to 3 months
☐ 4 to 6 months
☐ More than 6 months

☐ No

37. Did you start giving me soy formula within the first 2 months of my life?

☐ Yes
☐ No

GO TO QUESTION 38

- Participants interviewed their mother when possible (89%)
- Answers from relatives/family friends present during infancy (11%)

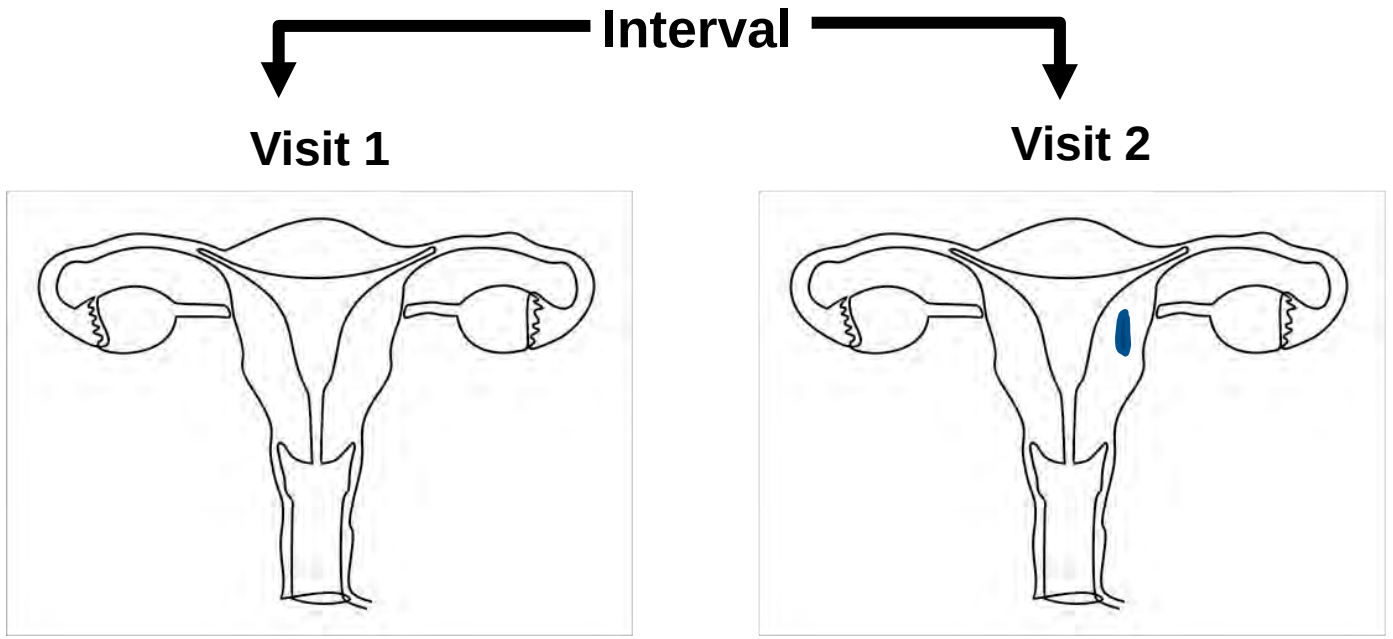
Composite Variable

Within 2 months & ≥4 months

↓

More exposed

Fibroid Incidence, n=1,121 participants



Eligible — No prior fibroid

Outcome — New fibroid case

Model — Cox regression, with age as time scale

Covariates

Model 1	Model 2	Model 3
Unadjusted	**Maternal and Early Life Factors**	**Time-varying Participant Factors**
§ Age as time scale	§ Mother's age at birth § Mother's education § Maternal pregnancy complications § Birth weight	§ BMI § Income § Smoking § Parity § Years since last birth § Years since last contraceptive injection

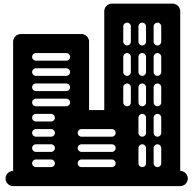
Demographics of SELF Cohort (n=1,610)

Mean age 29 Y (SD 3.4)

45% household income <$20,000

78% some college

60% had a birth



60% employed

13% fed soy formula

Image sources: Birthday cake, SBTS; Education, Imran Shaikh; Building, Ralf Schmitzer; Birth, David Khai.

Demographics by Soy Formula Feeding in Infancy*

Characteristic	Never Fed (n=971)	Ever Fed (n=150)
Participants' mothers:		
Age ≥30 years at participant's birth	19%	26%
≥4 years college	10%	17%
Participants at enrollment:		
Age 23-25 years	25%	33%
Current smoker	20%	13%
Household income <$20,000	46%	43%
Ever use of contraceptive injection	48%	42%

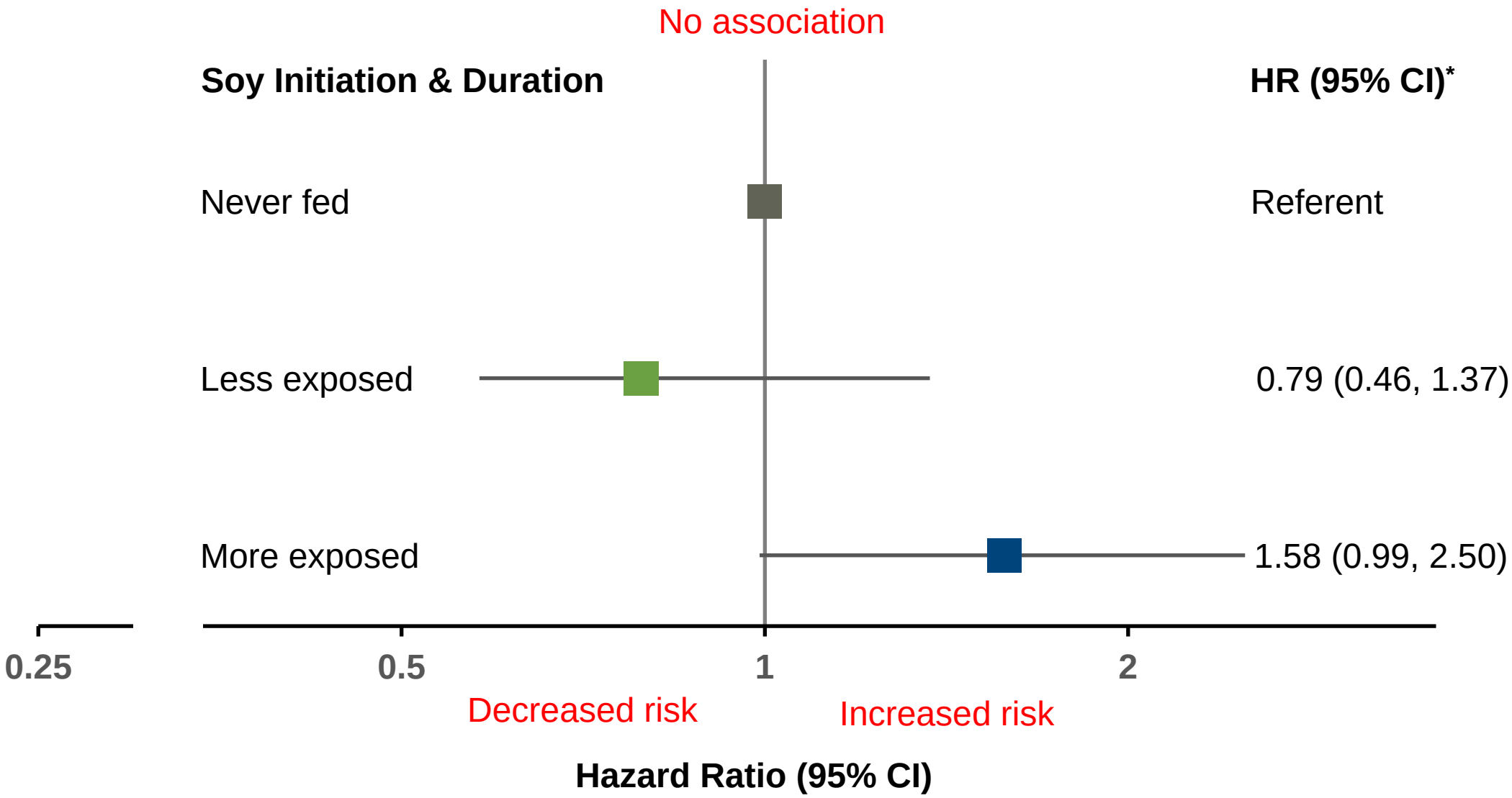
*Among 1,121 fibroid-free participants at enrollment.

Demographics by Soy Formula Feeding in Infancy*

Characteristic	Never Fed (n=971)	Ever Fed (n=150)
Participants' mothers:		
Age ≥30 years at participant's birth	19%	26%
≥4 years college	10%	17%
Participants at enrollment:		
Age 23-25 years	25%	33%
Current smoker	20%	13%
Household income <$20,000	46%	43%

*Among 1,121 fibroid-free participants at enrollment.

Risk of Incident Fibroids by Soy Formula Feeding

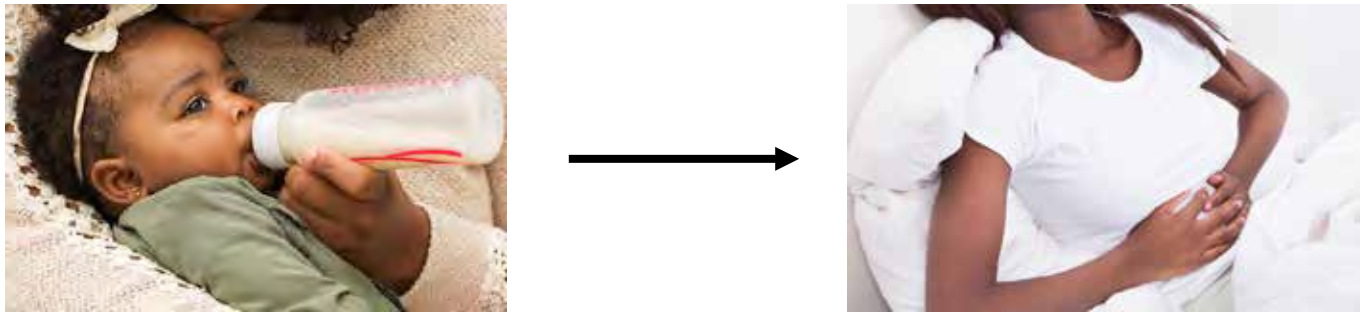


*Adjusted for age (time scale), maternal pregnancy complications, mother's age at participant's birth, mother's education, birth weight, and time-varying: BMI, income, smoking, parity, time since last birth, time since last contraceptive injection.

Conclusions

Soy-based infant formula and uterine fibroids

- **Increased risk** of ultrasound-identified incident fibroids in adulthood for those fed **soon after birth and for a longer duration**
- Consistent with prior animal and human studies
- Biological pathway is not established



Summary

- Prenatal/early life exposures affect reproductive tract and organs influencing health and disease later in life
- More research needed to understand mechanisms

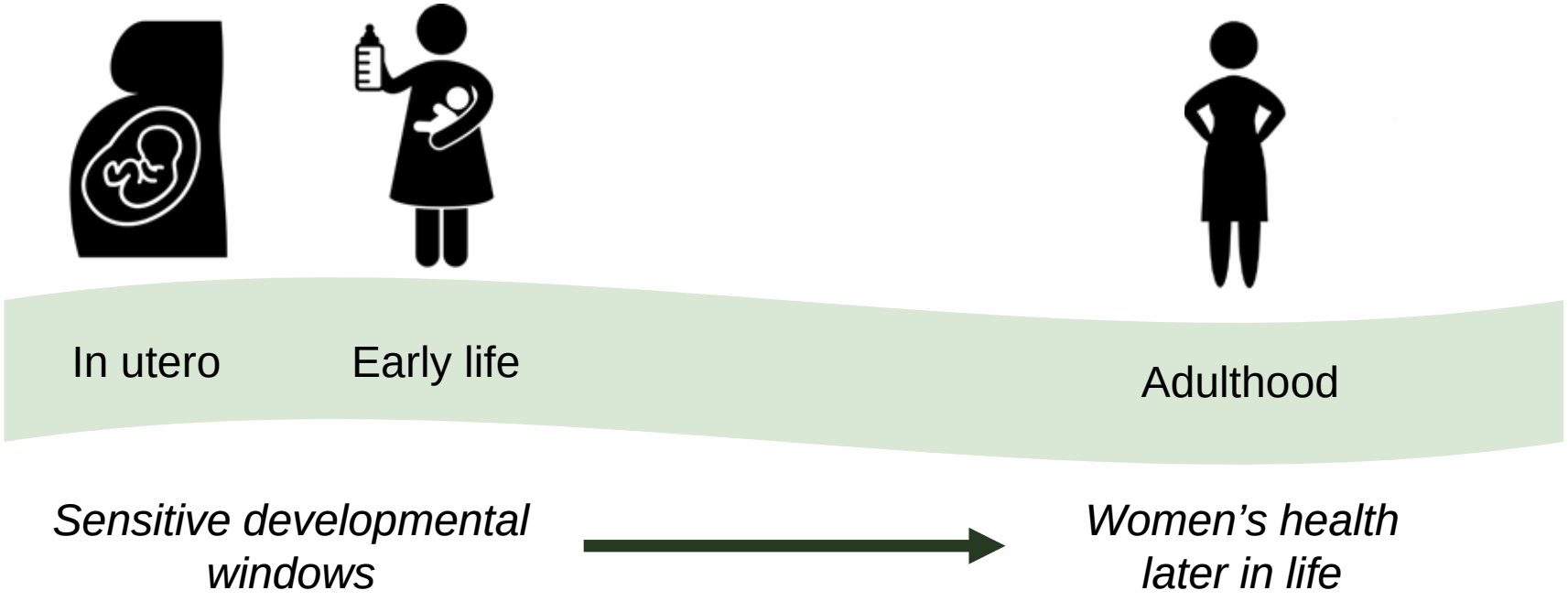


Image sources from Noun Project: Fetus, Lars Meiertoberens; Midwife, Gan Khoon Lay; Female aging, Marie Van den Broeck.

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Closing Remarks

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Thank you

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Join us tomorrow at 9am eastern for day 2 of the symposium.

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