Toxicant Effects on Mitochondria in Oocytes

A growing understanding of the intersection between metabolism and genetics

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TRDRP
Tobacco-Related Disease Research Program

Disclaimer

All of my research was performed at University of California, Irvine

Any views presented here are my own and do not represent my employers

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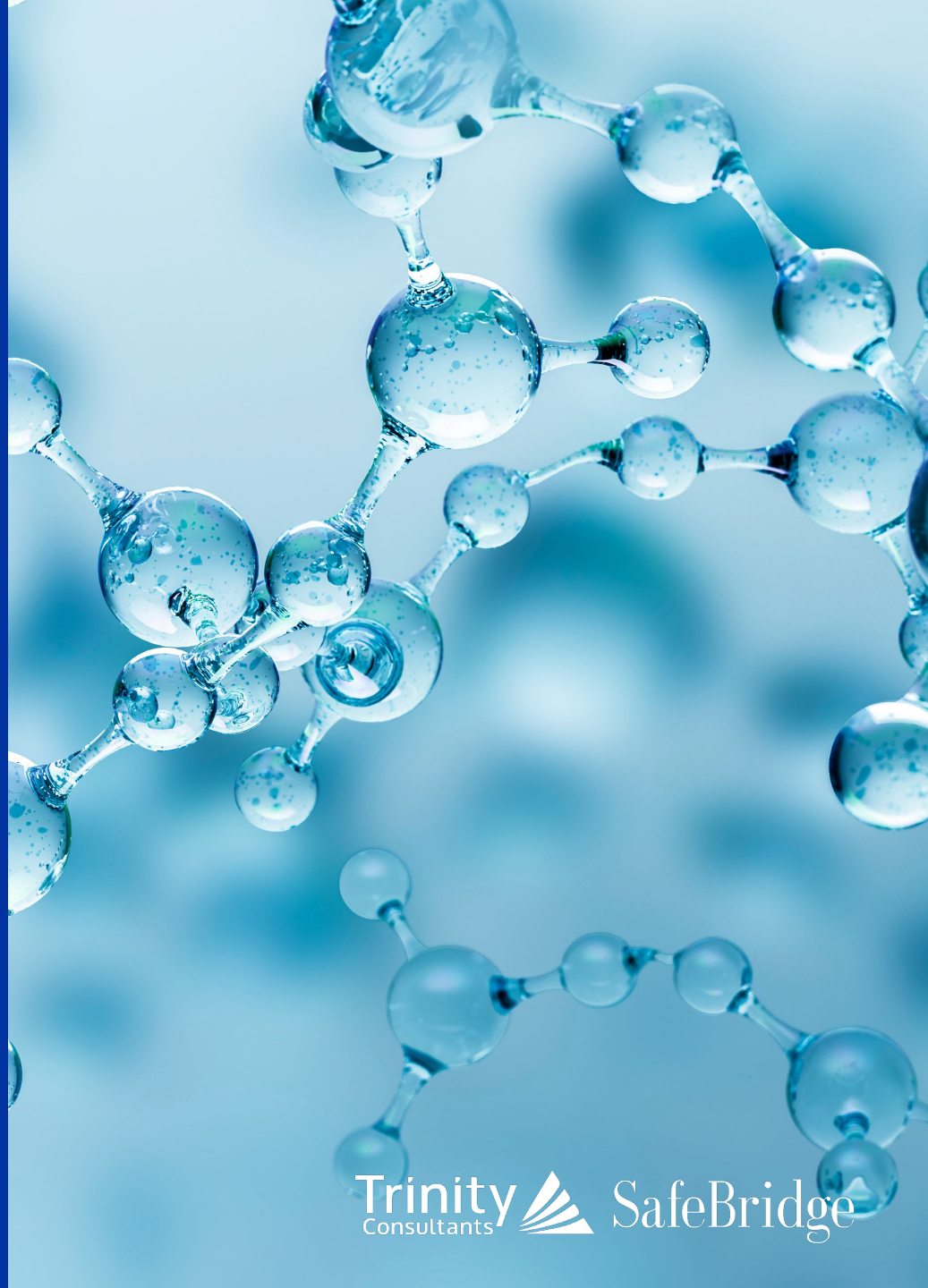
Outline

- Primordial germ cell development
- Germ cell mitochondria
- Zygote inheritance of mitochondria
- mtDNA and nDNA
- Toxicants and mitochondrial toxicity
- Maternal and gestational exposure to polycyclic aromatic hydrocarbons
- Conclusions



Pregnant Female Represents Multiple Generations of Exposure

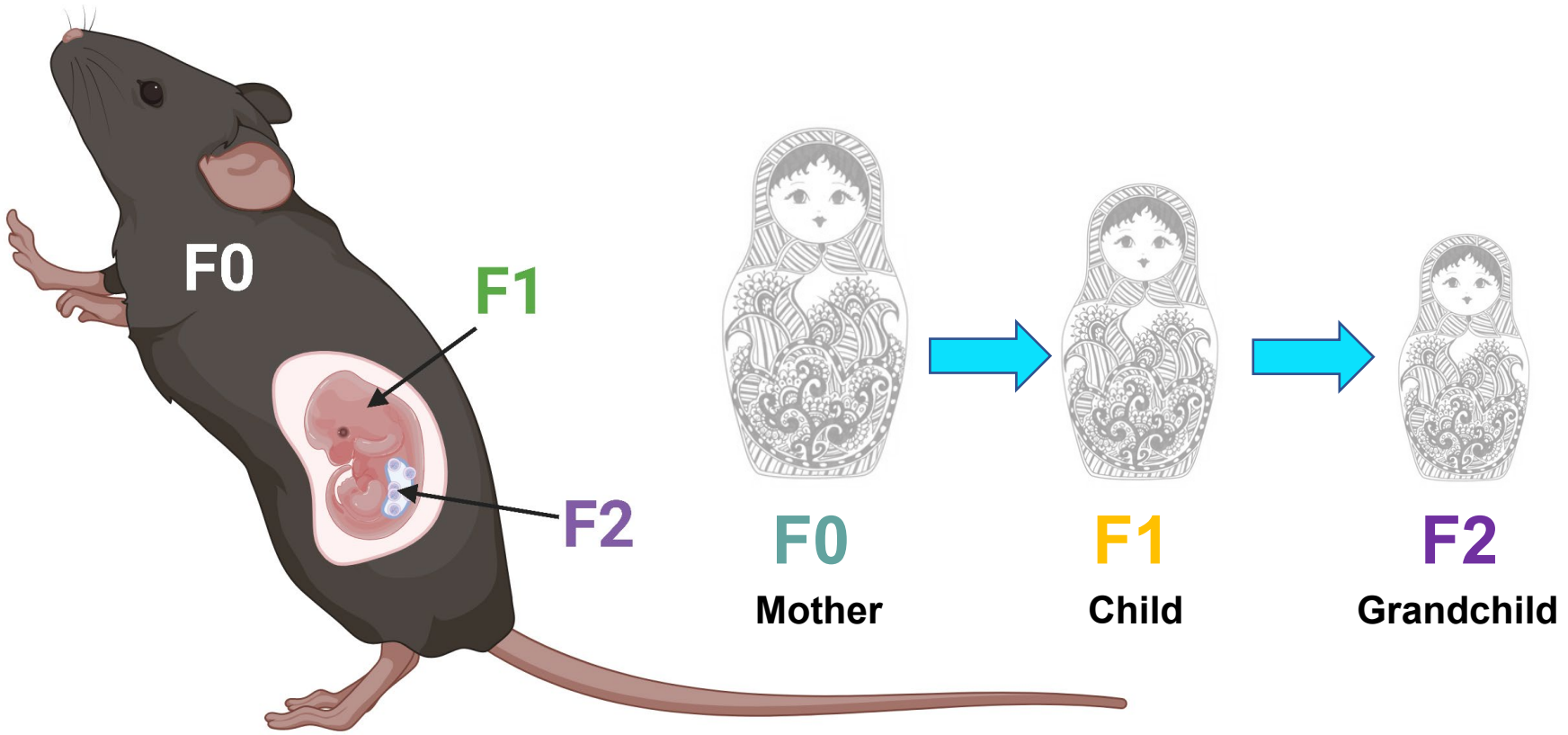
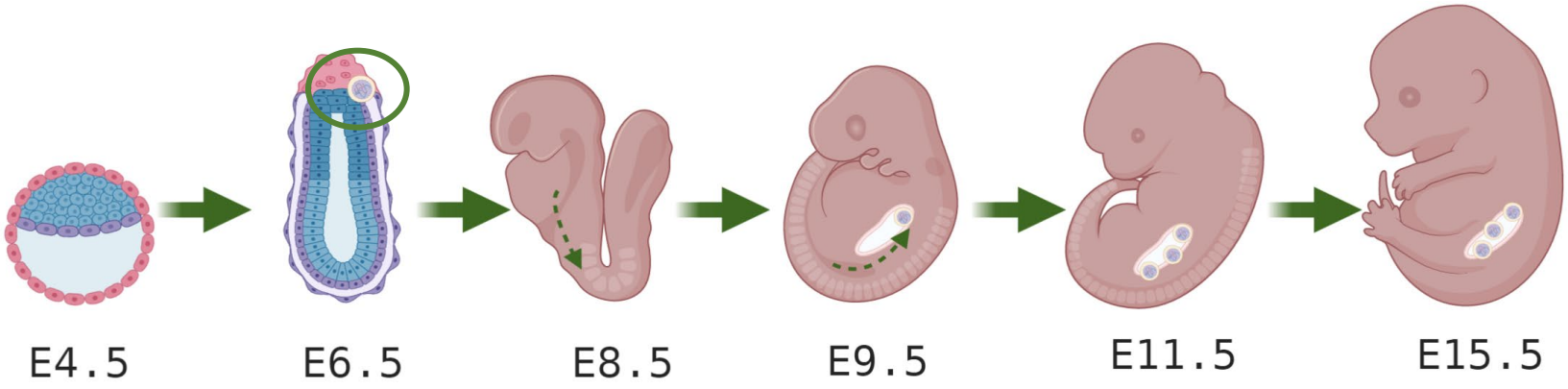


Image credit in part: Biorender.com

Mammalian Germ Cell Development

- Primordial germ cells (PGCs) arise around E5.0
- Proliferating rapidly, migrate to the developing gonad
- Progressively enter meiosis starting on E13.5
- Arrest in interconnected germ cell nests on E17.5
- Nests reorganized into follicles starting shortly before birth and completing a few days after



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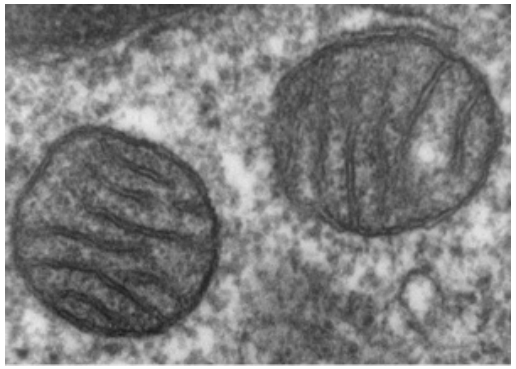
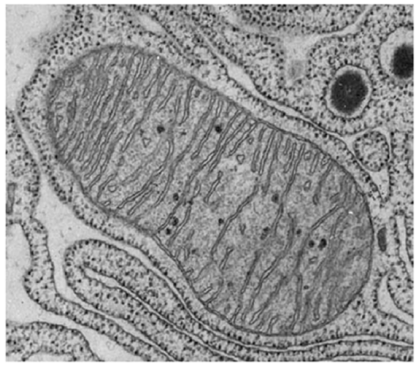
Germ Cell Mitochondria

- Mitochondria are the most abundant organelle in germ cells
- All mitochondria are derived from a “bottleneck” at the PGC stage

	PGC	Oocyte	Spermatocyte
Mitochondria per cell	~100	~300,000 – 400,000	50-70
mtDNA copy number per cell	~200	Estimates as high as 14,000,000 copies	1 – 1,000





- Important commonality: both the oocyte and sperm *rely solely on their own energy source* once released from their respective tissues
  - Very different in function

Ramalho-Santos et al., 2009

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Zygotic Inheritance of Mitochondria

- Accepted to be almost exclusively maternal
  - Sperm mitochondria are actively degraded by proteasomal or lysosomal pathways in the zygote upon ovulation
  - There are documented exceptions of mitochondrial heteroplasmy in individuals
  - Currently understood to be rare
- Consequently, this talk will be primarily focused on oocyte mitochondria

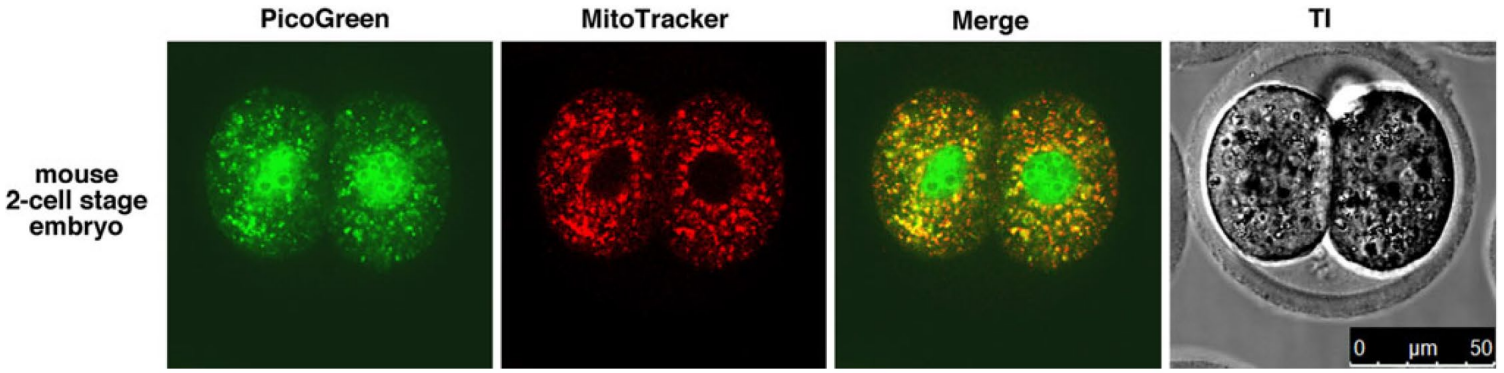

Image credit: Kasashima et al 2013

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Mitochondrial DNA and Nuclear DNA in Oocytes

- mtDNA consists of 16,569 base pairs encoding:
  - 22 tRNAs
  - 13 mitochondrial proteins involved in oxidative phosphorylation
  - 2 rRNAs
- Several mitochondrial proteins are encoded by nDNA
- mtDNA has a mutation rate 25X higher than nDNA
  - Lacks protective histones
  - Lacks repair mechanism

Otten et al 2015

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A Delicate Balance

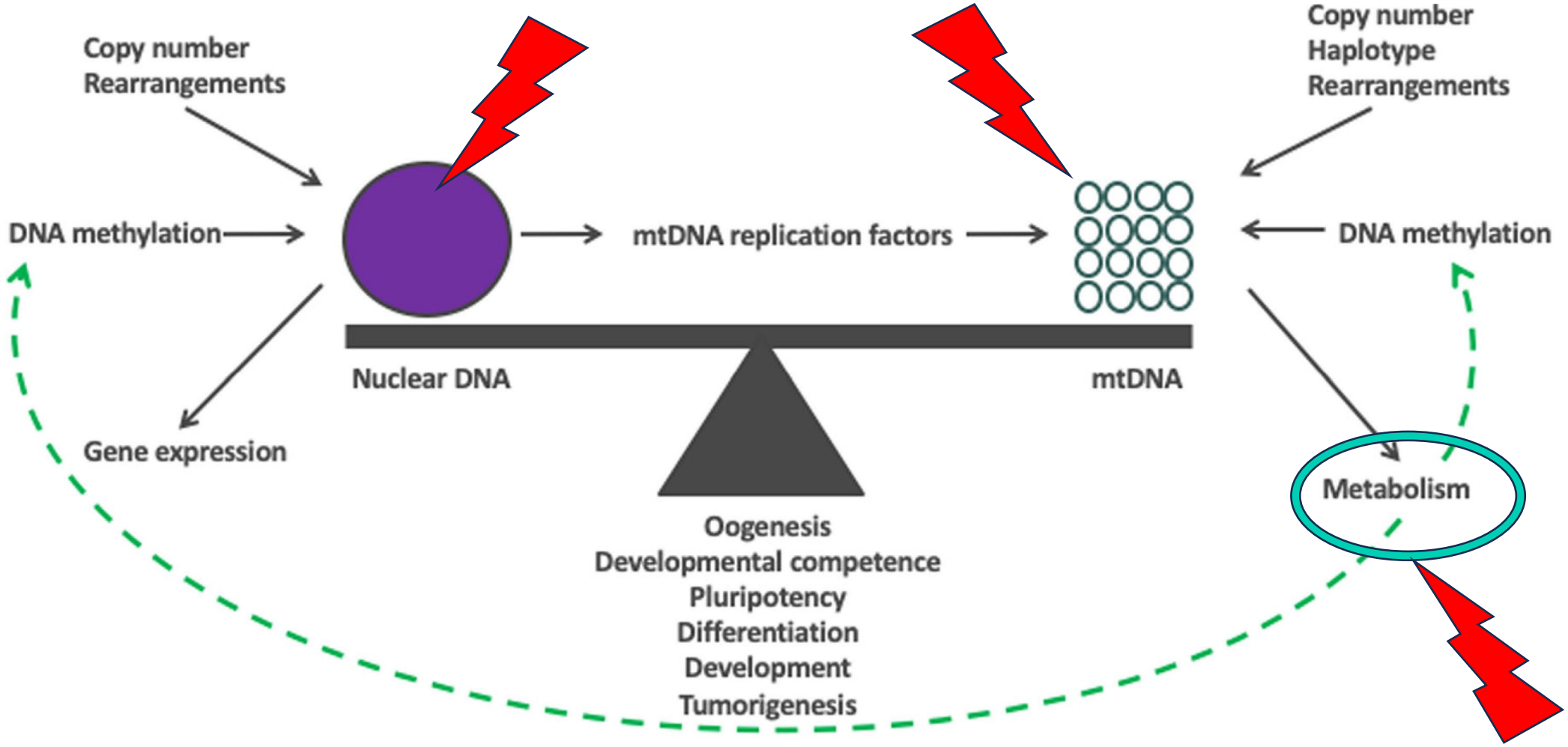

Image credit: St. John et al 2022

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Toxicants Implicated in Oocyte Mitochondrial Toxicity

- Chemotherapeutics
- Toxic Metals
- Pesticides
- Plasticizers
- Ionizing Radiation
- **Polycyclic Aromatic Hydrocarbons**

Malott and Luderer., 2021

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Common Findings in Ovarian Mitochondrial Toxicity

Malott & Luderer., 2021	Chemotherapeutics	Toxic Metals	Pesticides	Plasticizers	Ionizing Radiation	Polycyclic Aromatic Hydrocarbons
Mitochondrial Apoptosis	✓			✓	✓	
Mitochondrial Membrane Potential	↓	↓	↓	↓	↓	
ATP Content & Mitochondrial Content	Mitochondrial volume ↓	ATP content ↓	ATP content ↓	ATP Content ↓		Mito & ATP Content ↓
ROS Production	↑	↑	↑	↑		↑
Oocyte Meiotic Spindle Abnormalities		↑	↑	↑		↑
nDNA Effects & mtDNA Levels		Methylat[illegible]n ↓	Oxidative DNA damage	ETC genes mtDNA ↓	DNA DSBs	Oxidative DNA damage

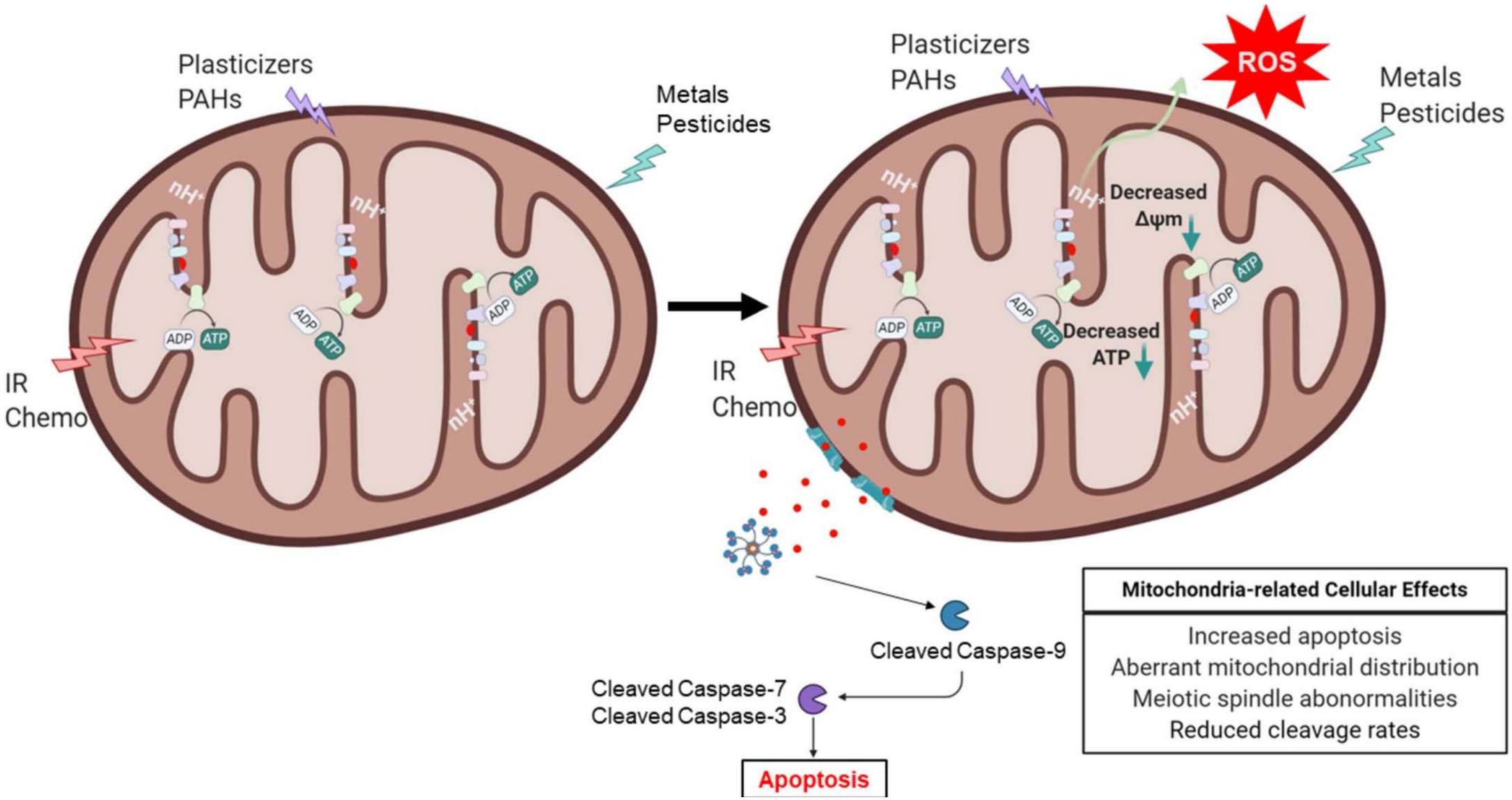

Image credit: Malott and Luderer., 2021

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Maternal and Gestational Exposure to Polycyclic Aromatic Hydrocarbons

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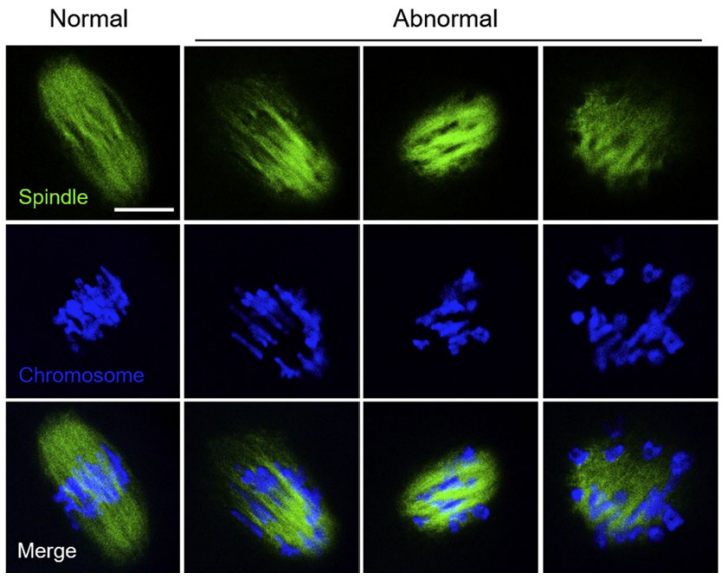
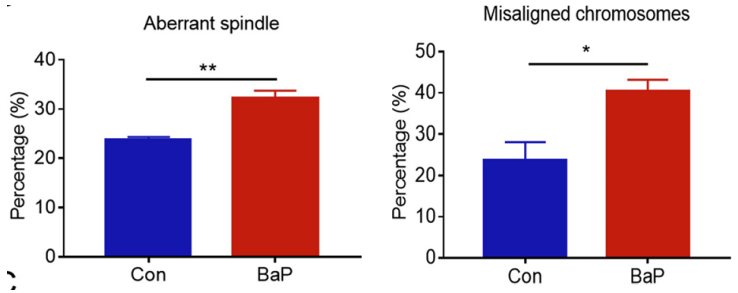
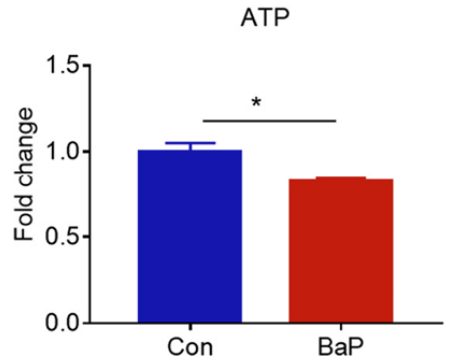
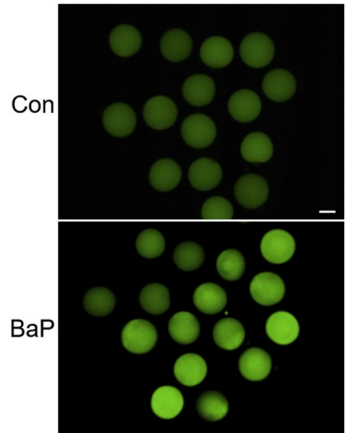
Maternal Exposure to BaP is Correlated with Mitochondrial Dysfunction in Mouse Oocytes

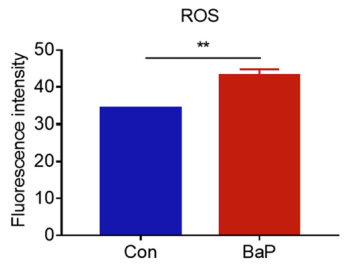
- 10 days, oral administration, 40 mg/kg/day, the mated overnight

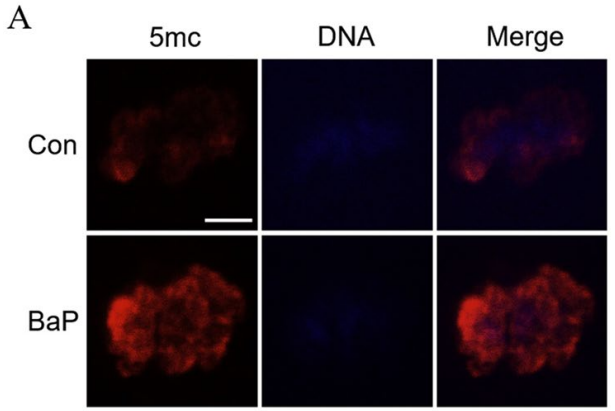


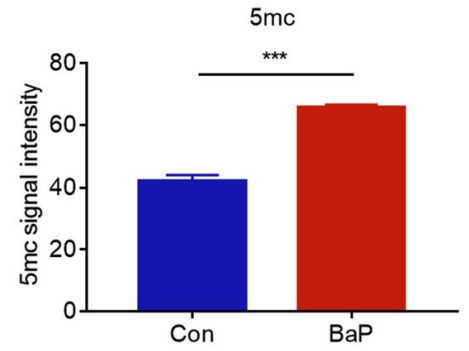












Sui et al., 2020

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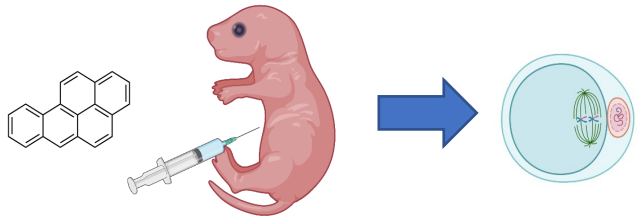
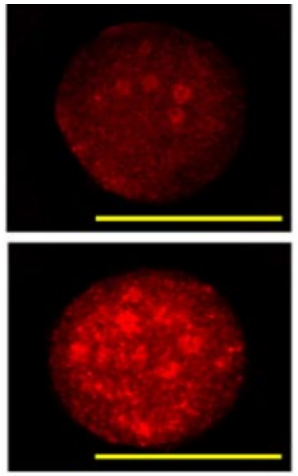
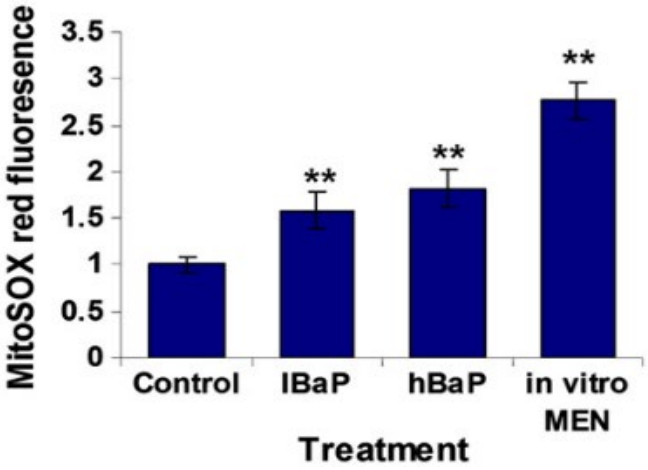
BaP Exposure Increases Oocyte Oxidative Stress and Decreased Developmental Competence



Molecular and cellular function	Up regulated	Down regulated
Cellular growth and proliferation	39	20
Tissue development	30	14
Cell-to-cell signalling and interaction	15	13
Gene expression	32	11
Cell death	30	13
Cell cycle	18	5
Genetic disorder	63	35
Cellular development	31	13
Cancer	51	22
Tissue morphology	25	9
Small molecule biochemistry	28	13





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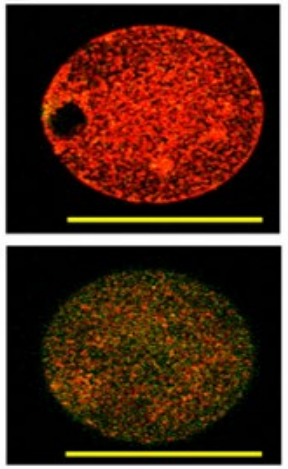
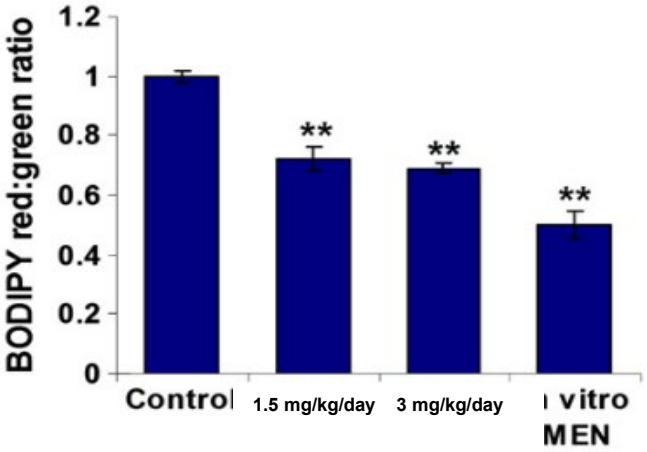
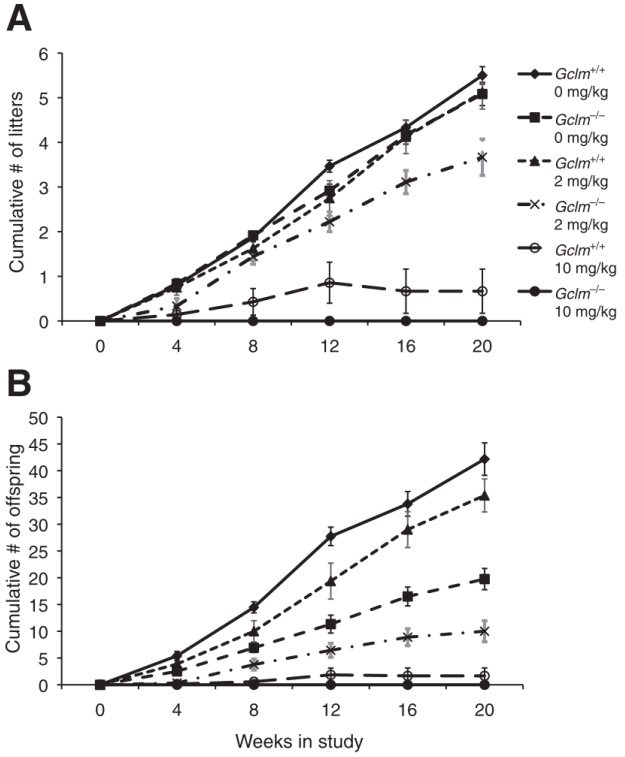
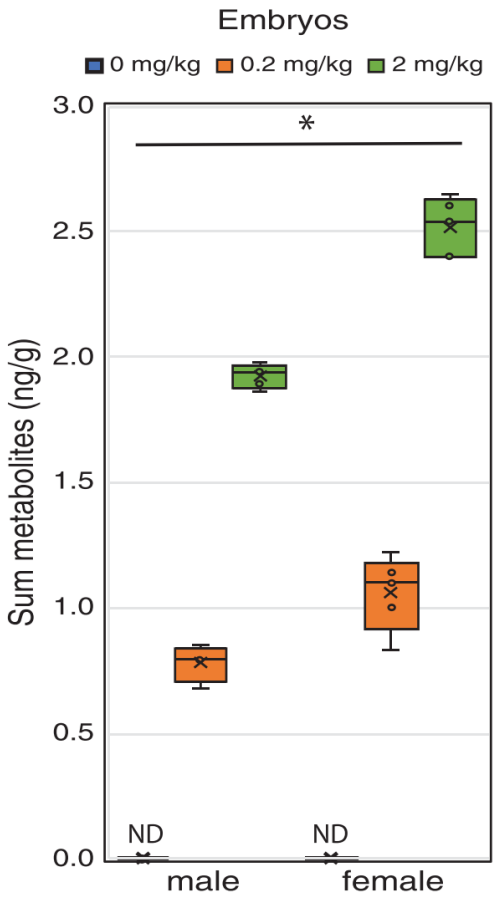


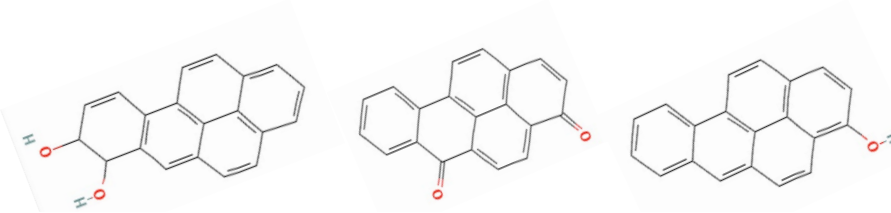


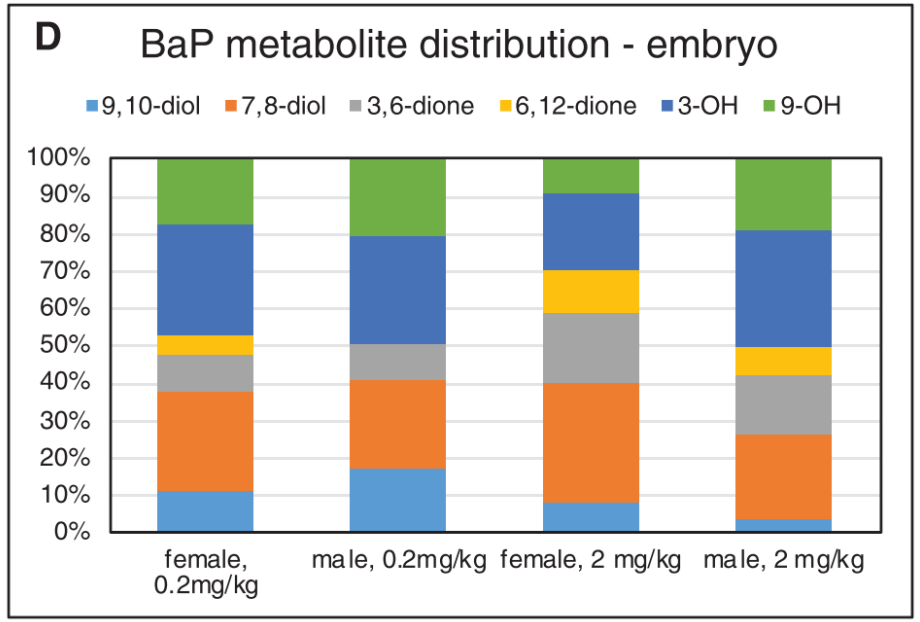
Sobinoff et al., 2012

Gestational BaP Exposure Reduces the Ovarian Reserve and Yields BaP Metabolite Production in the Embryo



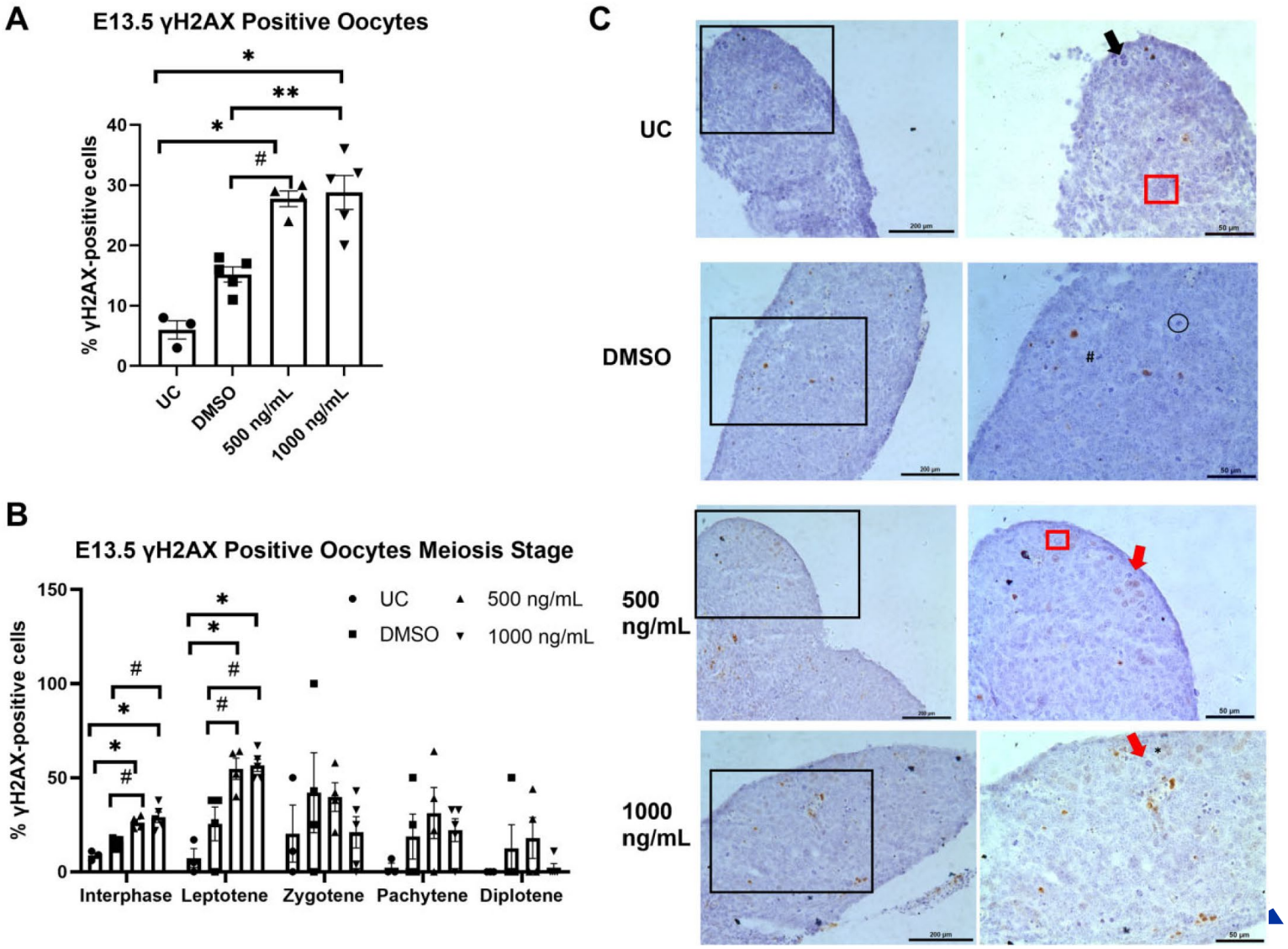






Lim et al, 2013; Lim et al., 2022

In Vitro BaP Exposure Increases Percentage of Oocytes Positive for gH2AX



Malott et al., 2022

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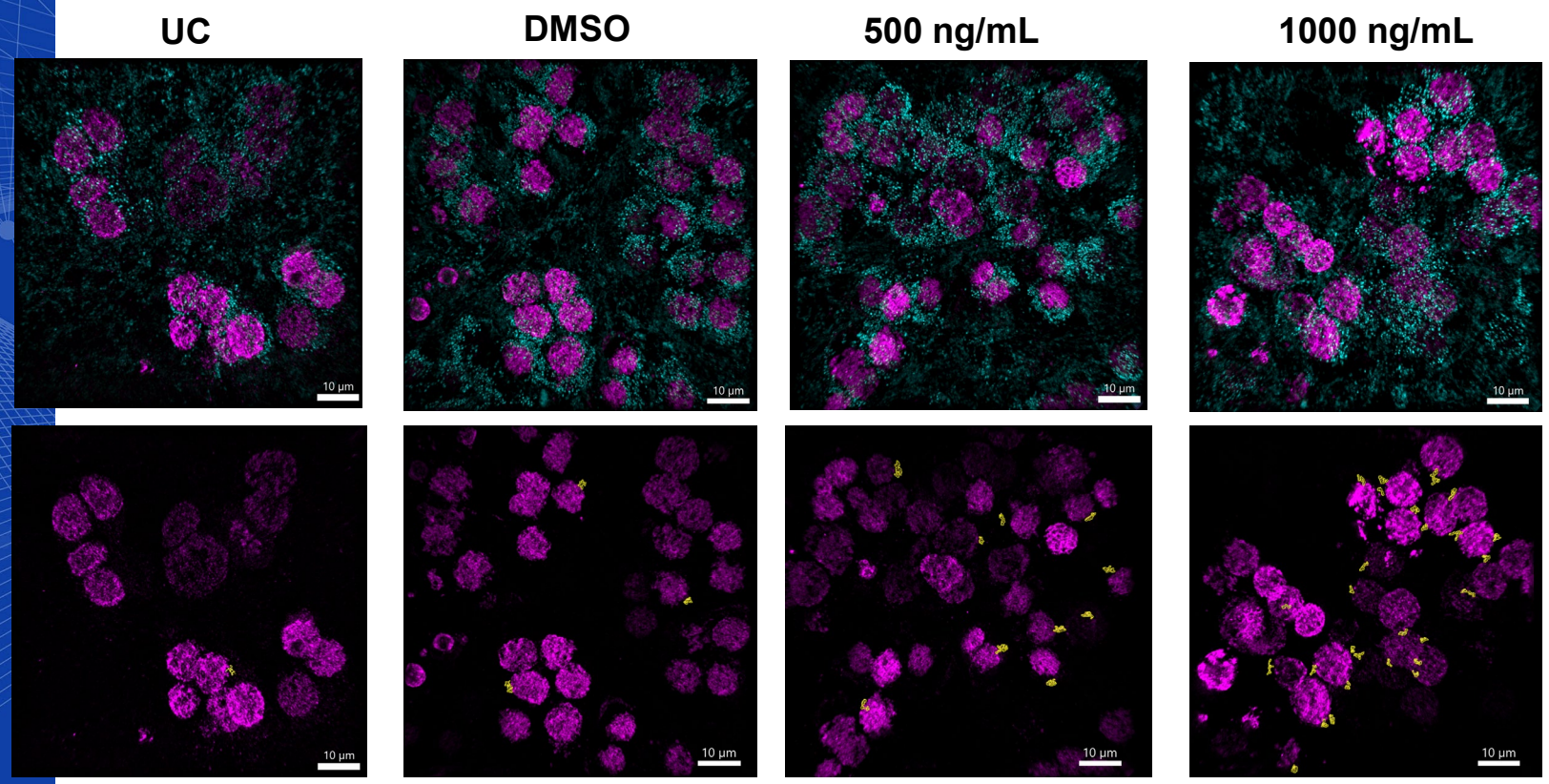
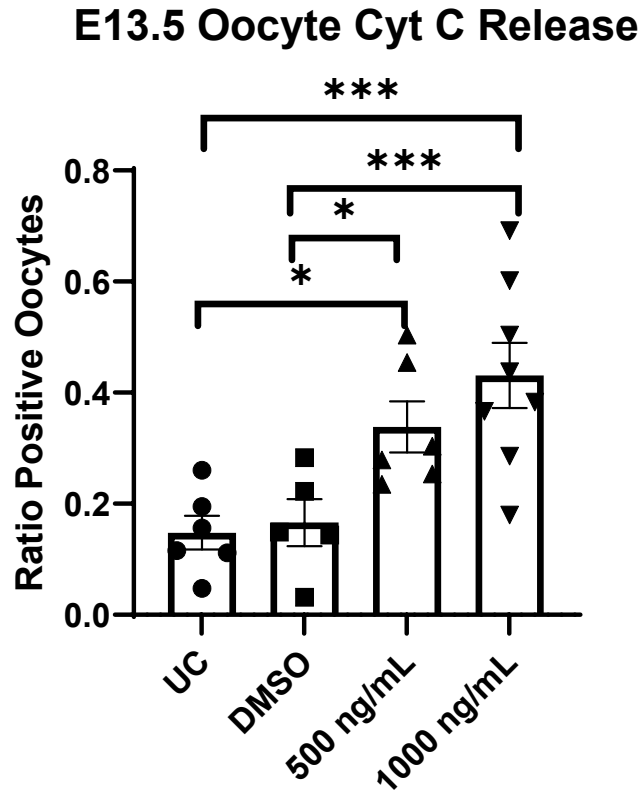
In Vitro BaP Exposure Increases Percentage of Oocytes Positive for Cyt C Release





Malott et al., 2022

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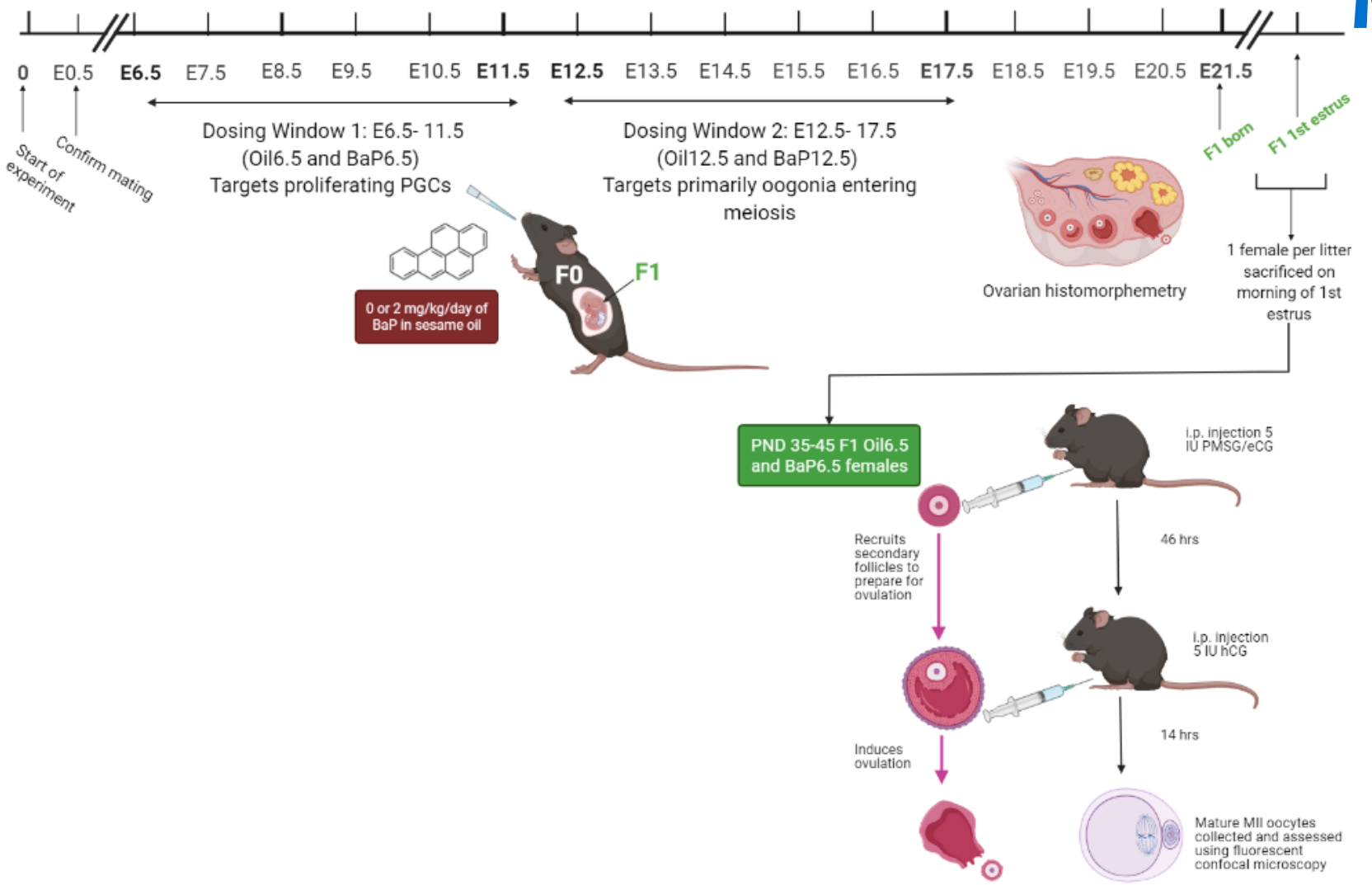
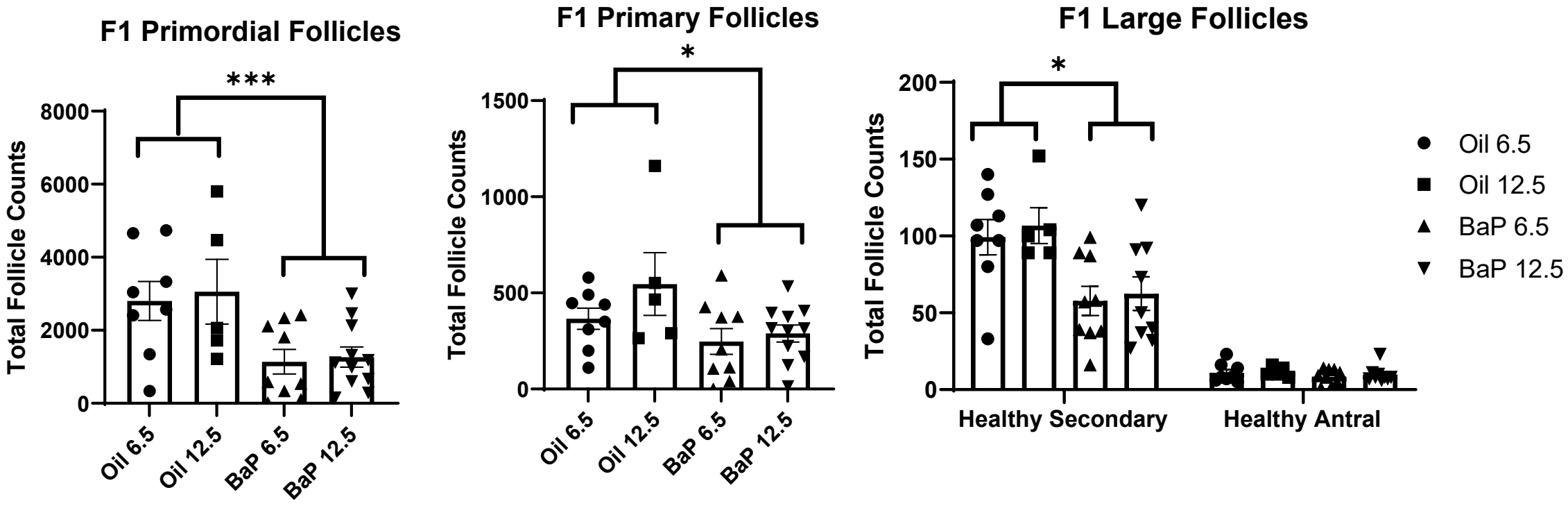
Developing Ovary Sensitivity to BaP-induced Germ Cell Death and Persistent Oxidative Damage in Mature Oocyte



Malott et al., 2022

Gestation BaP Exposure Decreased Ovarian Reserve in F1 Females



Malott et al., 2022

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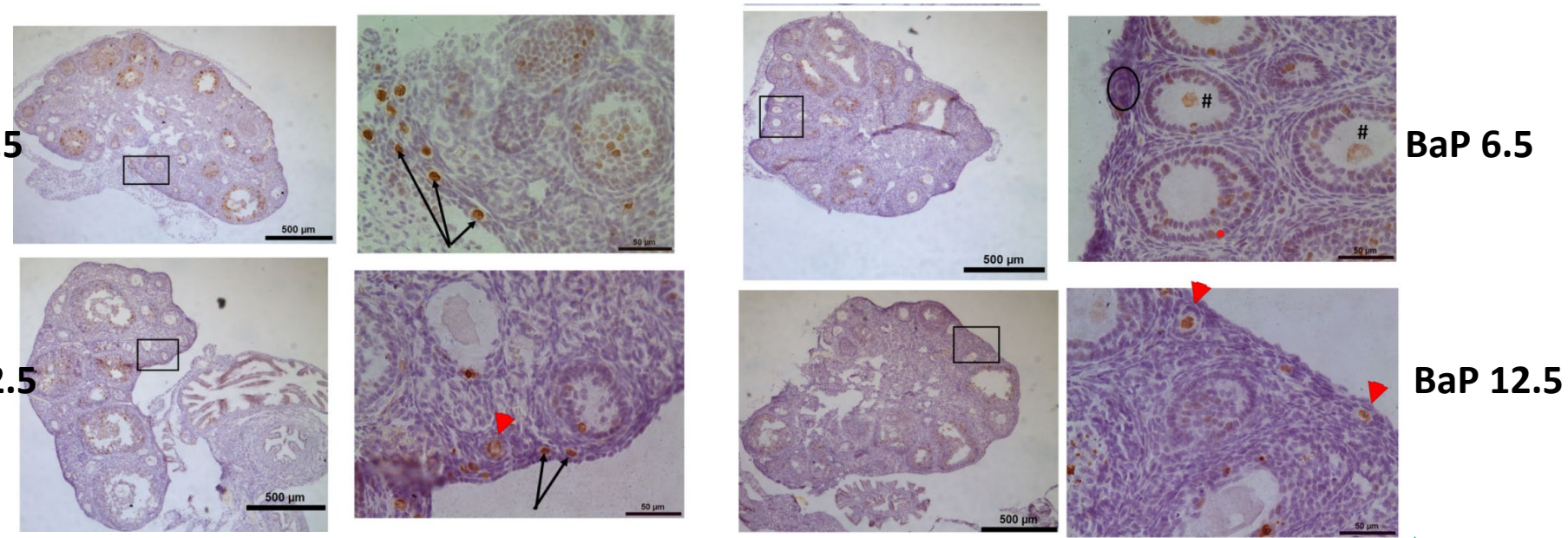
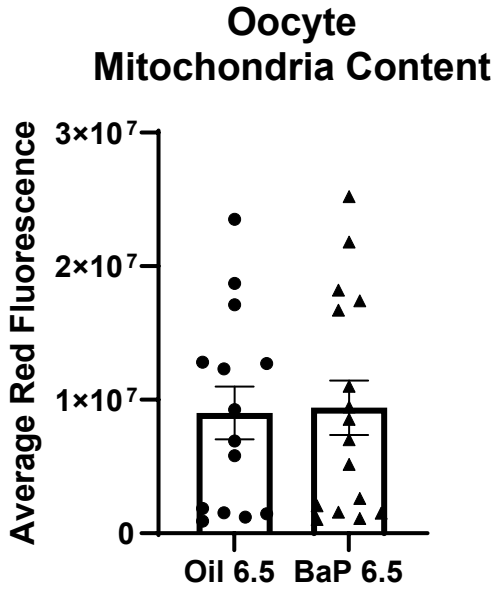
Ovaries of Gestationally-Exposed F1 Females Have Decreased Percentage of Oocytes Positive for gH2AX

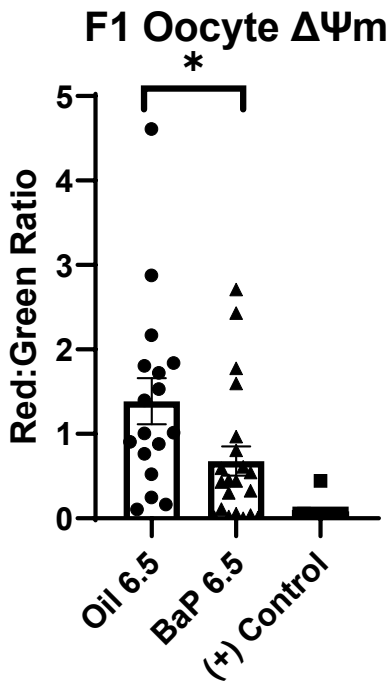
	% Follicles With Positive Granulosa Cells			% Follicles With Positive Oocytes			
	Primary ± SEM[a]	Secondary ± SEM	Antral ± SEM	Primordial ± SEM[b]	Primary ± SEM[c]	Secondary ± SEM[d]	Antral ± SEM
Oil 6.5	11 ± 5	72 ± 5	50 ± 29	60 ± 6	82 ± 6	74 ± 5	50 ± 29
Oil 12.5	22 ± 7	47 ± 14	57 ± 8	64 ± 3	84 ± 10	35 ± 9	37 ± 12
BaP 6.5	8 ± 3	47 ± 10	64 ± 11	46 ± 10	45 ± 15*	50 ± 12	51 ± 13
BaP 12.5	4 ± 3*	63 ± 7	47 ± 18	47 ± 4*	61 ± 9*	62 ± 11	33 ± 17

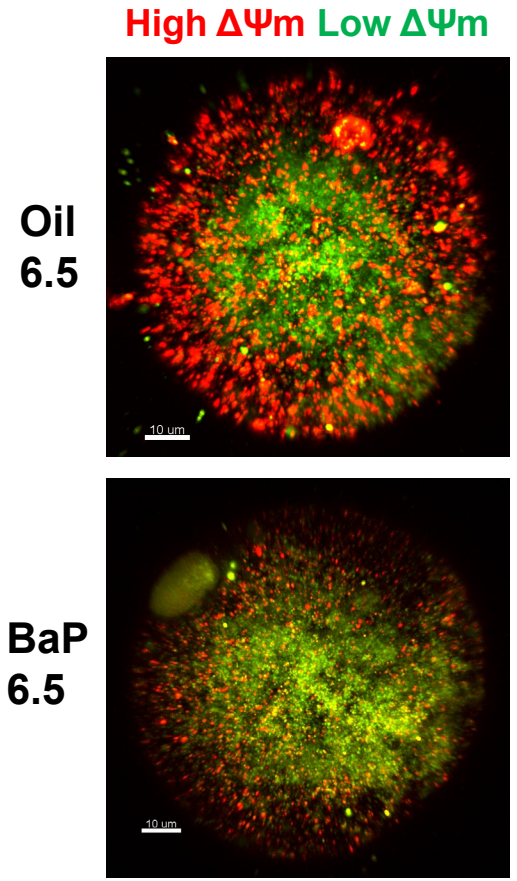


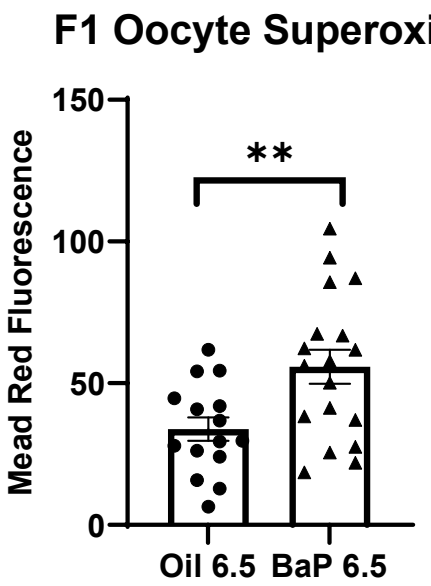
Malott et al., 2022

Gestation BaP Exposure has No Effect on Oocyte Mitochondria but Increases Oxidative Stress









Malott et al., 2022

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Conclusions

Common Findings in Ovarian Mitochondrial Toxicity

Malott & Luderer., 2021	Chemotherapeutics	Toxic Metals	Pesticides	Plasticizers	Ionizing Radiation	Polycyclic Aromatic Hydrocarbons
Mitochondrial Apoptosis	✓			✓	✓	
Mitochondrial Membrane Potential	↓	↓	↓	↓	↓	
ATP Content & Mitochondrial Content	Mitochondrial volume ↓	ATP content ↓	ATP content ↓	ATP Content ↓		Mito & ATP Content ↓
ROS Production	↑	↑	↑	↑		↑
Oocyte Meiotic Spindle Abnormalities		↑	↑	↑		↑
nDNA Effects & mtDNA Levels		Methylat[illegible]n ↓	Oxidative DNA damage	ETC genes mtDNA ↓	DNA DSBs	Oxidative DNA damage

Aberrant Mitochondria Pose a Real Concern for Development and Disease

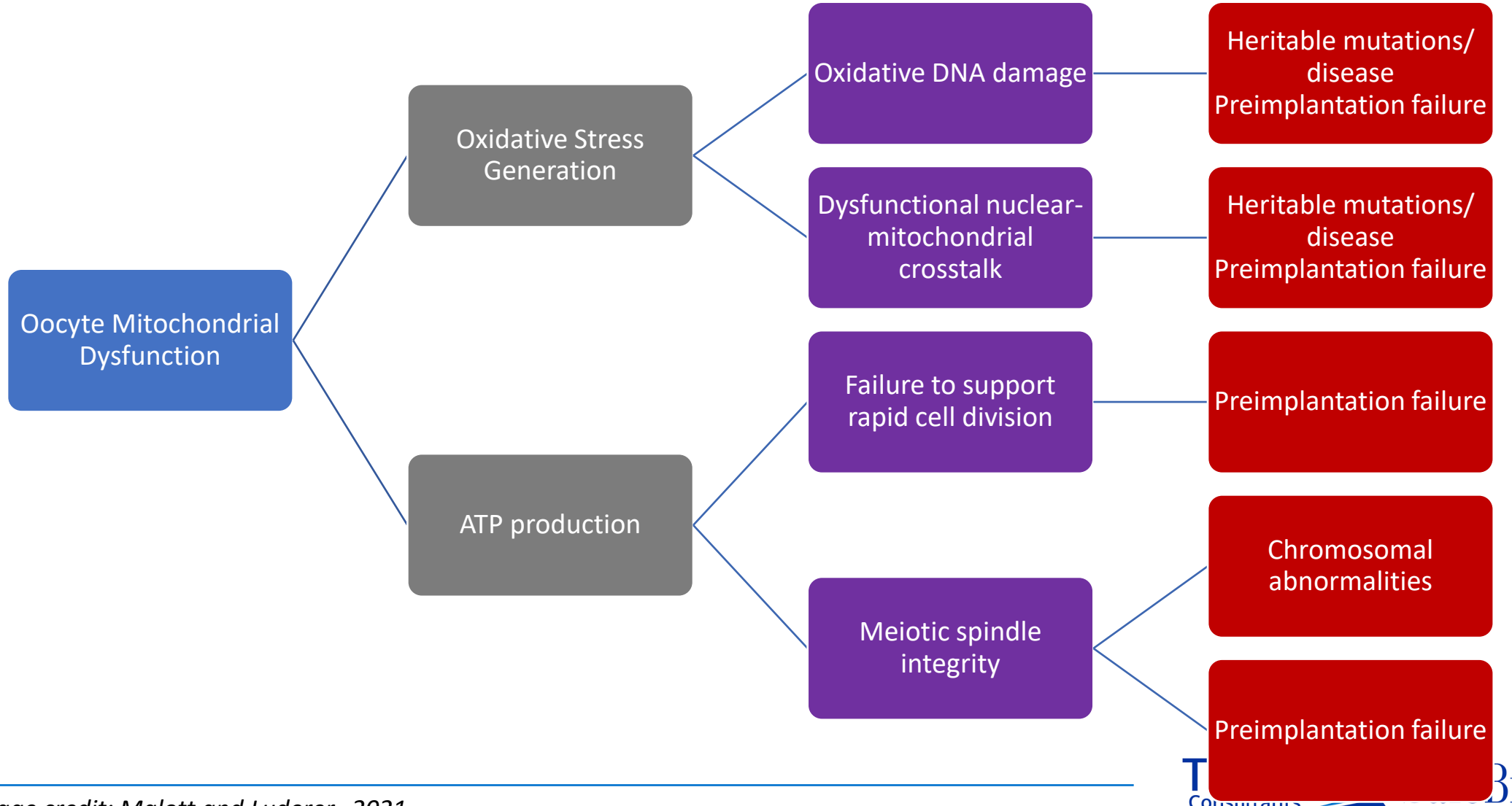

Image credit: Malott and Luderer., 2021

Call to Action

More research is needed to understand the link between gestational and maternal exposures, oocyte metabolism and genomics and the developmental origins of health and disease

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Thank you!
Questions?

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References

Ramalho-Santos, J. and Amaral, S. (2013) Mitochondria and mammalian reproduction. *Mol. Cell. Endocrinol.*, 379, 74–84.

Sobinoff AP, et al. (2013) Scrambled and fried: Cigarette smoke exposure causes antral follicle destruction and oocyte dysfunction through oxidative stress. *Toxicol Appl Pharmacol*. 271(2):156-167. doi:10.1016/j.taap.2013.05.009

Sui, L. et al., (2020) Maternal benzo[a]pyrene exposure is correlated with the meiotic arrest and quality deterioration of offspring oocytes in mice. *Reprod. Toxicol.*, 93, 10–18.

Lim J, et al. (2013) Glutathione-deficient mice have increased sensitivity to transplacental benzo[a]pyrene-induced premature ovarian failure and ovarian tumorigenesis. *Cancer Res*. 73(2):908-917. doi:10.1158/0008-5472.CAN-12-3636.

Lim J, et al., (2022) Sex Differences in Embryonic Gonad Transcriptomes and Benzo[a]pyrene Metabolite Levels After Transplacental Exposure.163(1):1-17.

Malott K, and Luderer U. (2021) Toxicant Effects on Mammalian Oocyte Mitochondria. *Biol Reprod*.1-5.

Malott K, et al., (2022) Gestational Benzo[a]pyrene Exposure Destroys F1 Ovarian Germ Cells through Mitochondrial Apoptosis Pathway and Diminishes Surviving Oocyte Quality. *Tox Sci.* 190(1): 23- 40.

Kasashima K. et al., (2014) Dynamic Regulation of Mitochondrial Genome Maintenance in Germ Cells. *Reprod Med Biol.* 13:11-20.

Otten et al., (2015) Evolutionary defined role of the mitochondrial DNA in fertility, disease, and ageing. *Human Reprod Update. 21(5): 671-689*

St. John, J.C. (2019) Mitochondria and female germline stem cells – A mitochondrial DNA perspective. *Cells* 8, 852.

Mechanisms for Safeguarding Mitochondrial Inheritance

Muller's Ratchet in mtDNA

- Mutation prevention: endosymbiotic gene transfer and antioxidants
  - Transfer of mtDNA to the nucleus as it is less prone to mutations
  - Oocytes have the highest concentration GSH
- Diluting the target: high copy number
  - Redundancy amongst the system
- Mutation recovery: the mtDNA bottleneck and bottleneck-mediated selection
  - Positive or negative selective forces to achieve homoplasmy
  - Only about ~200 mtDNA copies/PGC
    - Protects from pre-existing mutation accumulation due to rapid segregation

Otten et al., 2015