The Effects of Phthalates on Female Reproduction

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Overview

- Background
  - Phthalates
  - Phthalate mixture
- Effects of prenatal phthalate exposure on the ovary, sex steroid hormone levels, and fertility in the F1, F2, and F3 generations
  - Hypotheses
  - Experimental design
  - Results
- Conclusions

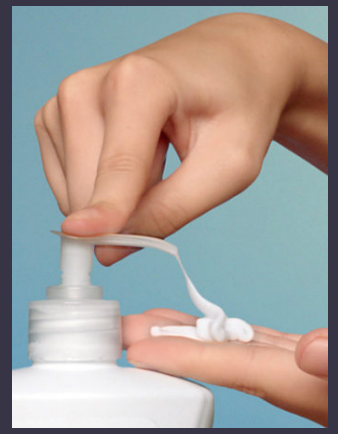
What are phthalates?

Phthalates

Commonly used as plasticizers and additives






Why the concern?

Phthalates

- Detected in human fluids and tissues
- High exposure in children and women
- Exposure estimates:
  - > 13 phthalates (NHANES)
  - Serum levels up to 450 ng/ml (single phthalate)
  - Up to 250 µg/kg bw/day (single phthalate)

Phthalates

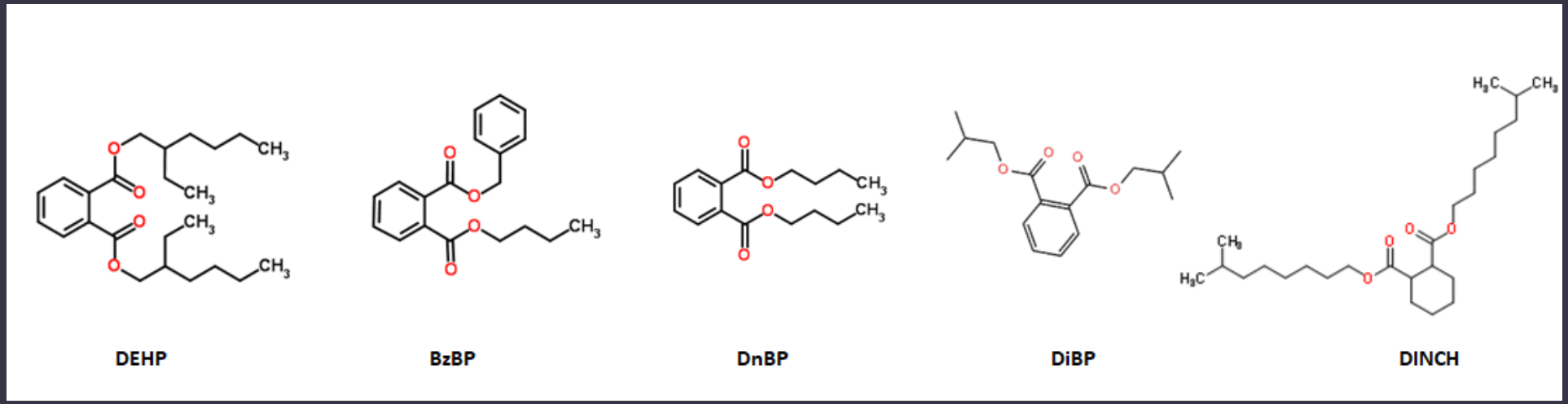
- Associated with human health risks
  - High blood pressure
  - Increased insulin resistance
  - Pregnancy loss
  - Preterm birth
  - Decreased sex steroid hormone levels
  - Fertility problems

Phthalates

- Cause adverse effects in animal models
  - Affect body weight
  - Disrupt development of reproductive organs
  - Disrupt puberty onset
  - Reduce fertility
  - Induce reproductive diseases

Single Phthalates vs. Phthalate Mixtures

- Previous studies focus on single phthalates
- Humans are exposed to phthalate mixtures



Phthalate Mixtures

- Limited information available
- Previous mixtures not environmentally relevant
  - Combinations of chemical classes
  - Very high doses
- Lack of information on female reproduction
- Lack of information on transgenerational effects

Phthalate Mixture

DEP: Diethyl Phthalate

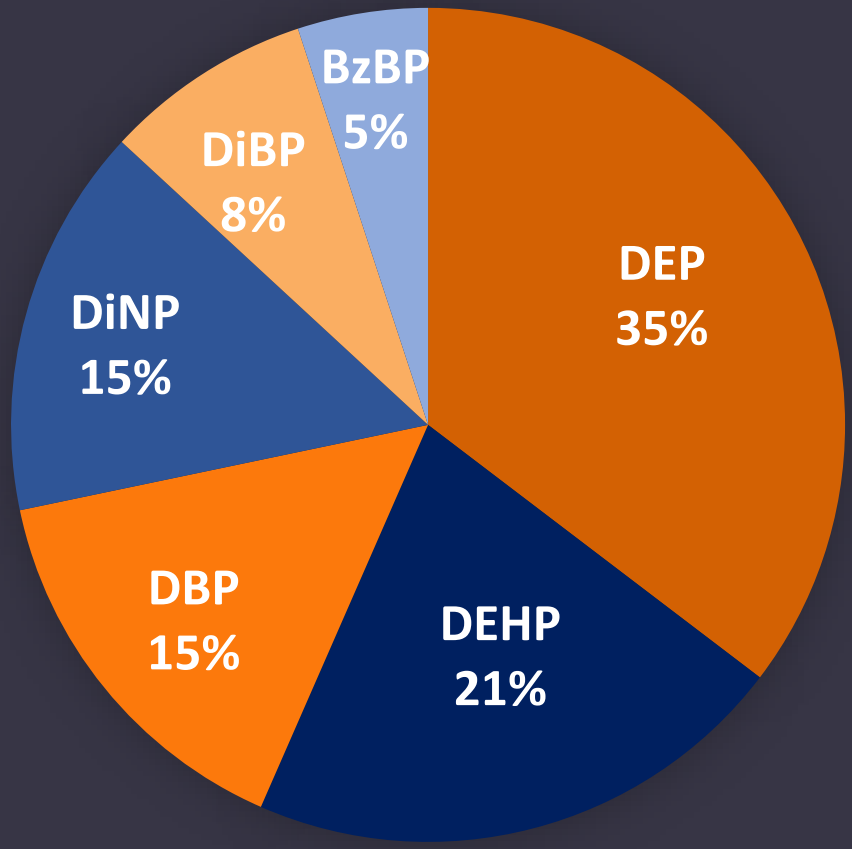
DEHP: Di(2-ethylhexyl) Phthalate

DBP: Dibutyl Phthalate

DiNP: Diisononyl Phthalate

DiBP: Diisobutyl Phthalate

BBzP: Benzylbutyl Phthalate



Based on levels detected in the iKids study

Phthalate Mixture

Phthalate	% of mixture	μg in 20 μg/kg dose	μg in 200 μg/kg dose	Median estimated μg/kg/day exposure in pregnant women (maximum)
Diethyl phthalate (DEP)	35	7.0	70	6.64 (1,263)
Dibutyl phthalate (DBP)	15	3.0	30	0.84 (5.86)
Diisobutyl phthalate (DiBP)	8	1.6	16	0.12 (2.90)
Benzylbutyl phthalates (BzBP)	5	1.0	10	0.50 (15.52)
Di(2-ethylhexyl) phthalate (DEHP)	21	4.2	42	1.7 (144)
Diisononyl phthalate (DiNP)	15	3.0	30	Information not available

What are the effects of the mixture on the ovary?

Functions of the Ovary (Antral Follicle)

Ovulation (oocytes)
- Fertility

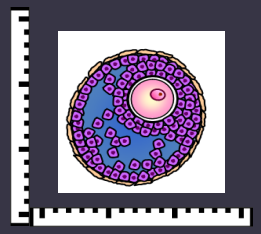
Synthesize and secrete sex steroid hormones
- Maintenance of the reproductive tract
- Development of ova
- Implantation
- Menstrual/estrous cyclicity
- Fertility
- Female health (cardiovascular, brain, bones)

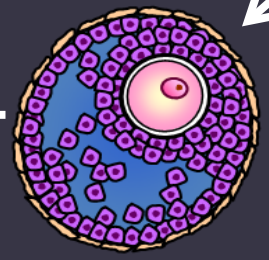
Hypothesis

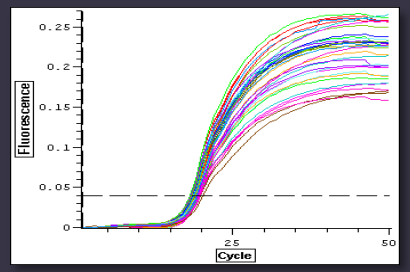
Phthalate mixture exposure decreases antral follicle growth, induces atresia, and compromises steroidogenic capacity

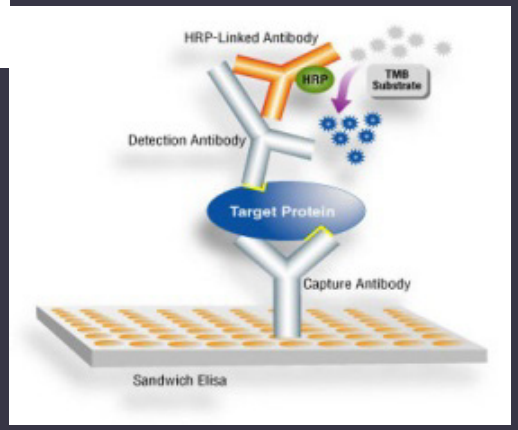
Experimental Design

- Vehicle (DMSO)
- Mixture (1 - 500 µg/ml)



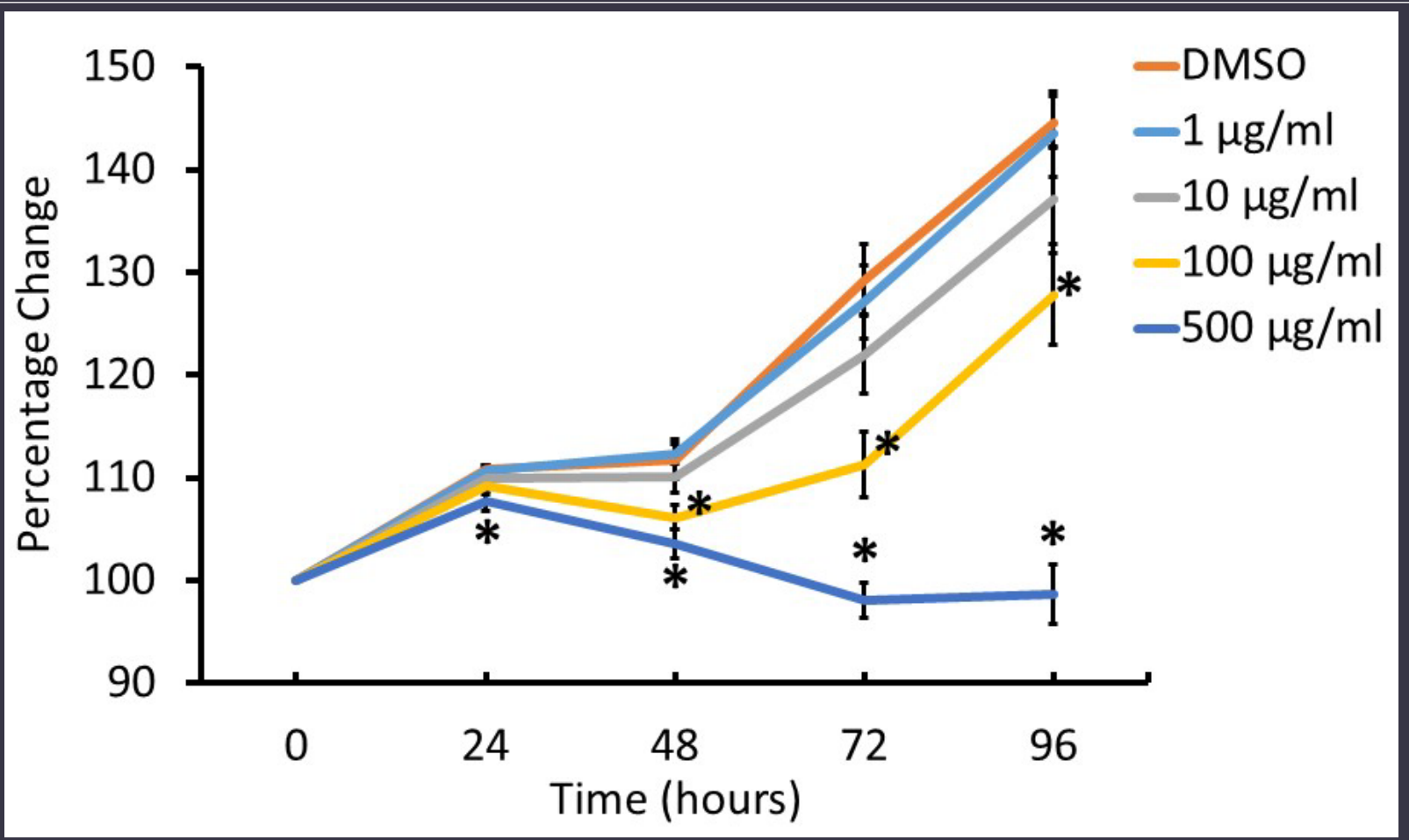






Apoptosis, cell cycle, steroidogenic enzymes

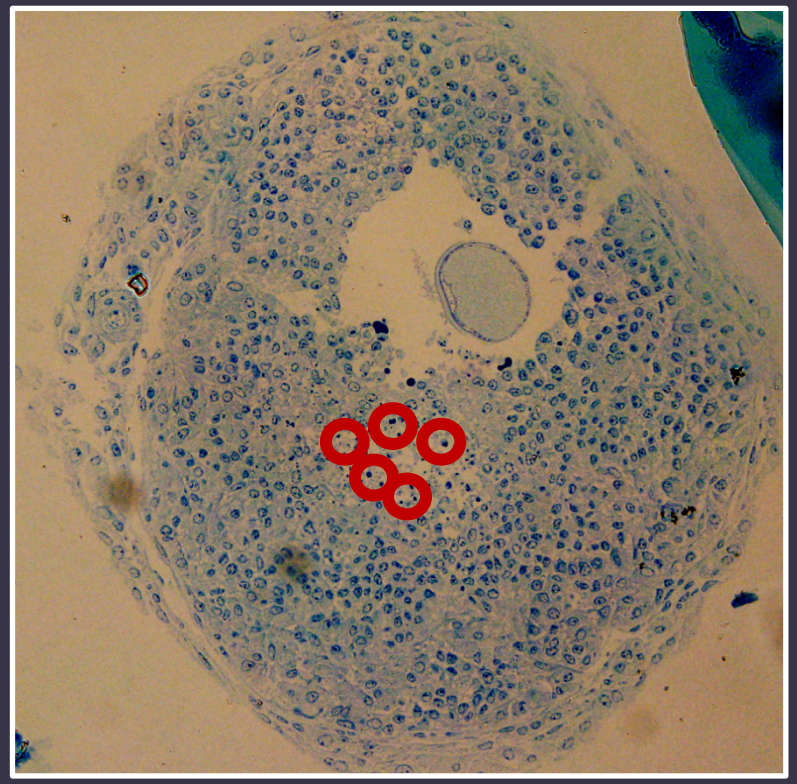
Effect of the Mixture on Follicle Growth



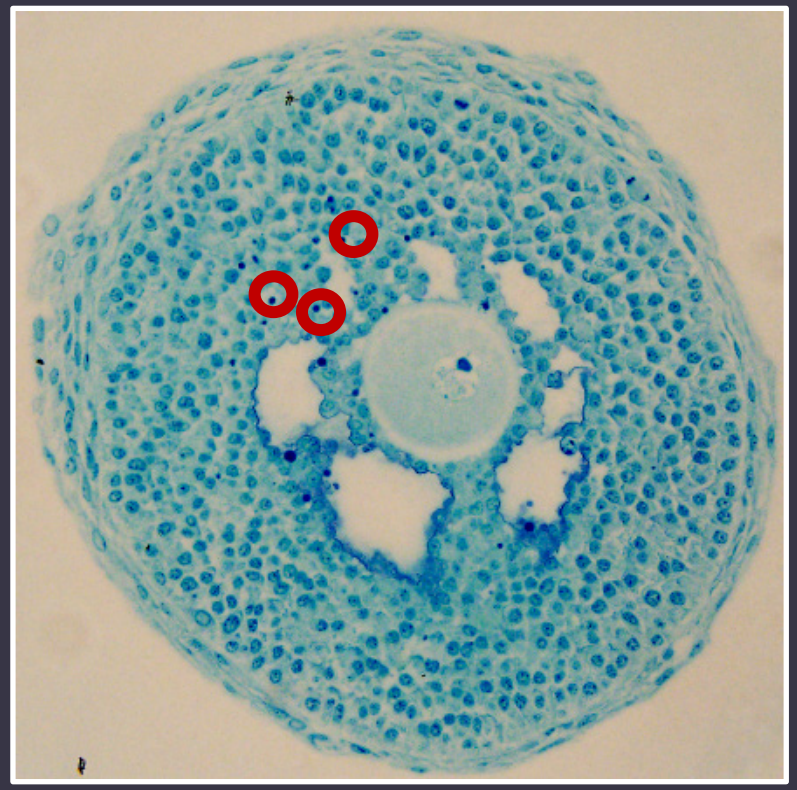
n = 3-4 cultures, with 6-12 follicles/treatment/culture, *p < 0.05

Effect of the Mixture on Atresia

Control

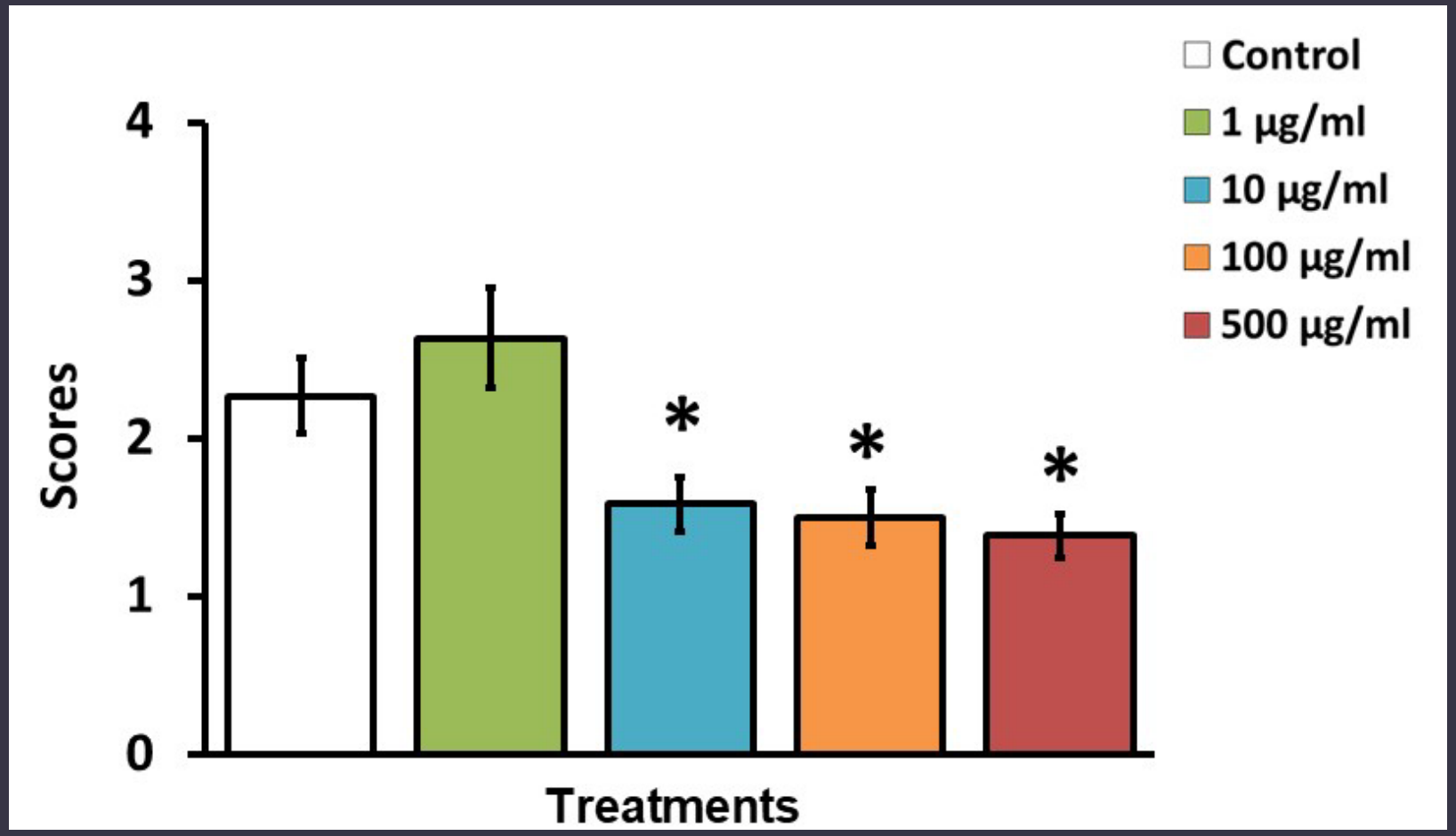


10 µg/ml



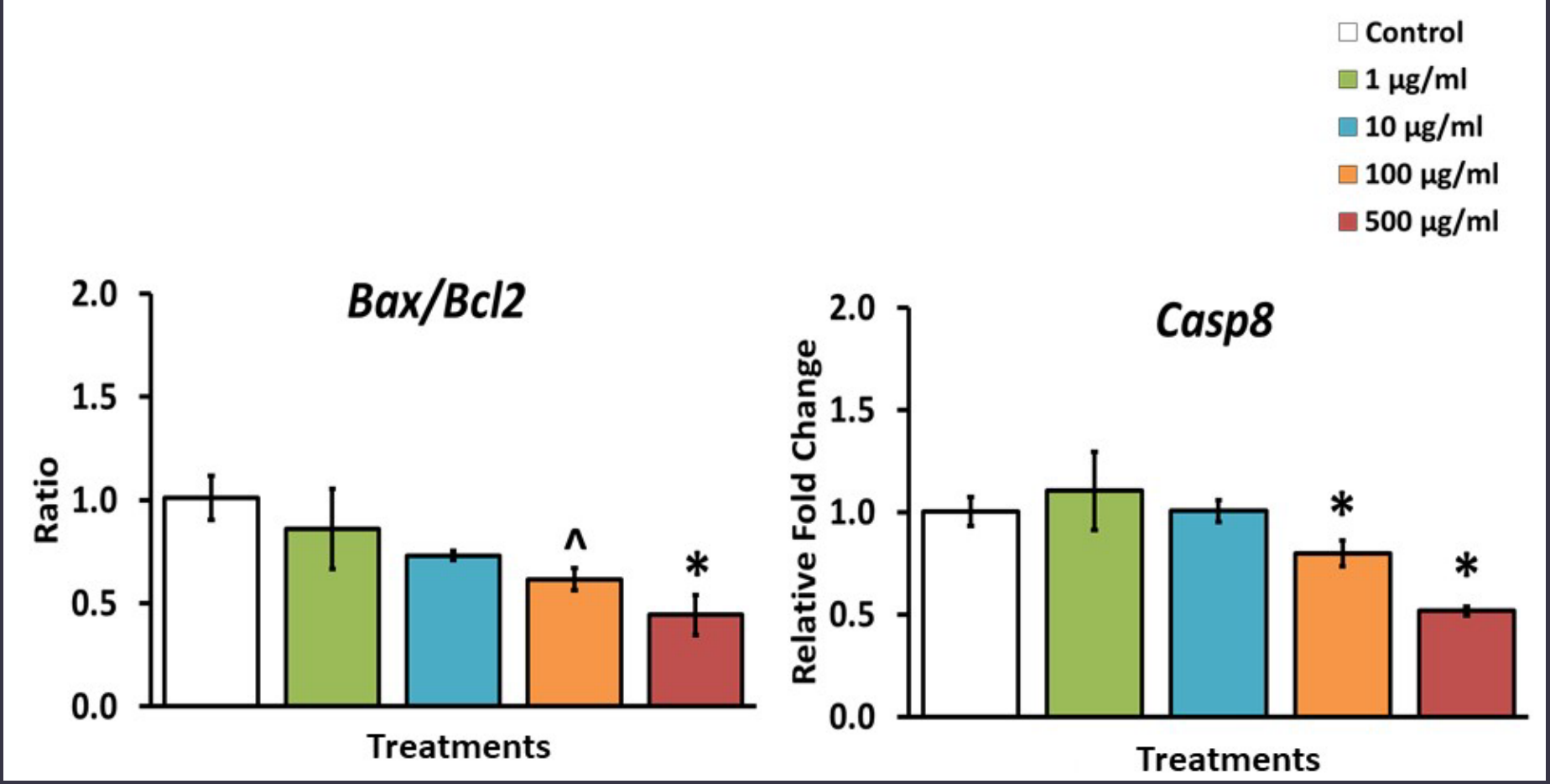
Atresia scores 1: < 3%; 2: 4-10%; 3: 11-30%; 4: >30%

Effect of the Mixture on Atresia Scores



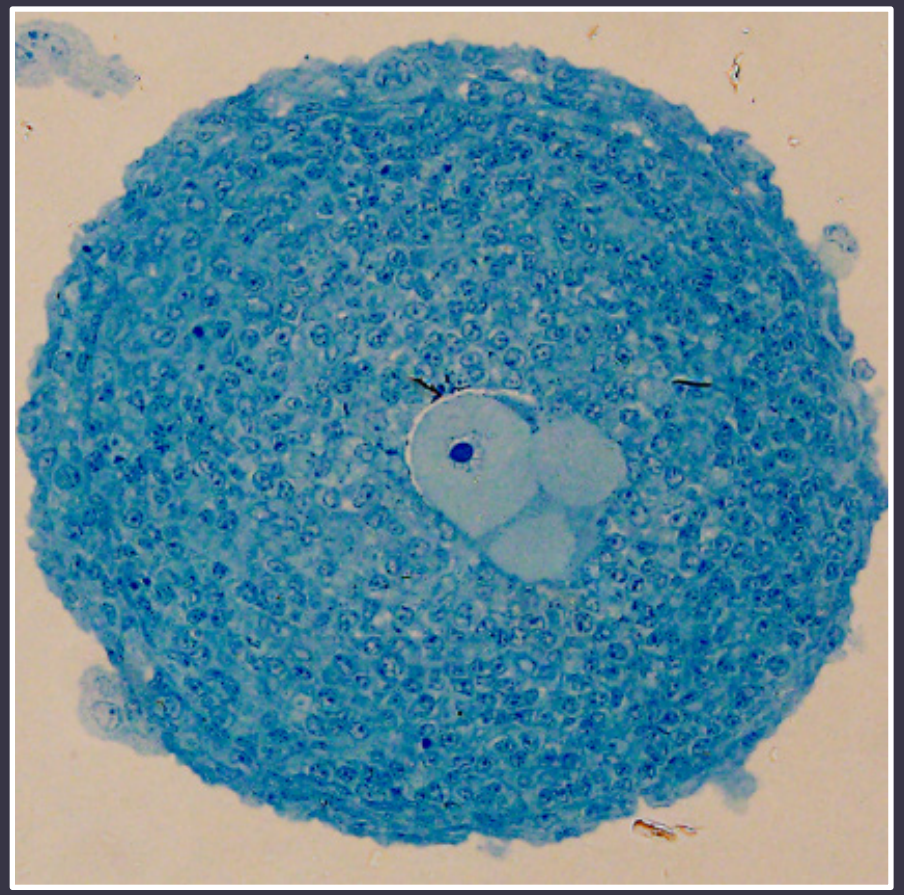
n = 3 follicles/treatment/culture, *p < 0.05

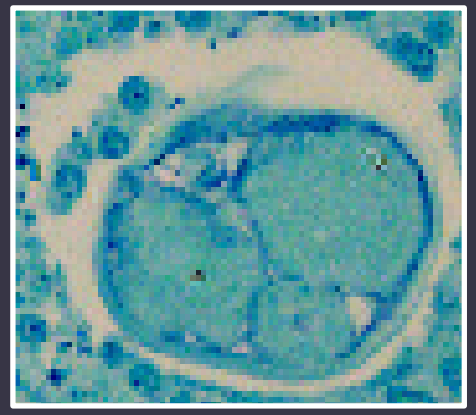
Effect of the Mixture on Apoptotic Gene Expression

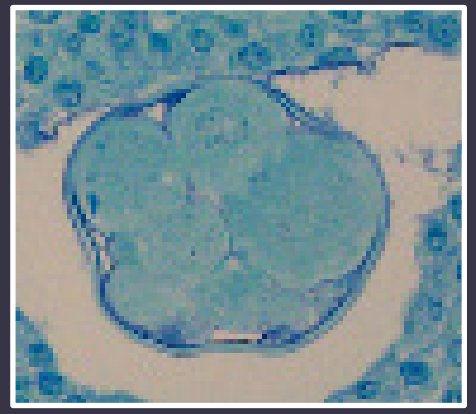


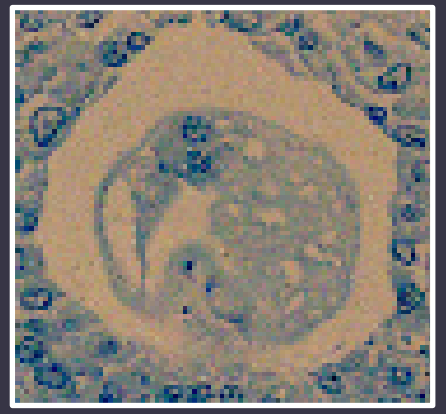
n = 3-4 cultures, with 6-12 follicles/treatment/culture, *p < 0.05

Effect of the Mixture on the Oocyte

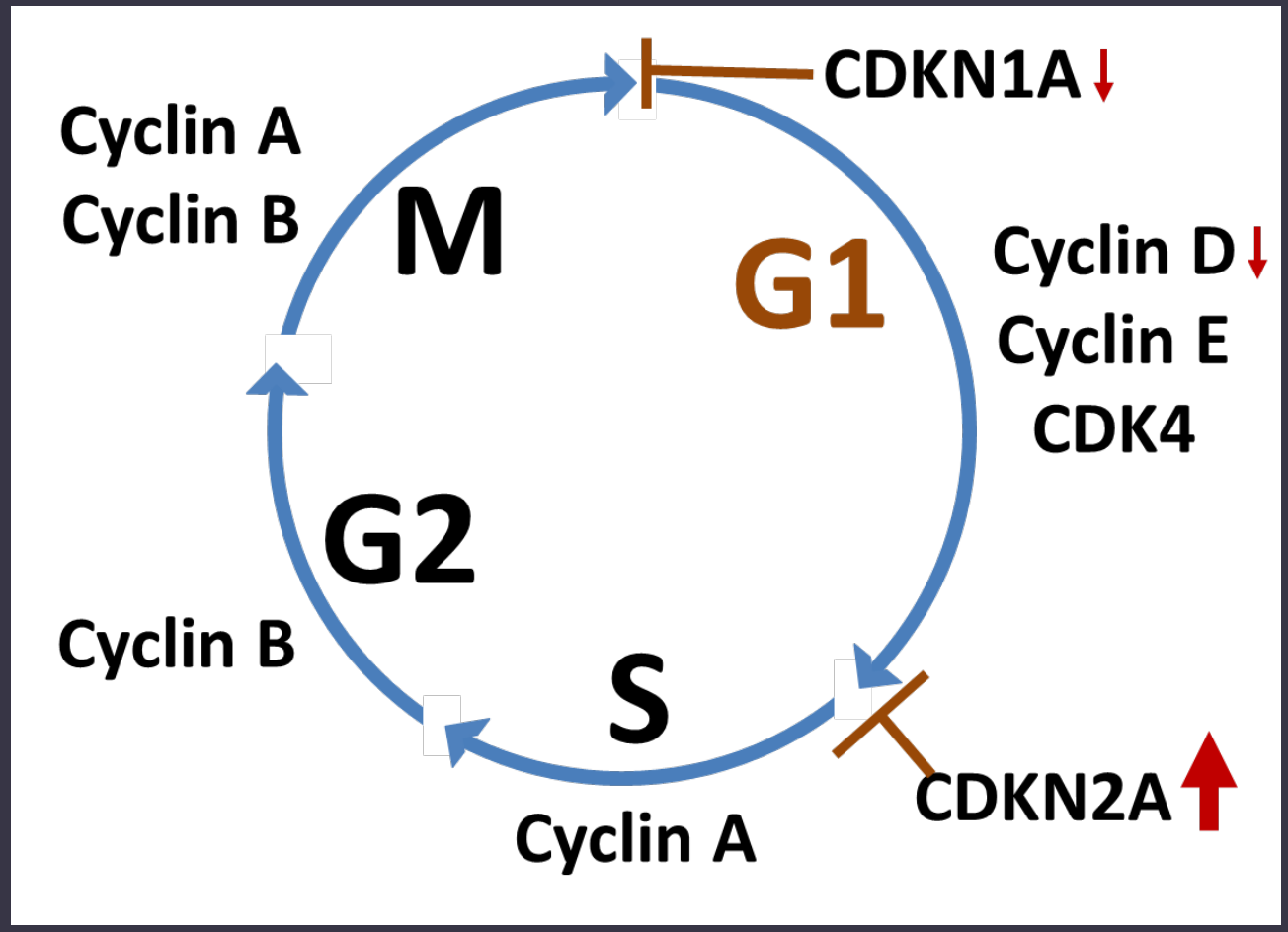




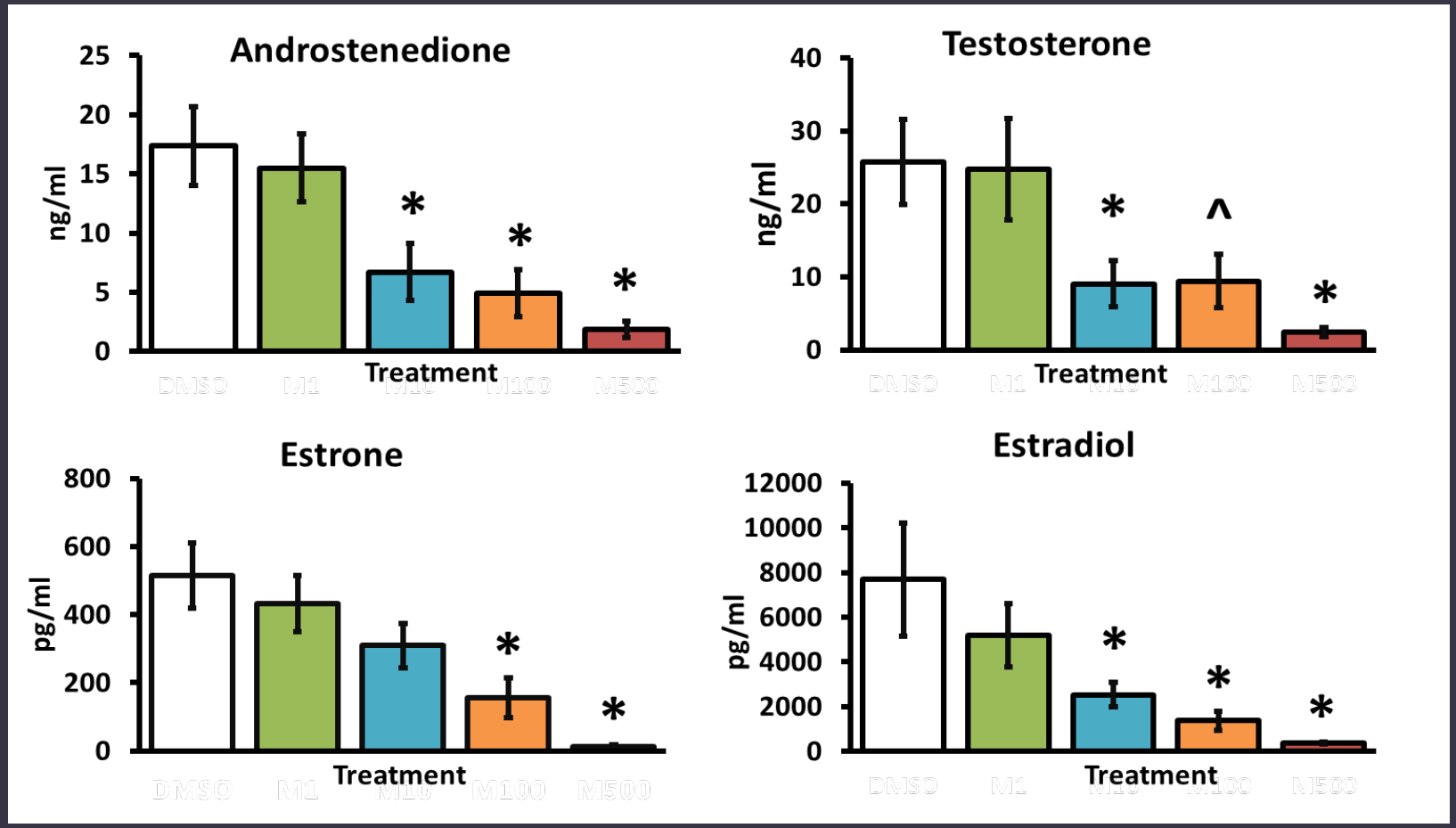




Effect of the Mixture on the Cell Cycle

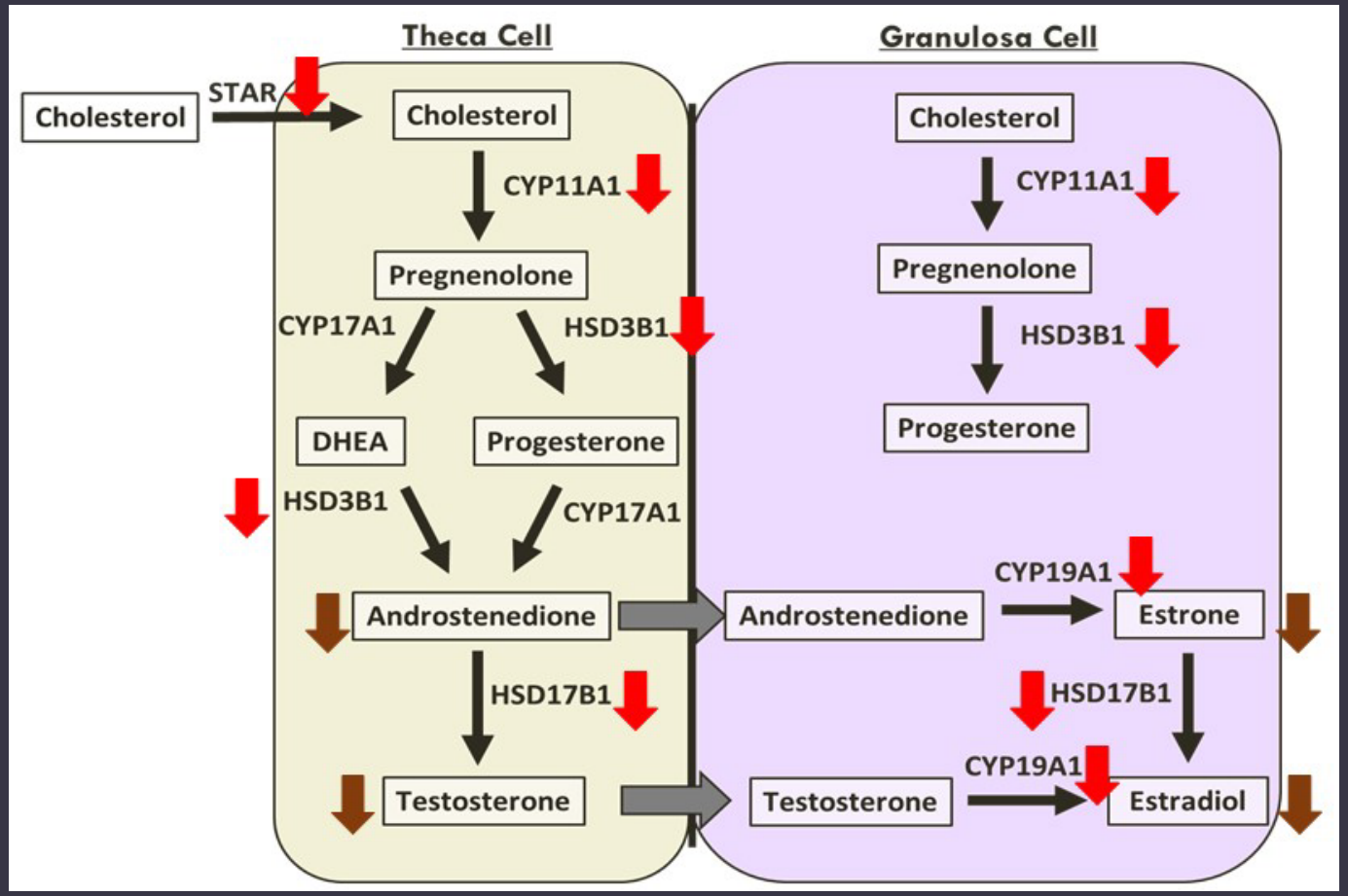


Effects of the Mixture on Steroidogenesis

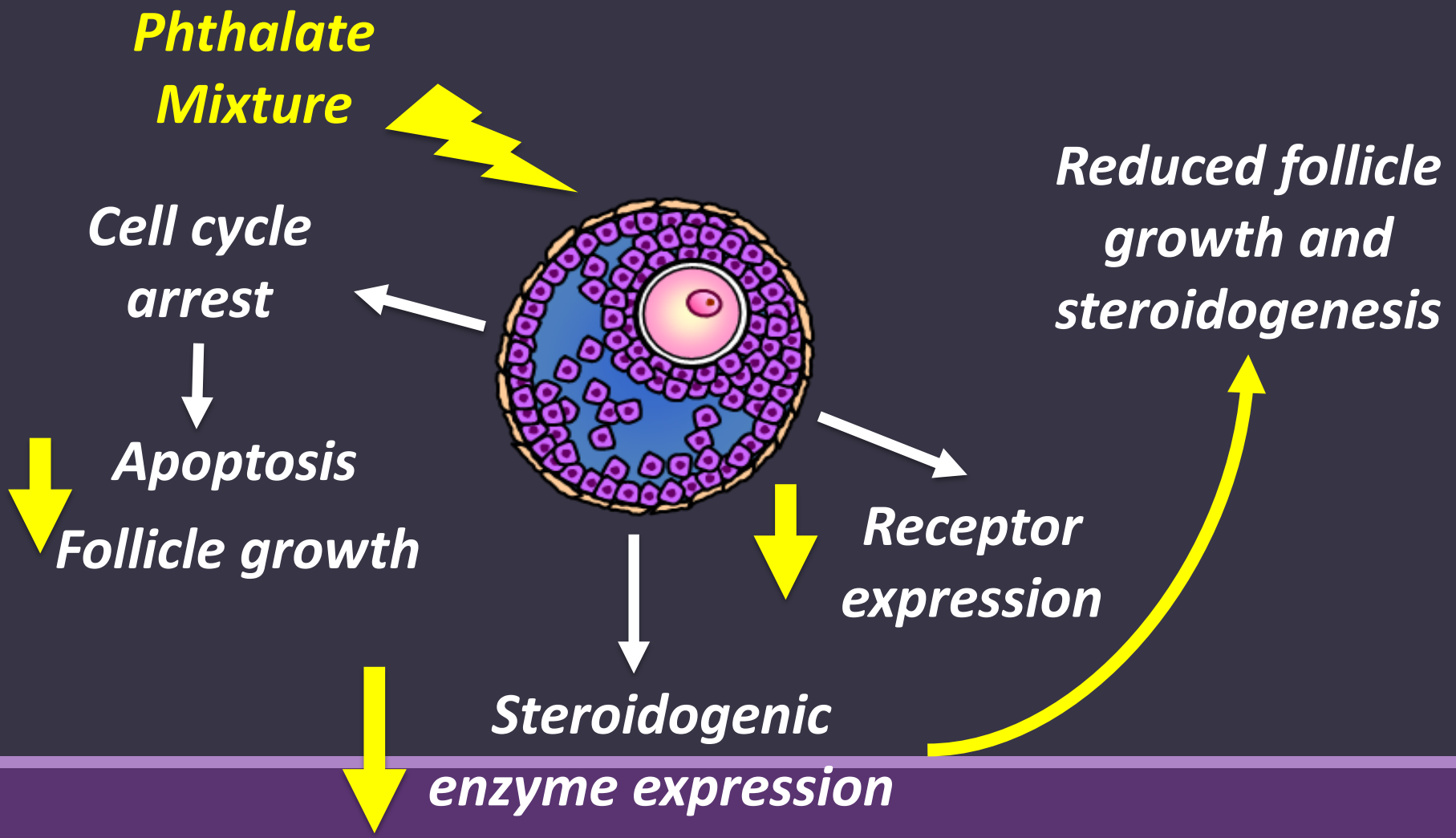


n= 3-5 cultures; ^p<0.1; *p < 0.05

Effects of the Mixture on Steroidogenesis



Summary of the In Vitro Results

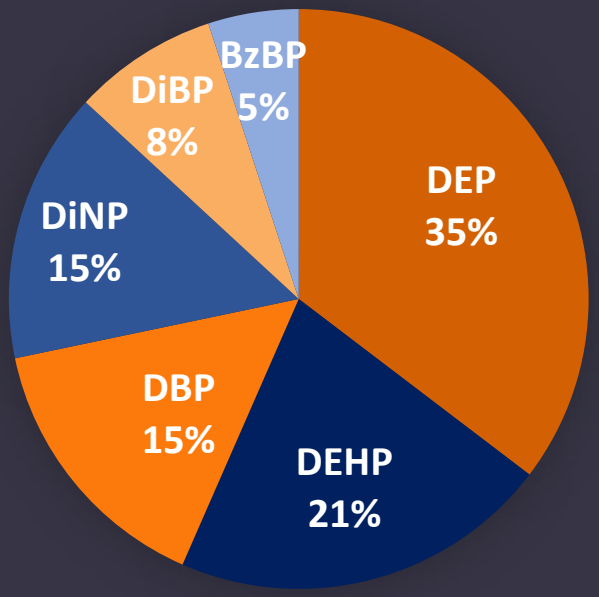


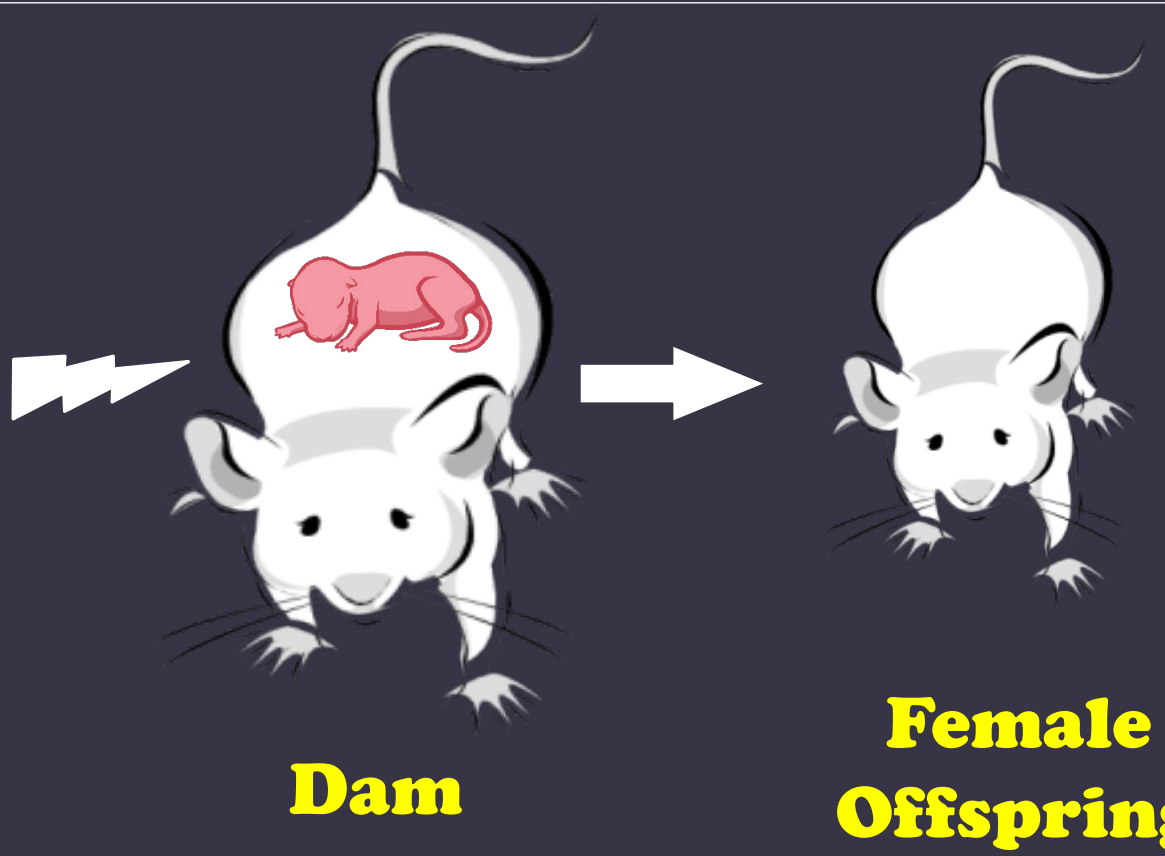
What are the effects of the mixture on female reproductive outcomes in vivo?

Hypothesis

Prenatal exposure to an environmentally relevant phthalate mixture adversely affects female reproduction in mice

Experimental Design





Dam

Female Offspring

Experimental Design

Oral dosing

Control: Tocopherol stripped corn oil

Mixture: 20 µg/kg/day
200 µg/kg/day
200 mg/kg/day
500 mg/kg/day

Tissue collection

PND 1, 4, 8, 21, 60, and 13 M:
- Body weights
- Tissue weights
- Anogenital distance (AGD)
- Sera

PND 21:
- Vaginal opening
- Estrous cyclicity

Fertility tests

- Estrous cyclicity
- Fertility indices
- Birth outcomes

Effects of Prenatal Exposure to the Mixture on F1 Females

- ↓ Anogenital distance (AGD)
- ↑ Uterine weights
- No changes in body and liver weights

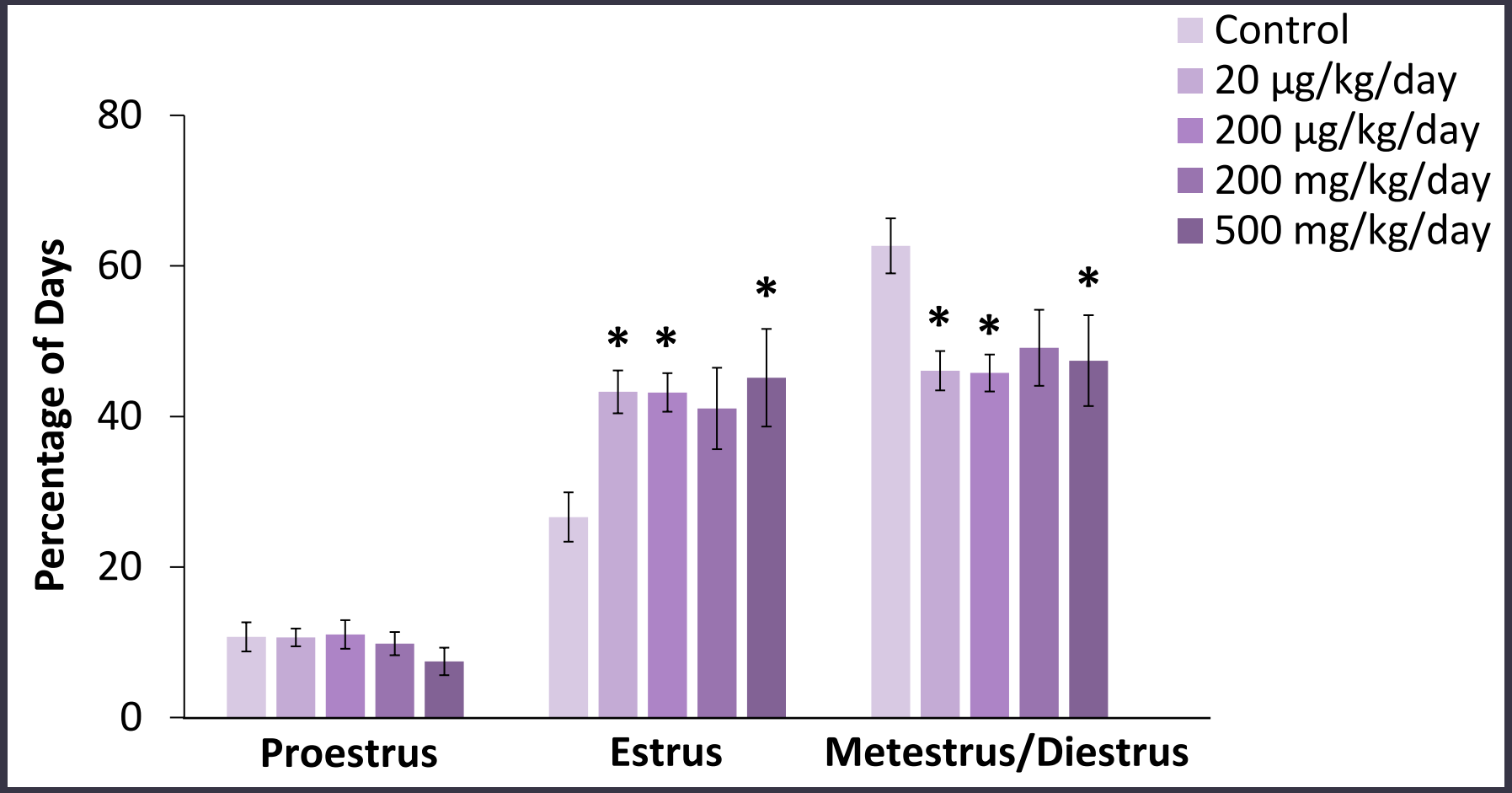
n = 11-12 dams/treatment group

Effects of Prenatal Exposure to the Mixture on F1 Ovaries

Treatment	% Females with Cystic Ovaries
Control	0
20 µg/kg/day	56 *
200 µg/kg/day	78 *
200 mg/kg/day	50 *
500 mg/kg/day	22

n = 6-11 females/treatment group, *p < 0.05

Effects of Prenatal Exposure to the Mixture on F1 Cyclicity



n = 6-12 dams/treatment, * $p < 0.05$

Fertility Related Indices

Mating index = # females with sperm plugs / # breeding females× 100

Fertility index = # pregnant females / # females with sperm plugs × 100

Gestational index = # females who delivered / # pregnant females × 100

% Produced live pups = # females delivered live pups / # breeding females

Effects of Prenatal Exposure to the Mixture on F1 Indices

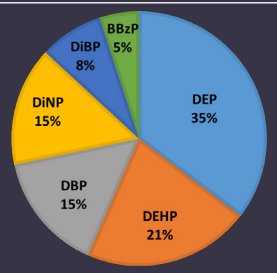
- No significant differences in:
  - Mating index
  - Fertility index
  - Gestational index
  - Percent of females that produced live pups

Effects of Prenatal Exposure to the Mixture on F1 Birth Outcomes

- ↓ Litter size
- No effects on average pup birth weight
- No effects on sex ratio

n = 7-12 dams/treatment

Summary of F1 Results



Female Offspring

Decreased AGD

Increased uterine weight

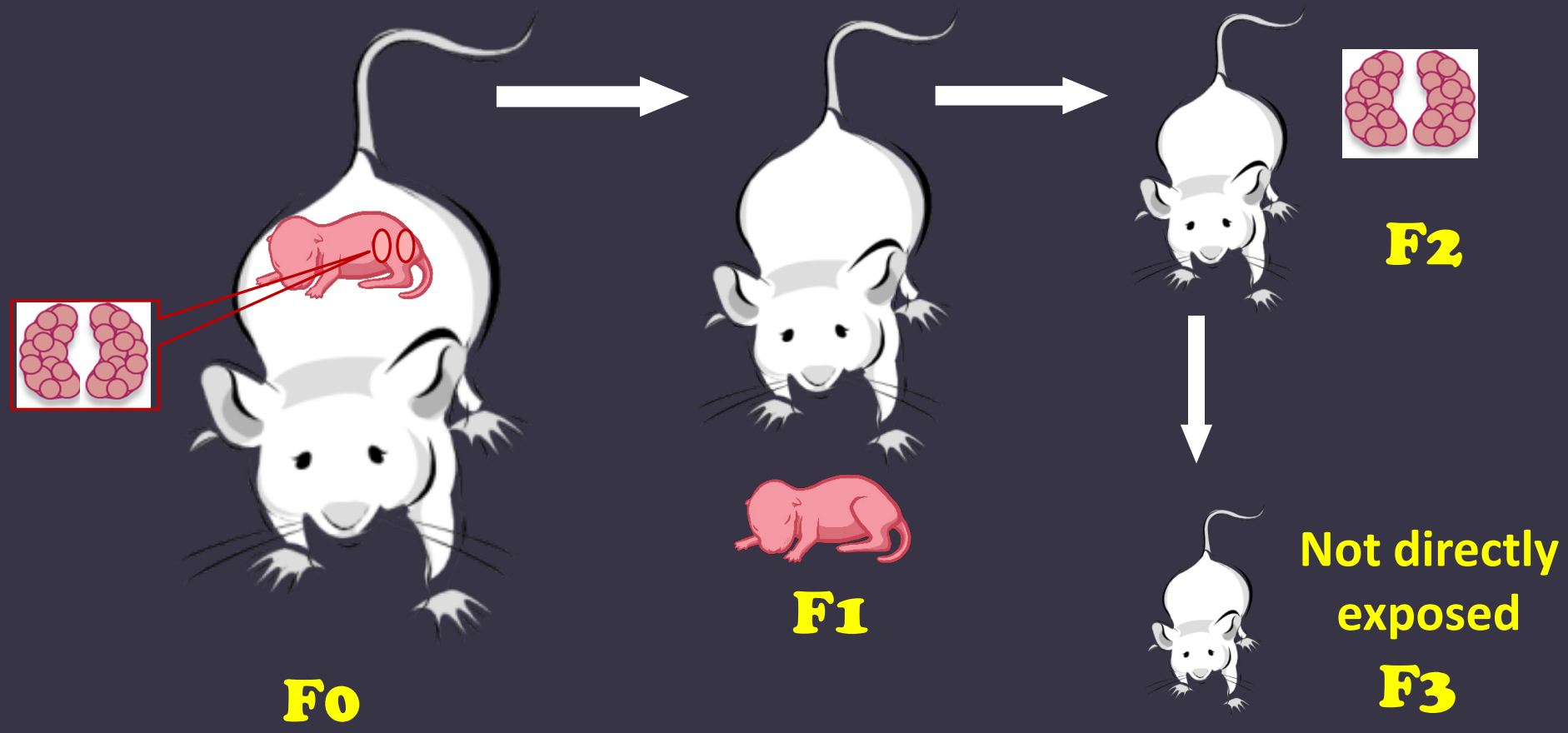
Induced cystic ovaries

Disrupted estrous cyclicity

Reduced litter size

Does the phthalate mixture cause multigenerational or transgenerational effects?

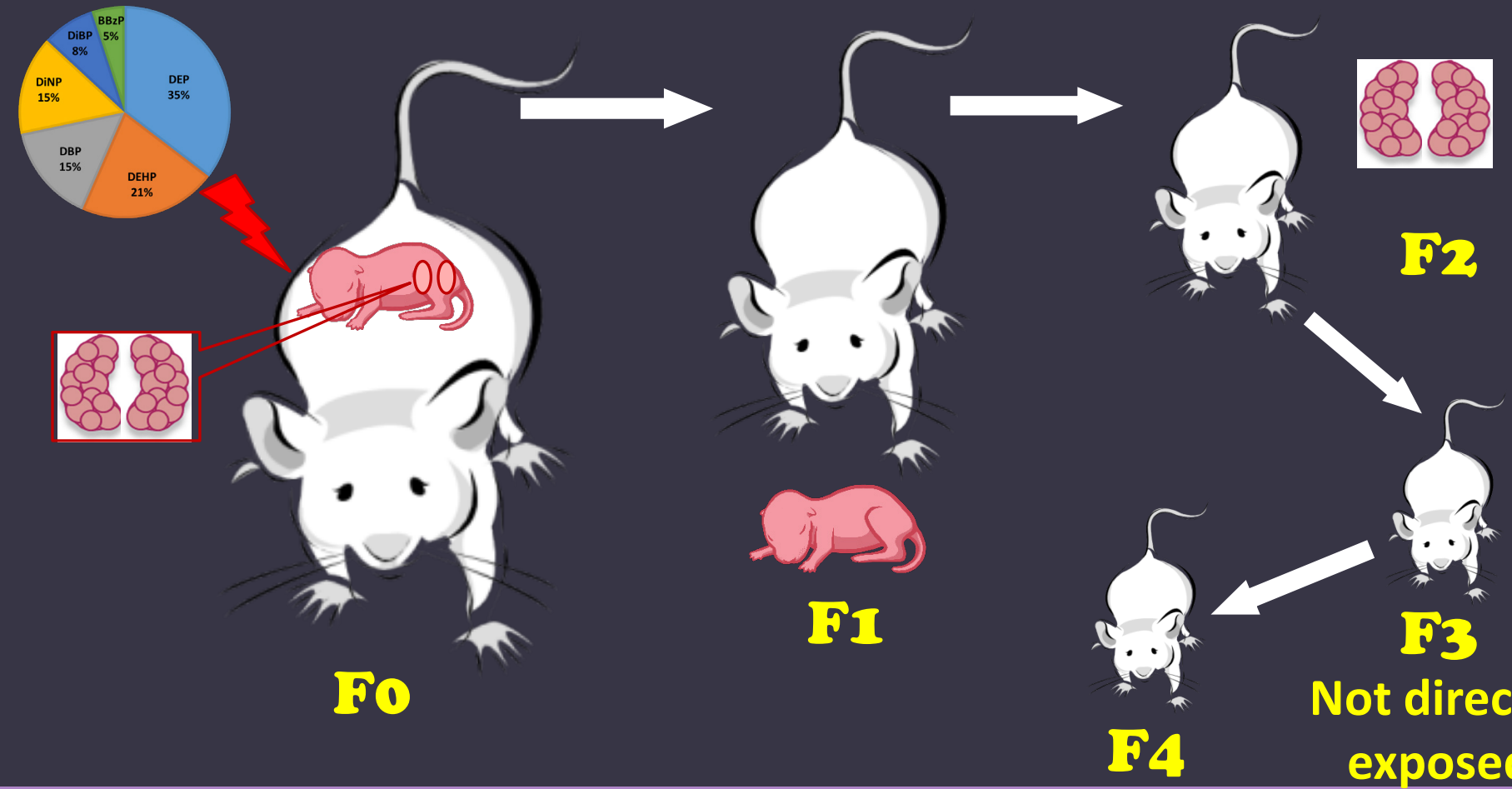
Multigenerational and Transgenerational Effects



Hypothesis

Prenatal exposure to an environmentally relevant phthalate mixture induces multigenerational or transgenerational effects on female reproduction in mice

Experimental Design



Effects of Prenatal Exposure to the Mixture on F2 Females

The mixture:

- ↑ Body weight
- ↑ Uterine weight
- ↑ Cystic ovaries

n = 6-12 dams/treatment

Effects of Prenatal Exposure to the Mixture on F2 Female Fertility

The mixture:

- ↑ Time to pregnancy
- No effects on fertility related indices
- No effects on litter size, pup weight, or sex ratio

n = 6-12 dams/treatment

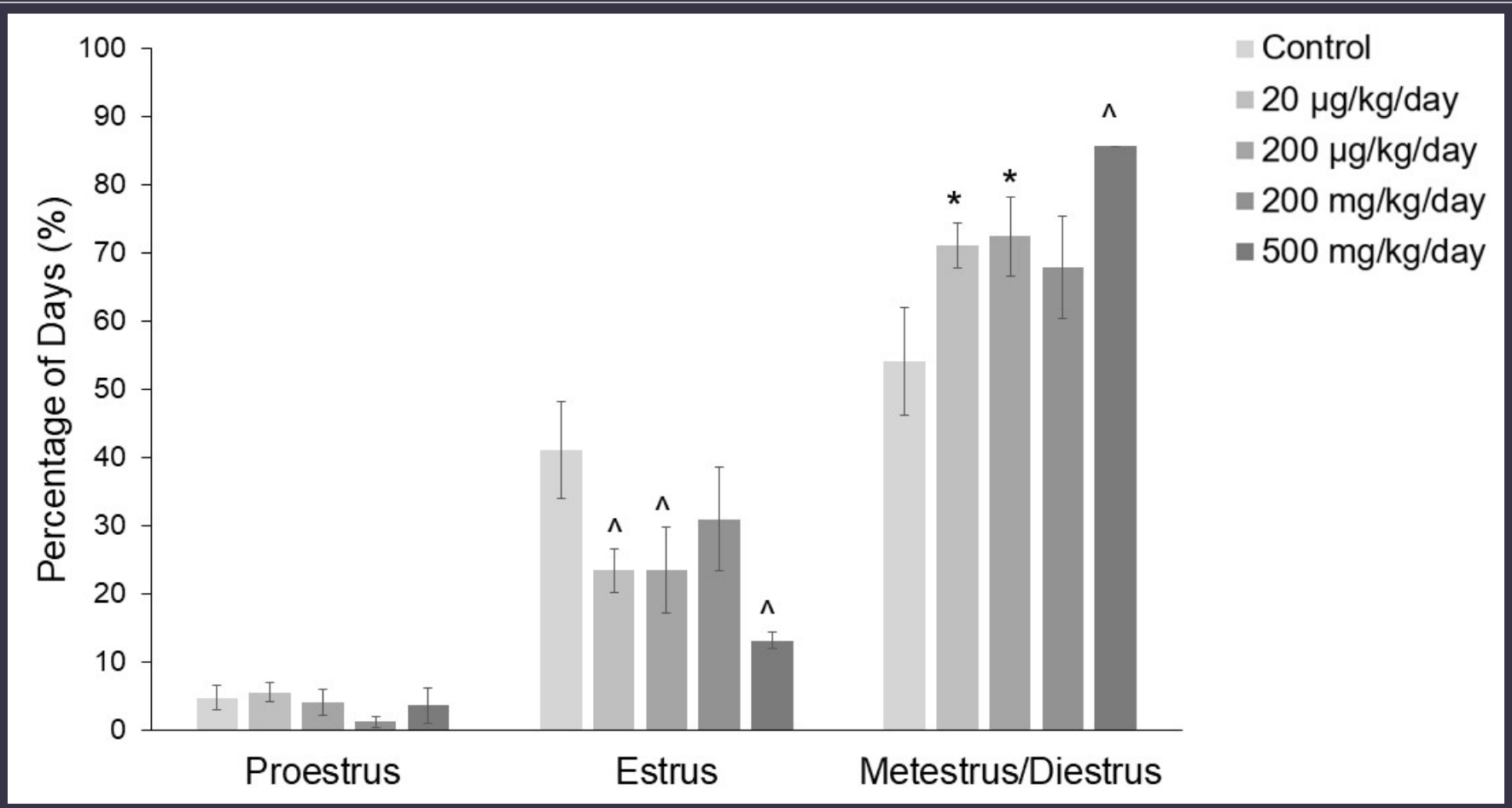
Effects of Prenatal Exposure to the Mixture on F3 Females

The mixture:

- ↑ Body weight
- ↓ AGD
- ↑ Uterine weight
- ↑ Cystic ovaries

n = 6-12 dams/treatment

Effects of Prenatal Exposure to the Mixture on F3 Estrous Cyclicity



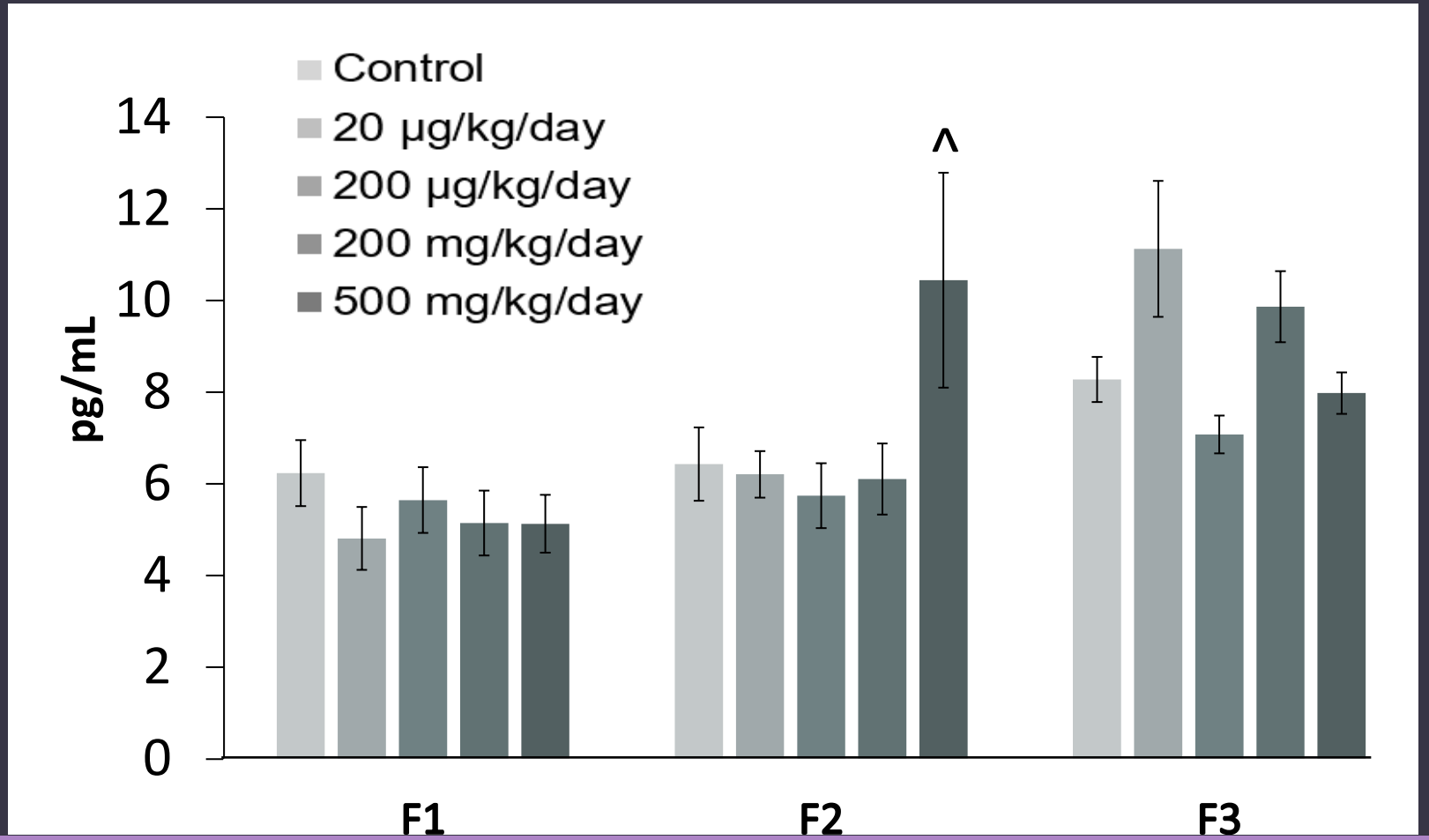
n = 4=11; * $p \leq 0.05$, ^ = $0.05 < p < 0.1$

Effects of Prenatal Exposure to the Mixture on F3 Female Fertility

Treatment	Total female	Mating index	Fertility index	Gestational index	% Produced Live Pups
Control	8	100	100	100	100
20 µg/kg/day	10	100	100	100	100
200 µg/kg/day	8	100	88	100	88
200 mg/kg/day	10	90	100	89	80
500 mg/kg/day	5	100	80	100	**40 ***

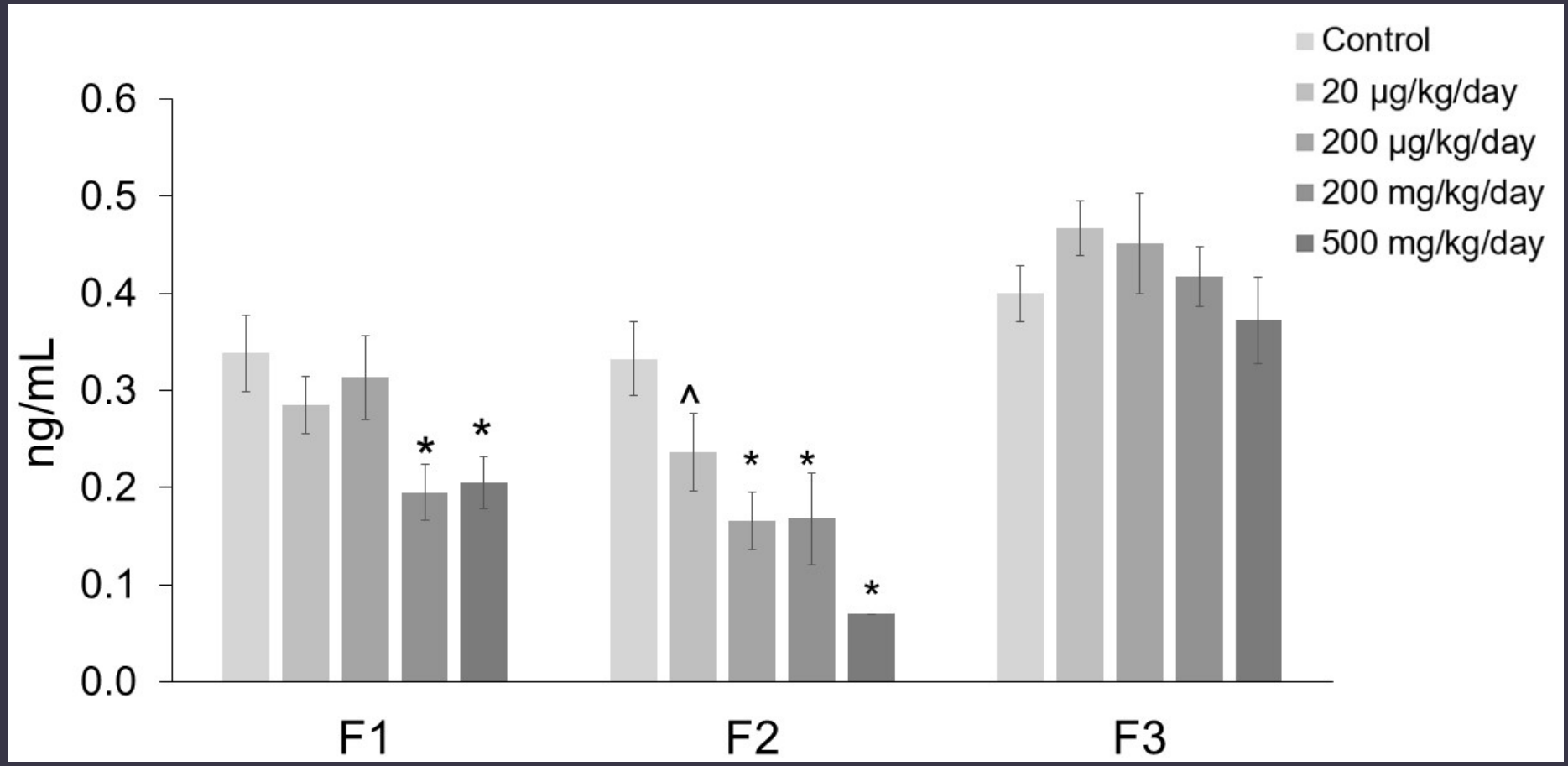
n = 4=11; * $p \leq 0.05$

Effects of Prenatal Exposure to the Mixture on Estradiol Levels in the F1, F2, and F3 Generations



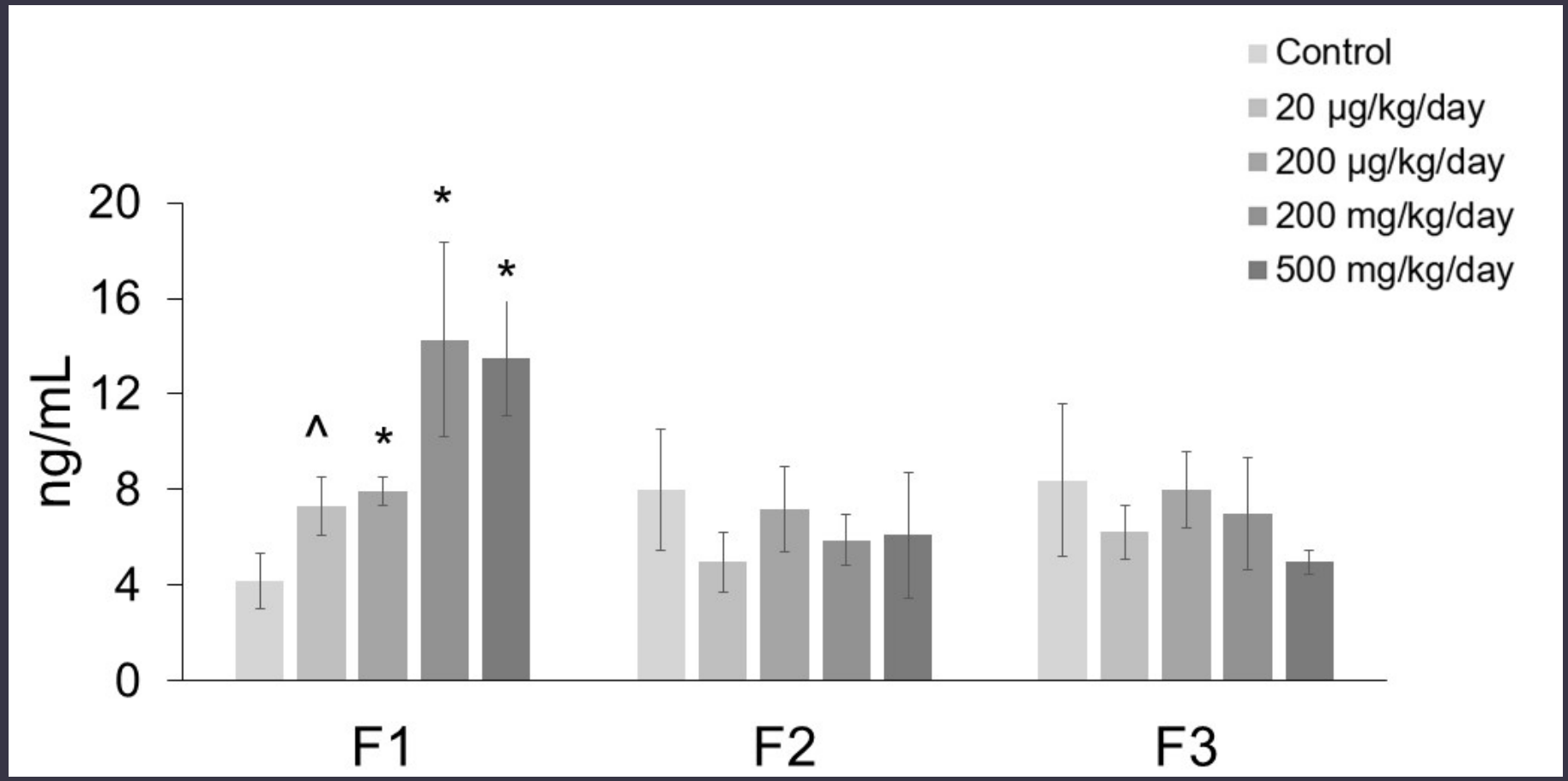
n = 4=11; ^ = 0.05 < p < 0.1

Effects of Prenatal Exposure to the Mixture on Testosterone Levels in the F1, F2, and F3 Generations



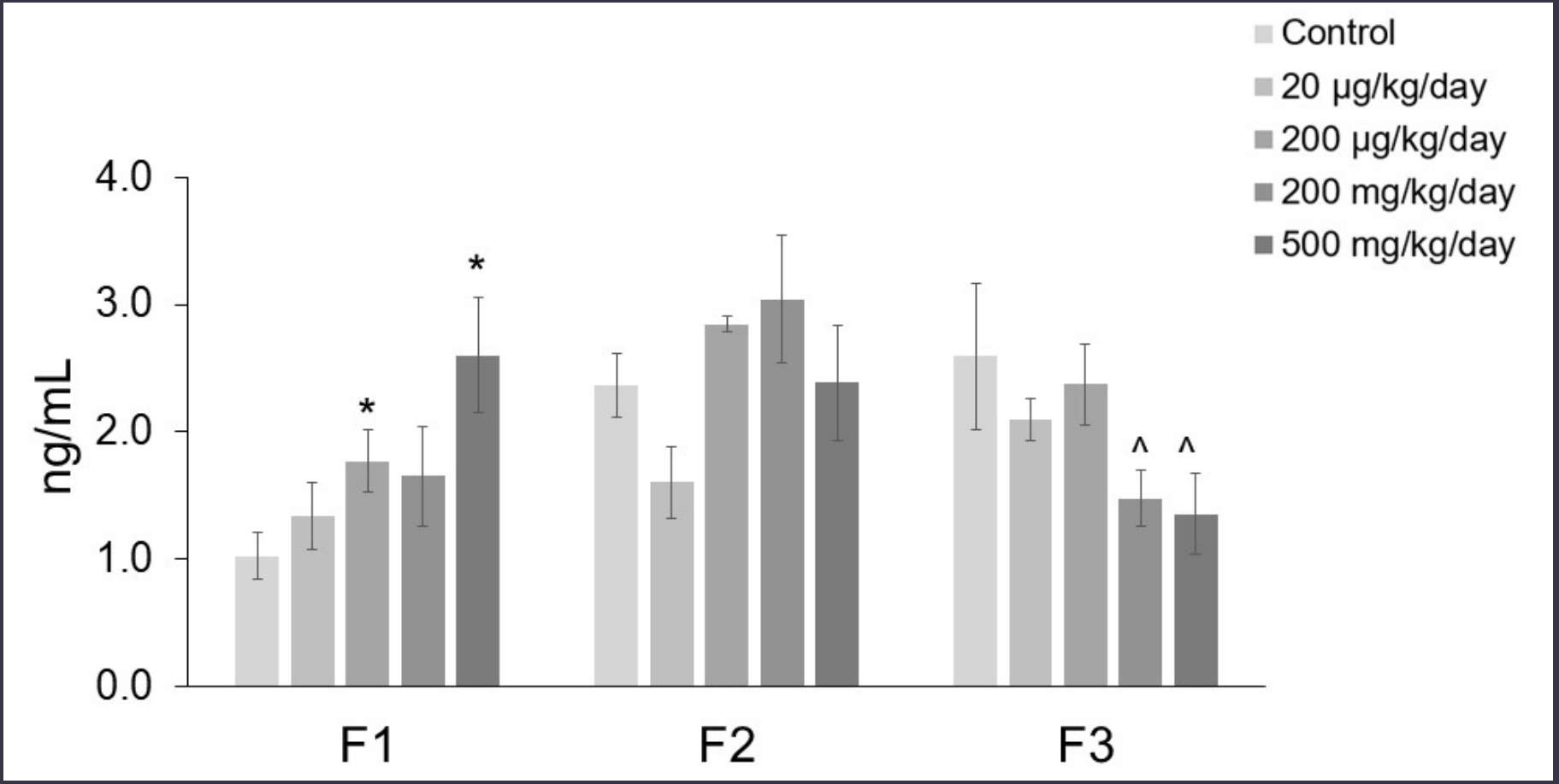
n = 4=11; * p ≤ 0.05, ^ = 0.05 < p < 0.1

Effects of Prenatal Exposure to the Mixture on FSH Levels in the F1, F2, and F3 Generations



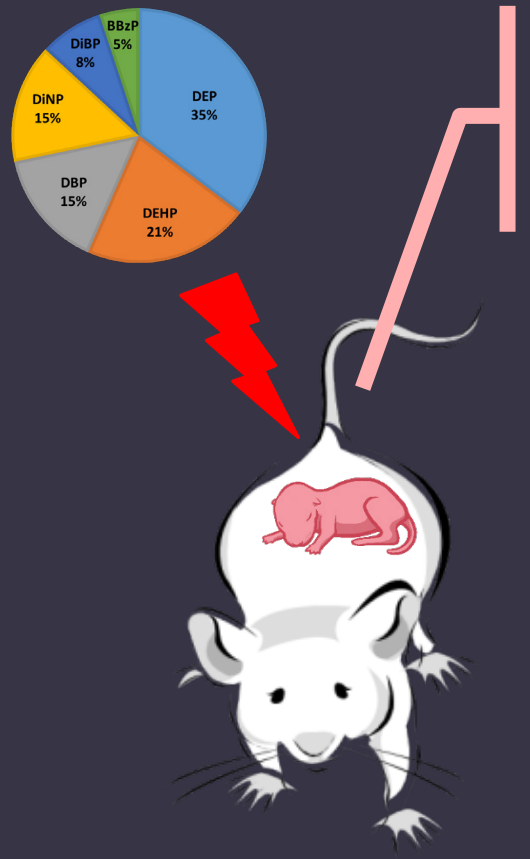
n = 4=11; * p ≤ 0.05, ^ = 0.05 < p < 0.1

Effects of Prenatal Exposure to the Mixture on LH Levels in the F1, F2, and F3 Generations



n = 4=11; * p ≤ 0.05, ^ = 0.05 < p < 0.1

Summary



Female Offspring
F2 ( ) and F3 (not exposed)

Increased F2 and F3 body weight

Decreased F2 and F3 AGD

Increased F2 and F3 uterine weight

Increased F2 and F3 cystic ovaries

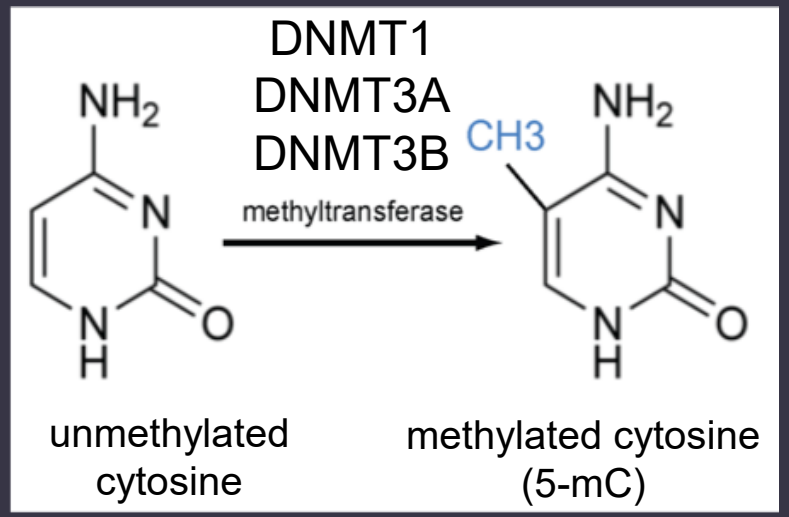
Reduced F3 fertility-related indices

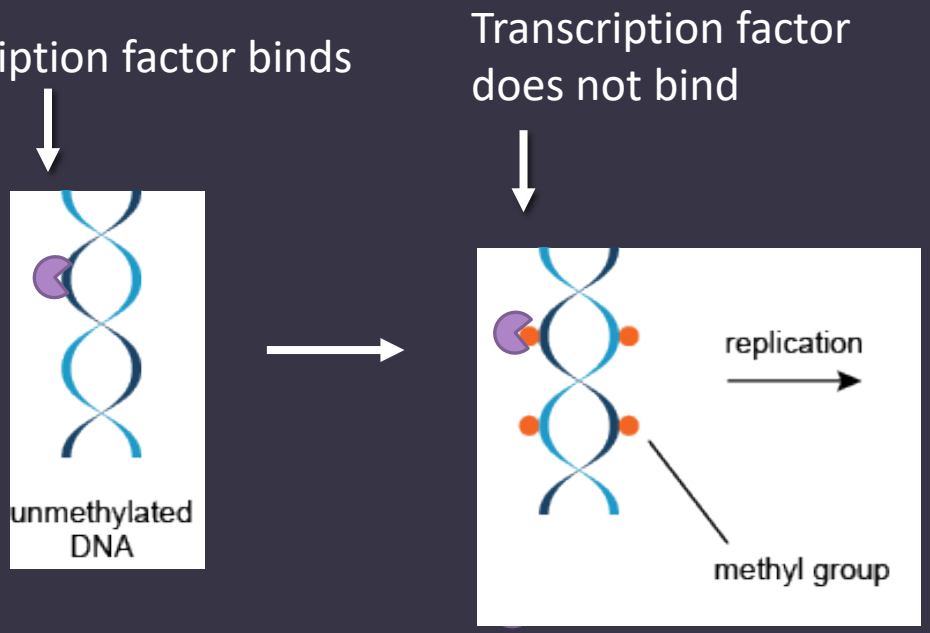
Altered F1 and F2 hormones

How does the phthalate mixture cause multigenerational or transgenerational effects?

Hypothesis

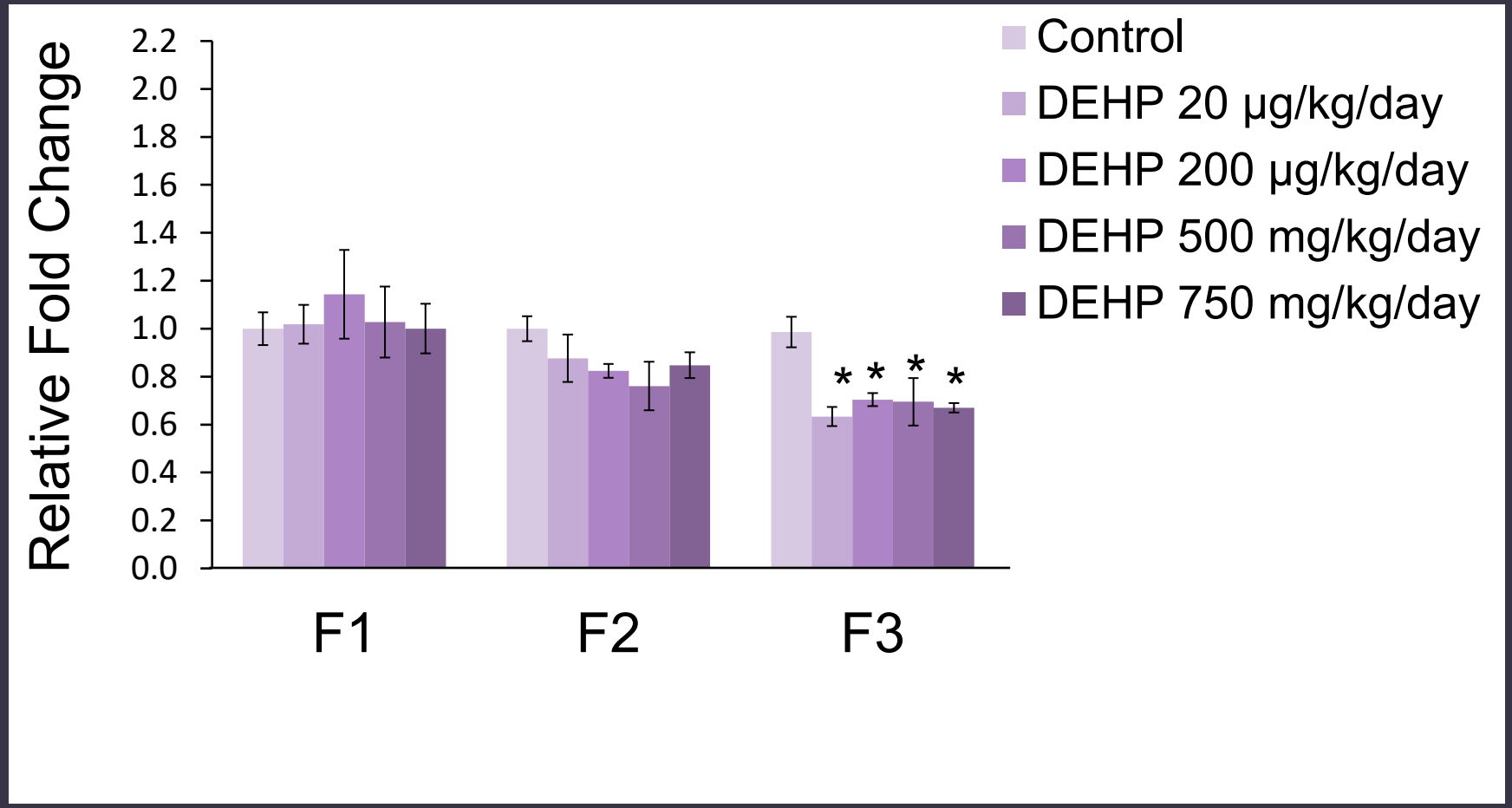
Prenatal phthalate exposure alters DNA methylation





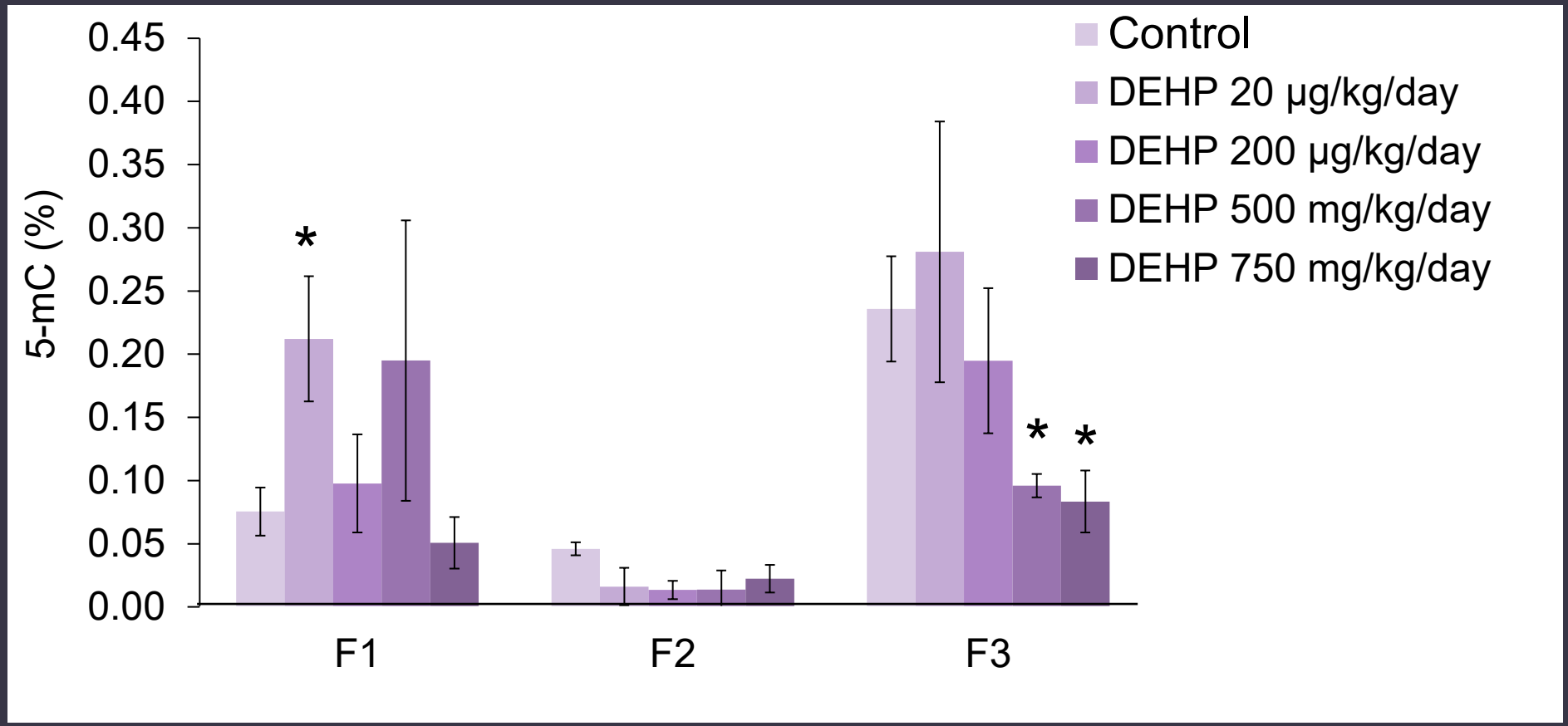
Images courtesy of umich.edu and https://www.atdbio.com/content/56/Epigenetics

Effects of Prenatal Exposure to DEHP on *Dnmt3b* Expression in the F1, F2, and F3 Generations



* $p \leq 0.05$

Effects of Prenatal Exposure to DEHP on 5-mC Percentage in the F1, F2, and F3 Generations



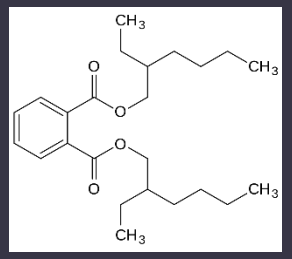
* $p \leq 0.05$

Effects of Prenatal Exposure to DEHP on Gene Expression in the F3 Ovary

DEHP exposure affected expression of:

- Steroid hormone regulators and receptors
- Cell cycle regulators
- Phosphoinositide 3-kinase signaling
- Anti-apoptotic factors

Summary



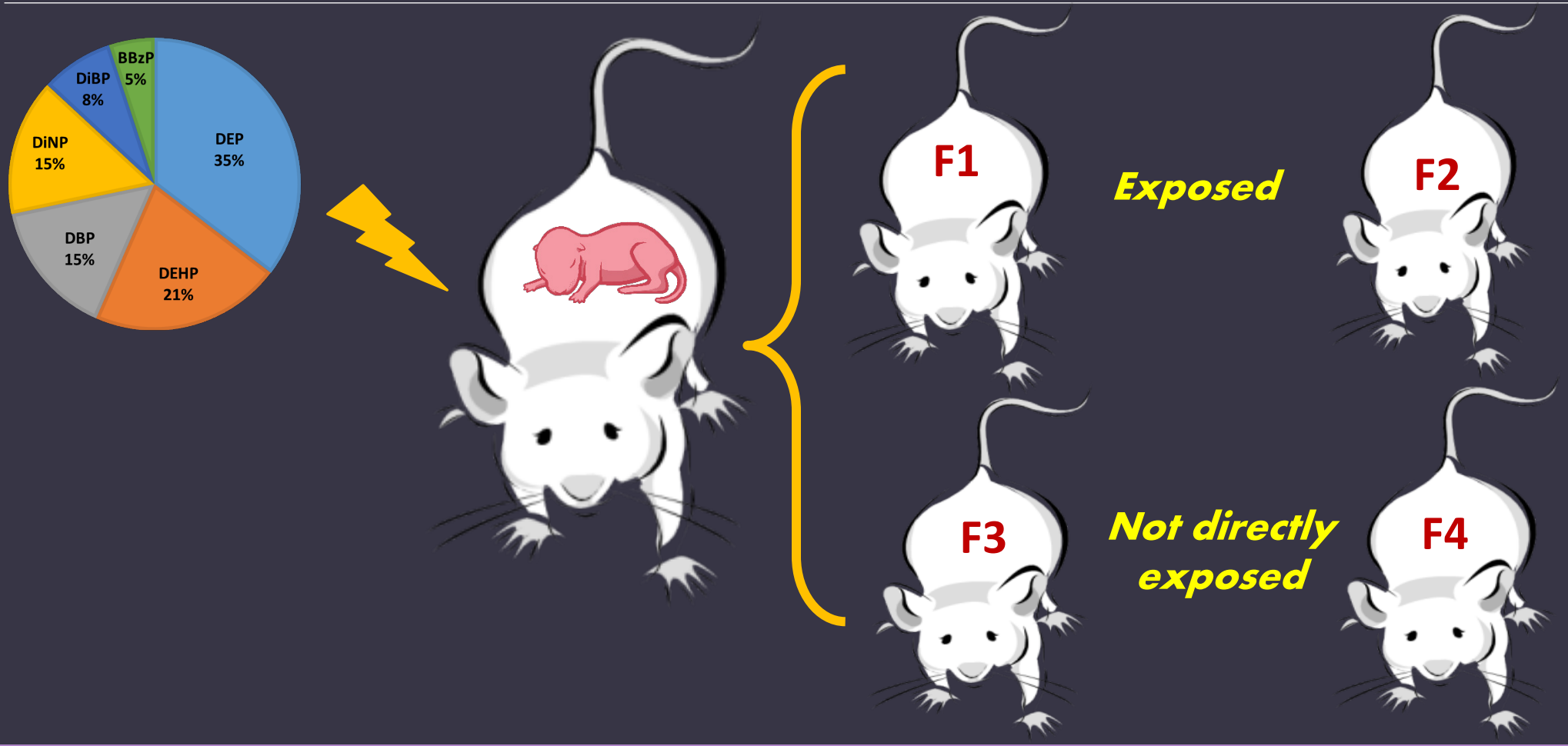
Female Offspring
F3 (not directly exposed)

Decreased *Dmnt* expression in the F3 ovaries

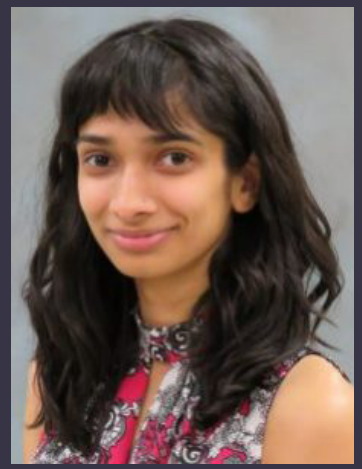
Decreased 5-mC percentage in the F3 ovaries

Altered gene expression in the F3 ovaries

Conclusions



Acknowledgments



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