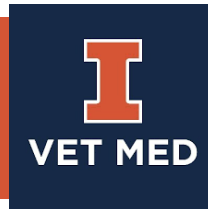


The Effects of Neonicotinoid Pesticides on Reproductive Health



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Department of Comparative Biosciences
University of Illinois Urbana-Champaign

- **Background**
 - Neonicotinoid pesticides (imidacloprid)
 - Ovary
- **Effects of imidacloprid on the ovary**
 - Hypothesis
 - Experimental Design
 - Results
 - Summary
- **Published effects of other neonicotinoid pesticides on reproduction**
- **Conclusions**

What are neonicotinoids?



Neonicotinoids

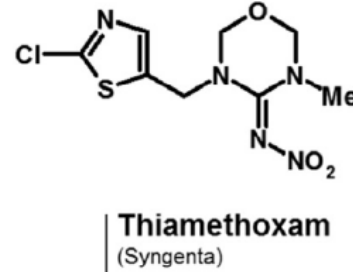
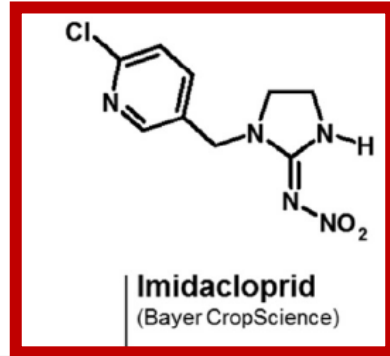
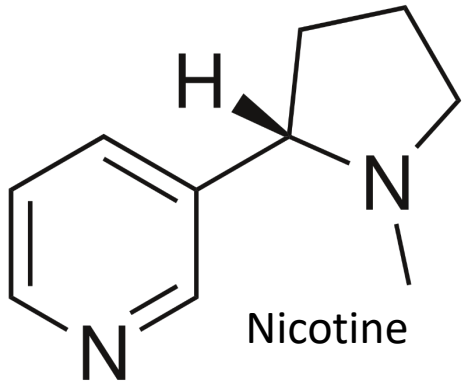
Targets: sucking and chewing insects

Uses: commercial agriculture, homes, and veterinary pharmaceuticals

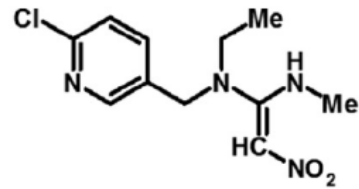
Human exposure: ingestion of contaminated food and water, and contact with pets



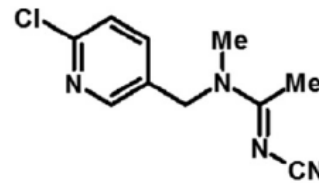
Neonicotinoids



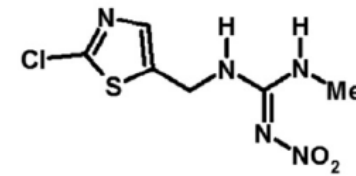
Nitenpyram
(SumiTake)



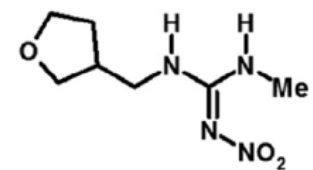
Acetamiprid
(Nippon Soda)



Clothianidin
(SumiTake / Bayer CropScience)

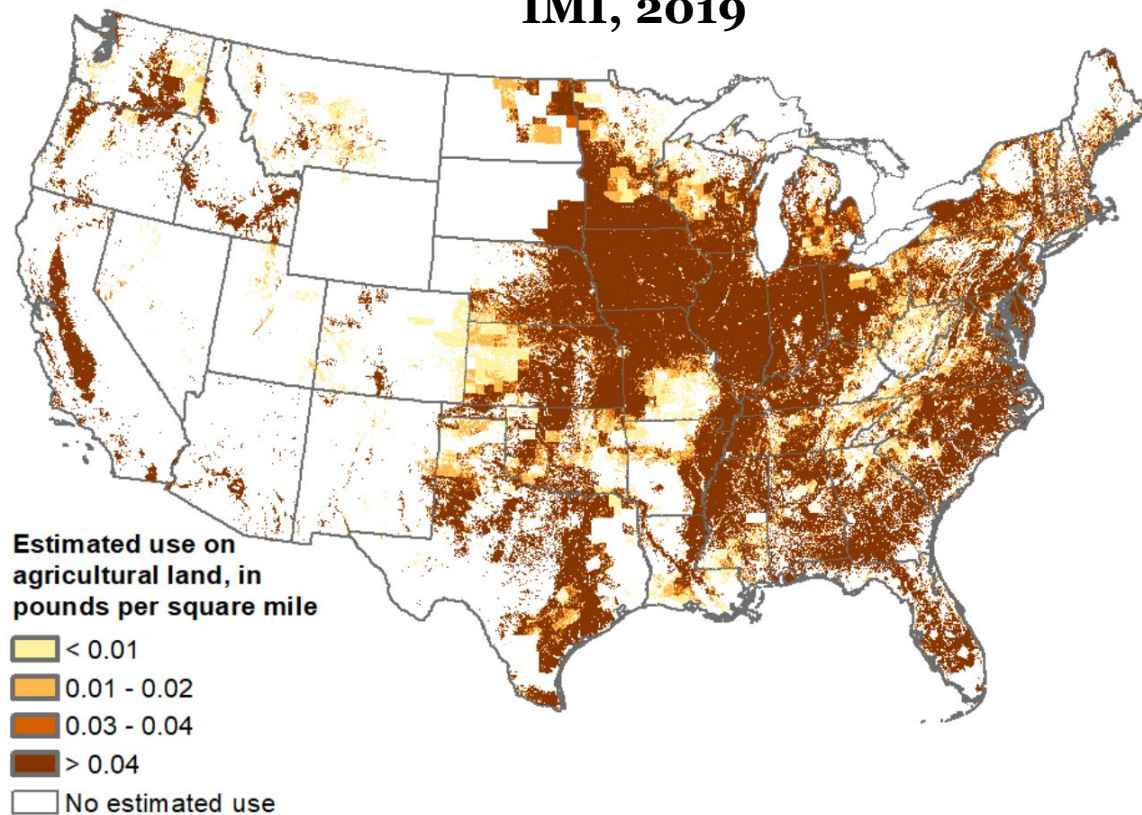


Dinotefuran
(Mitsui Chemicals)



Imidacloprid (IMI)

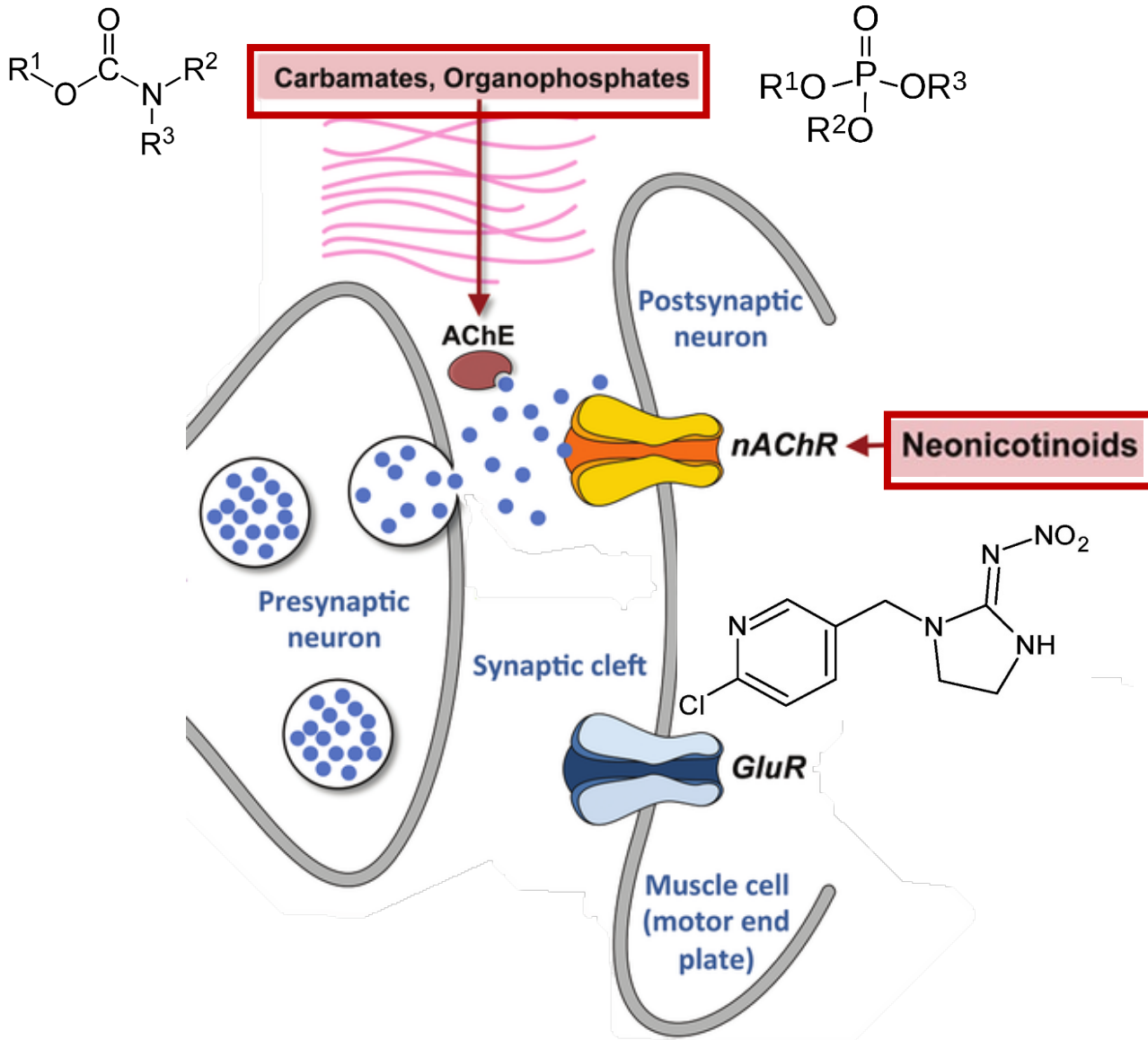
Estimated Agricultural Use for IMI, 2019



Environmental Contamination:

- Compared to other neonicotinoids, IMI has the highest detection frequency on domestic and imported produce
- IMI has been detected in drinking water sources and finished drinking water in the USA and China

Mechanism of Action

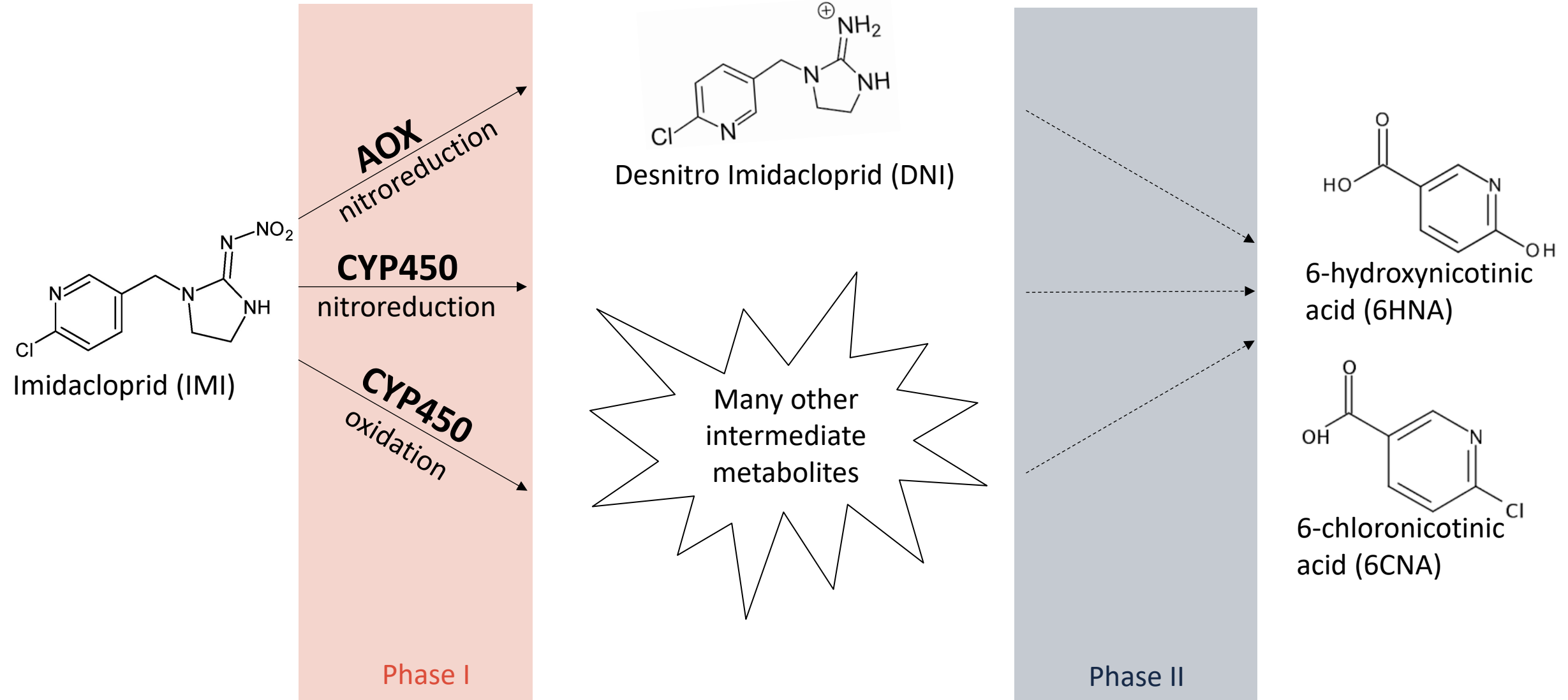


- IMI binds to nicotinic acetylcholine receptors (nAChR)
 - Causes neurotoxicity
- Insect nAChR has higher affinity for IMI than mammalian nAChR
 - Insects thought to be the primary target, but are mammals safe?

- Mammalian nervous system contains nAChR
 - Potential mammalian neurotoxicant
- IMI can be converted to toxic metabolites by the liver
 - Metabolites bind the nAChR
 - Metabolites have a higher affinity for the nAChR than IMI
 - Could cause toxicity in mammals

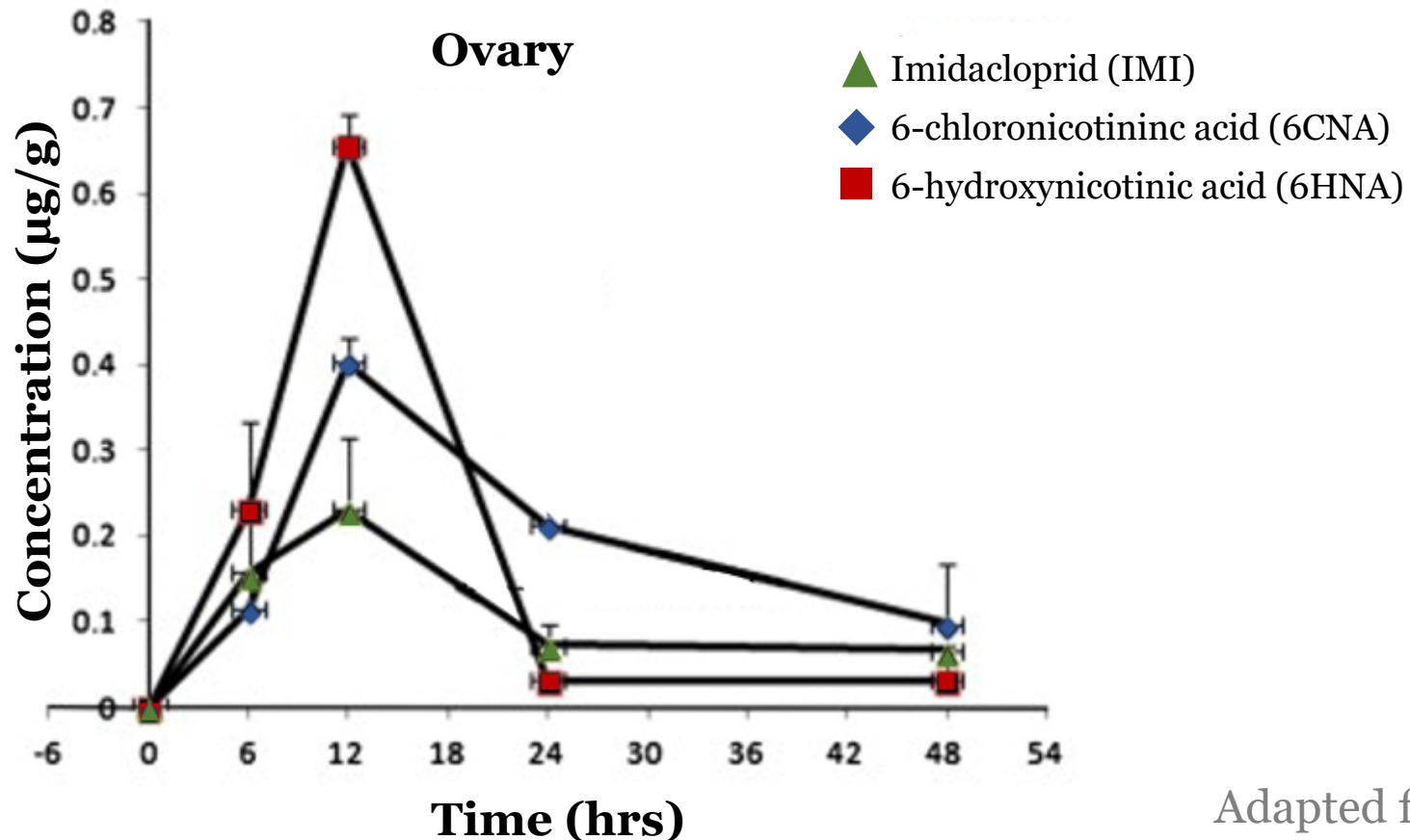


Liver Metabolism of IMI



Potential Concerns

- IMI and its metabolites reach the ovary
 - Concentrations of IMI, 6CNA, and 6HNA in the ovary after single oral administration of imidacloprid (20mg/kg):



Questions

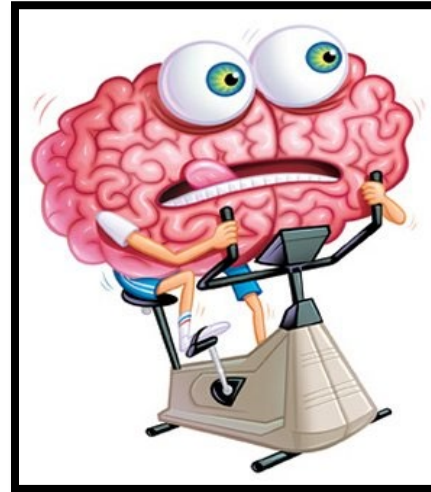
- Does the ovary have nAChR?
- Can the ovary convert IMI to potentially toxic metabolites?
- Are IMI and its metabolites toxic to the ovary?

Why care about IMI reaching the ovary?

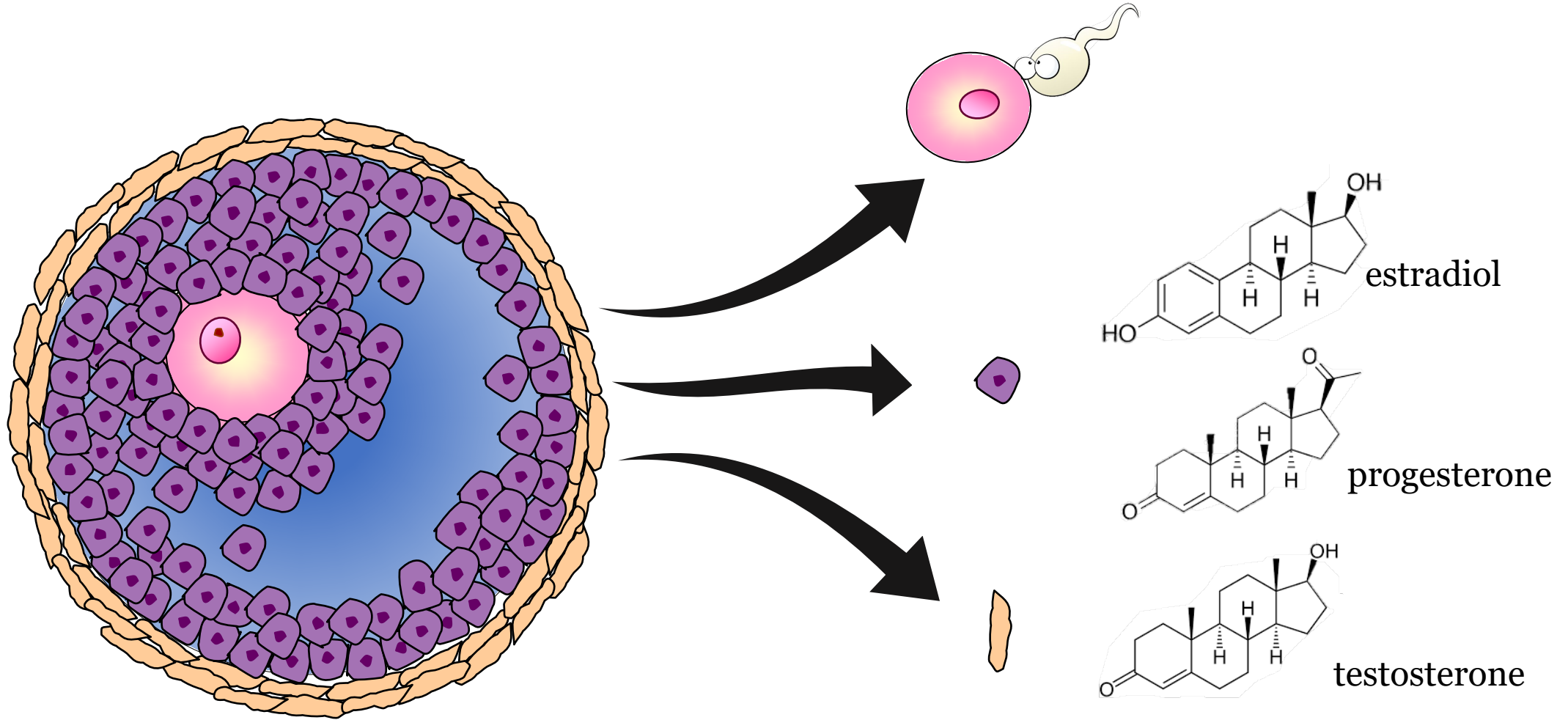


The Ovary

- Required for fertility and producing healthy offspring
 - Produces oocytes (eggs)
 - Required for making hormones that regulate reproduction and many other processes in the body
 - Heart, brain, and bones



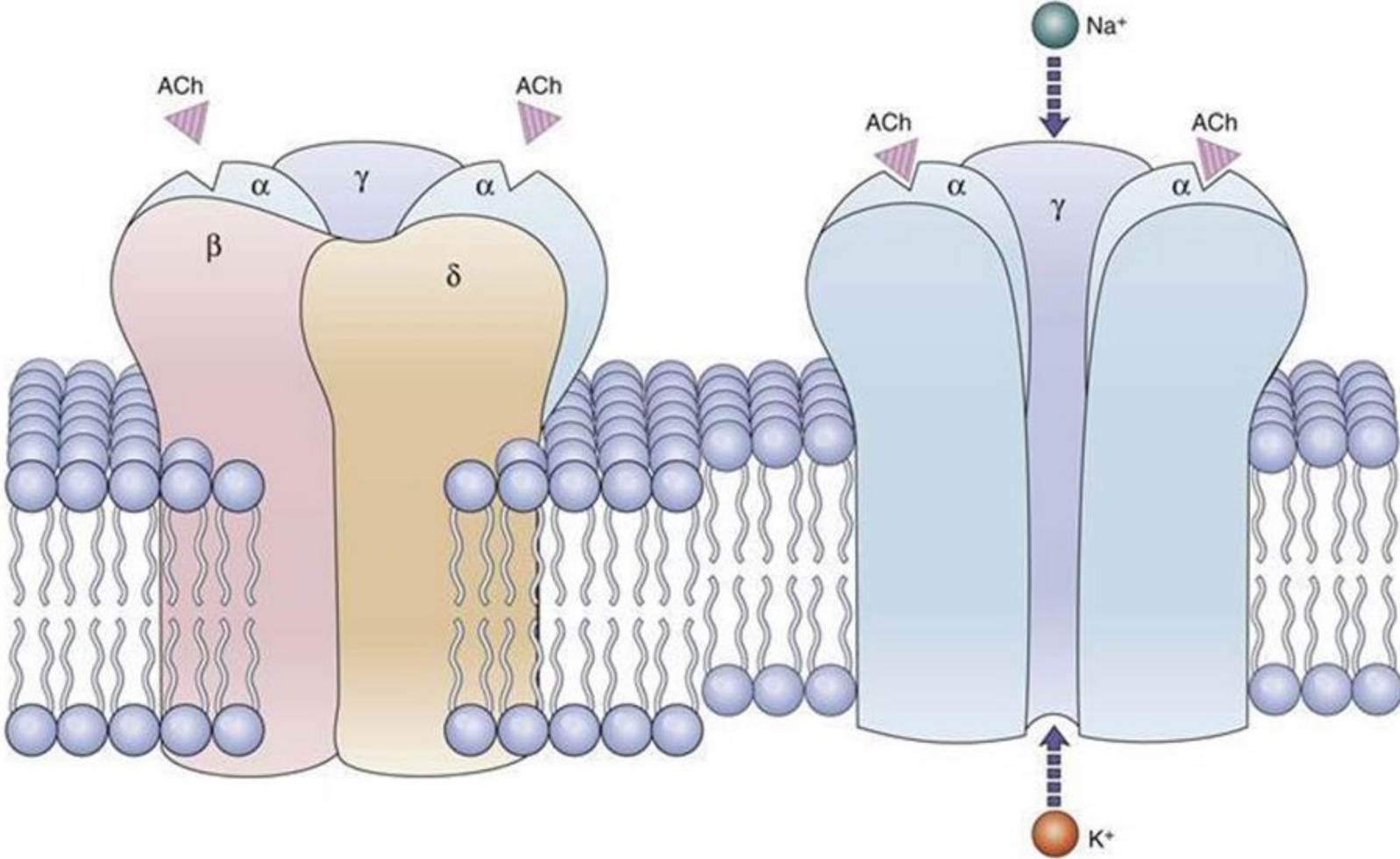
The Ovary: Antral Follicles



Does the ovary have nAChRs?



Nicotinic Acetylcholine Receptors (nAChRs)

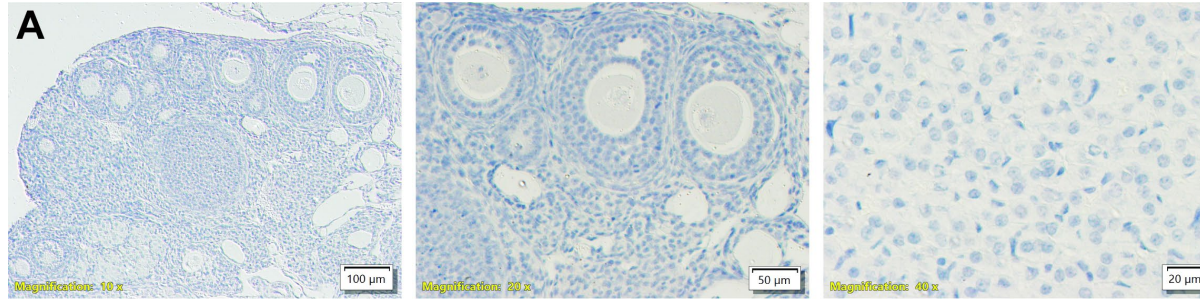


Hypothesis

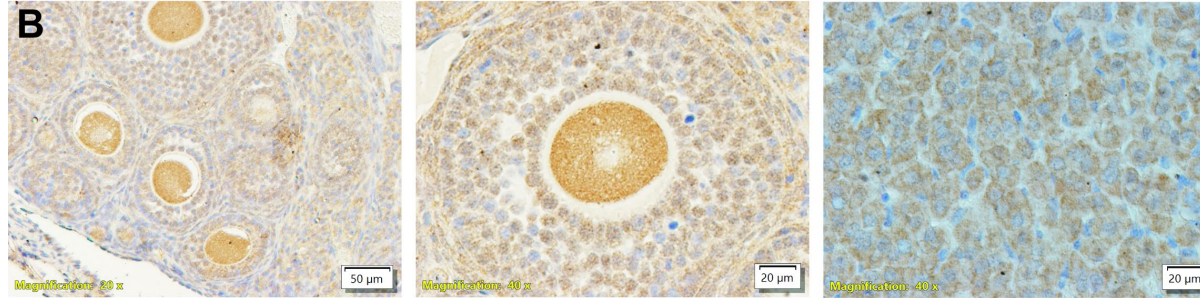
The mammalian ovary contains
nAChR subunits

Protein Localization of nAChR in the Ovary

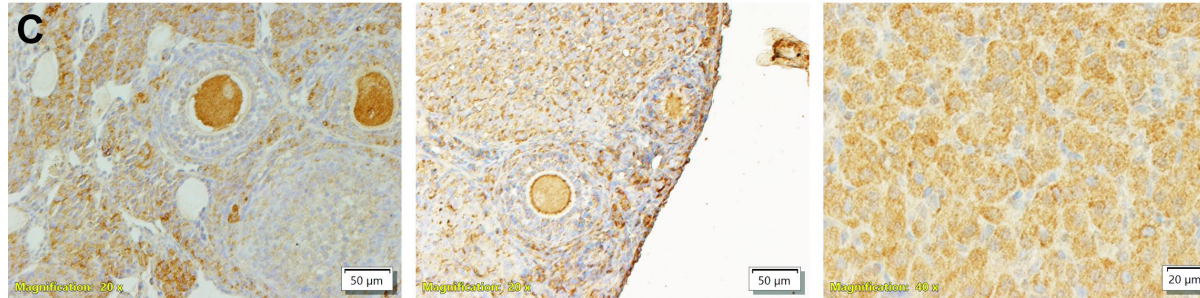
Negative control



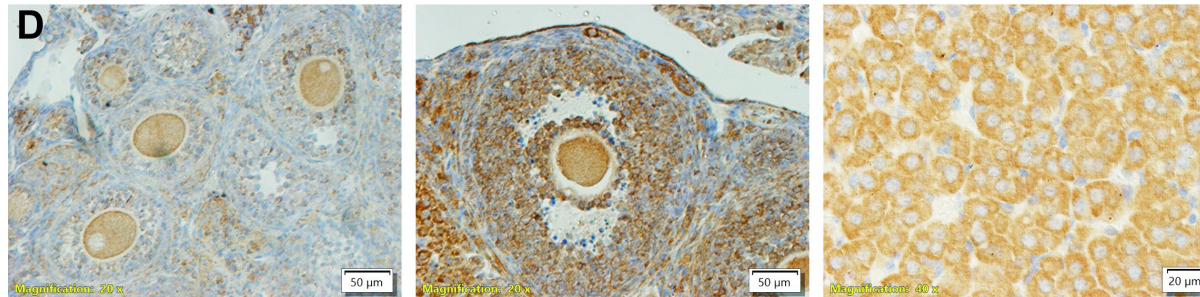
CHRNA2



CHRNA4



CHRNA7



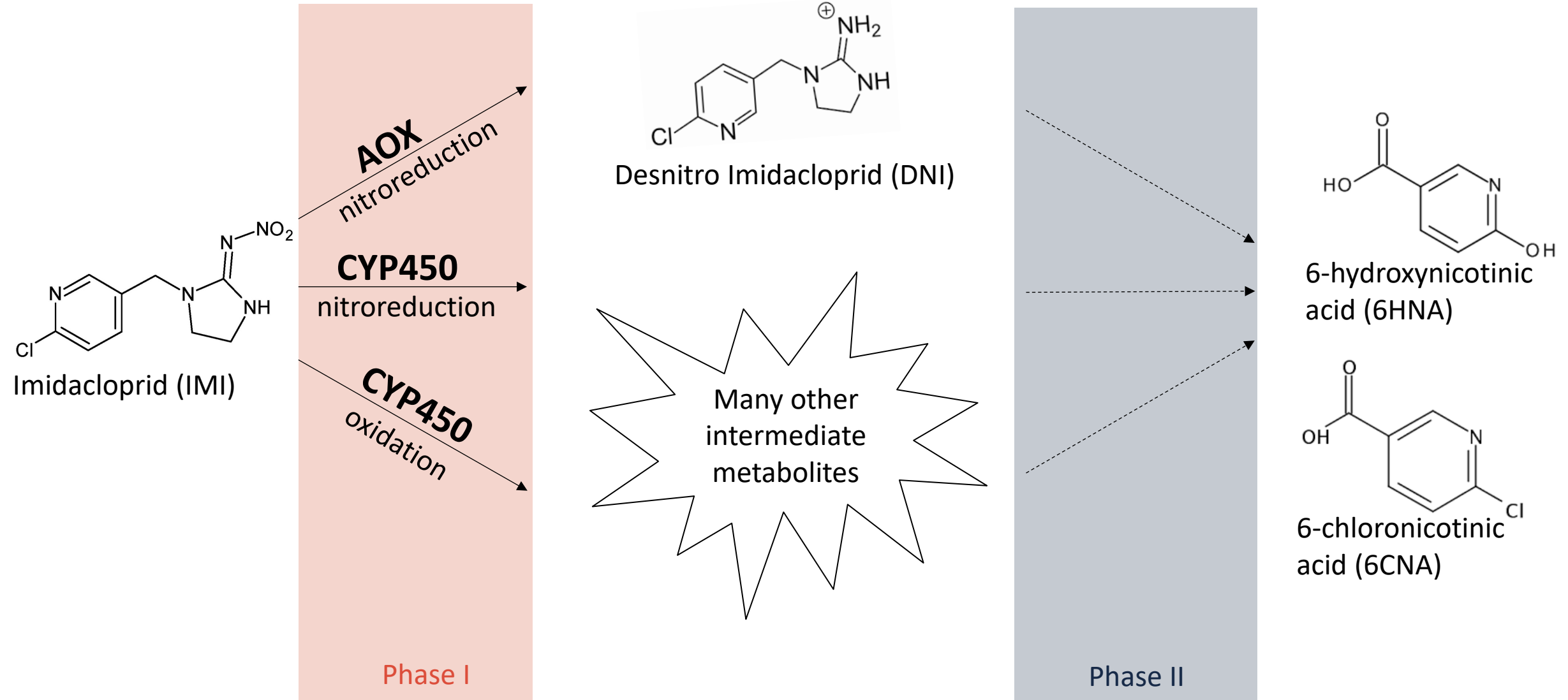
Does the ovary metabolize IMI?



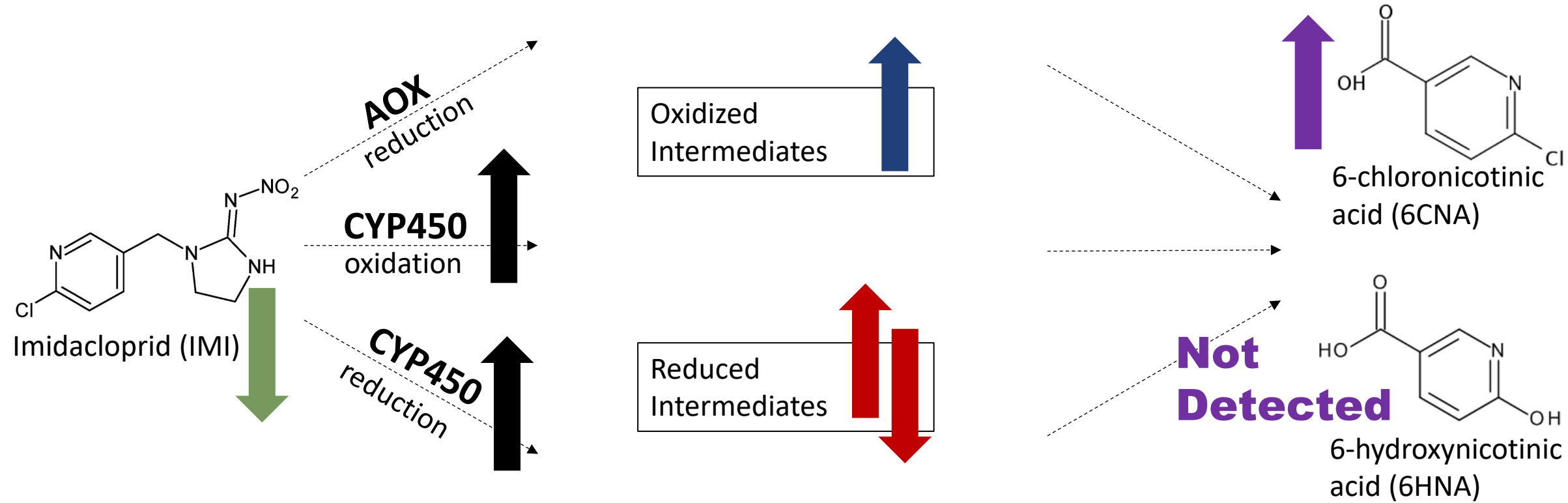
Hypothesis

Ovarian follicles metabolize IMI

Liver Metabolism of IMI



Summary: Ovarian Metabolism of IMI



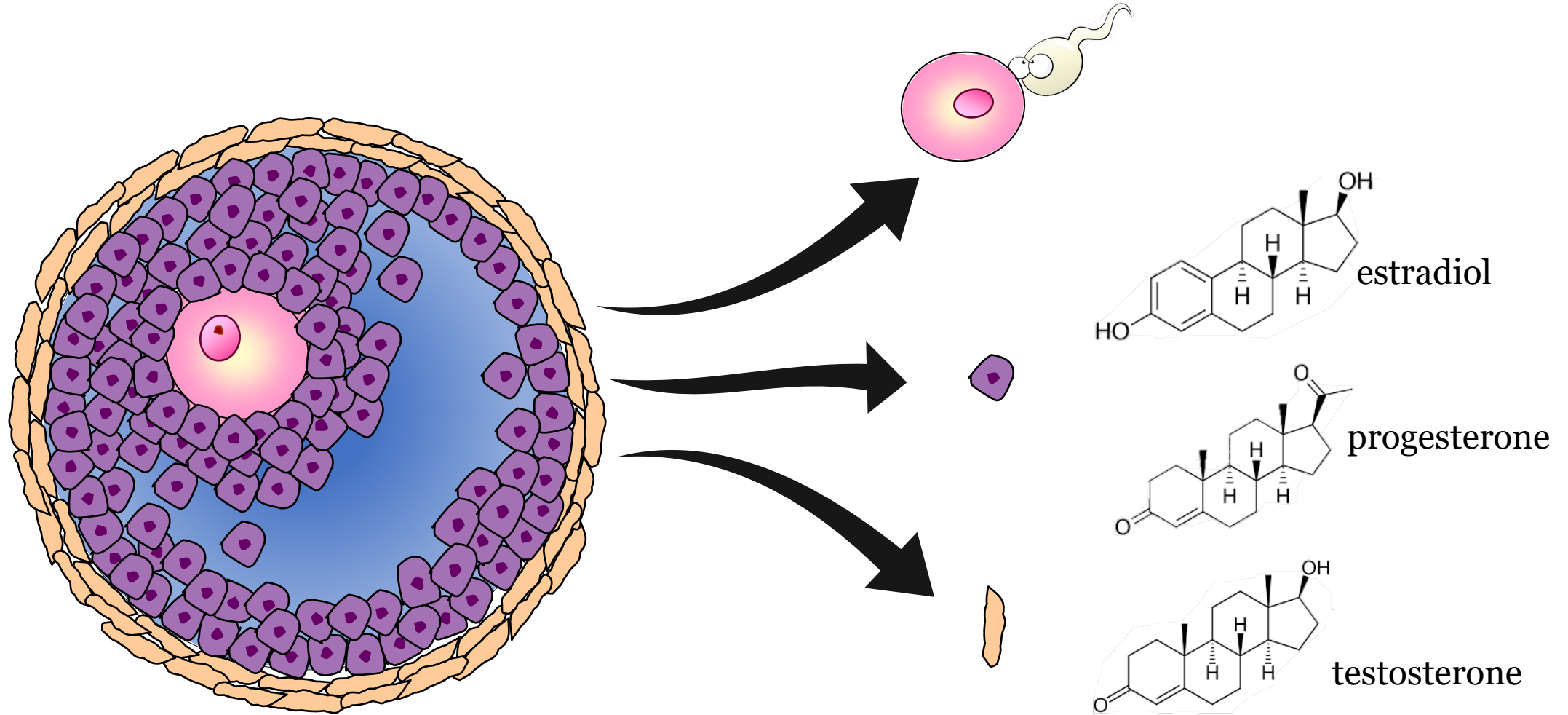
Are IMI and its metabolites toxic to the ovary?



Hypothesis

IMI and DNI are toxic to the ovary

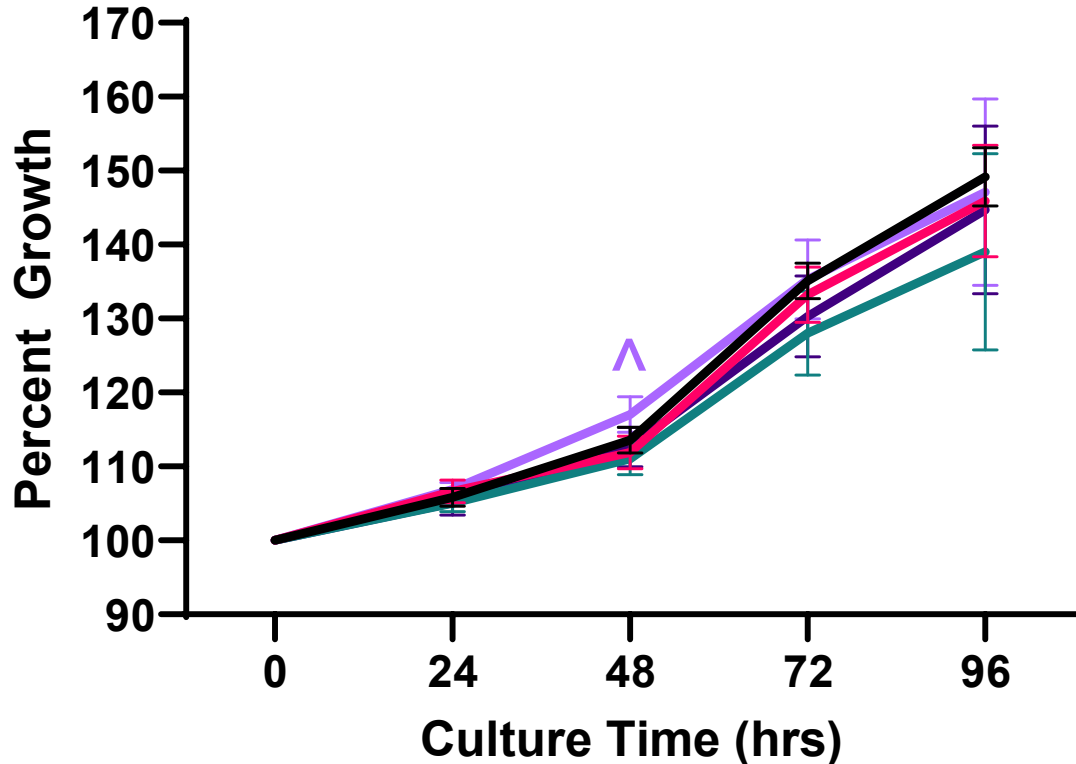
The Ovary: Antral Follicles



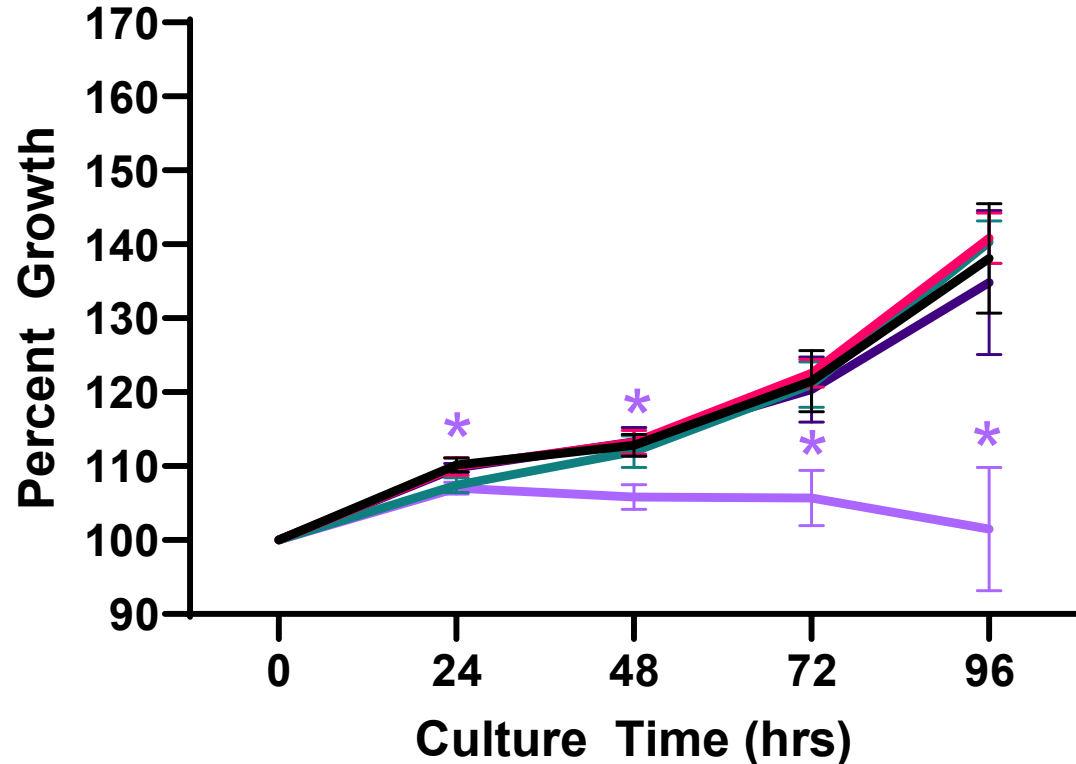
Effects of IMI and DNI on Follicle Growth



IMI



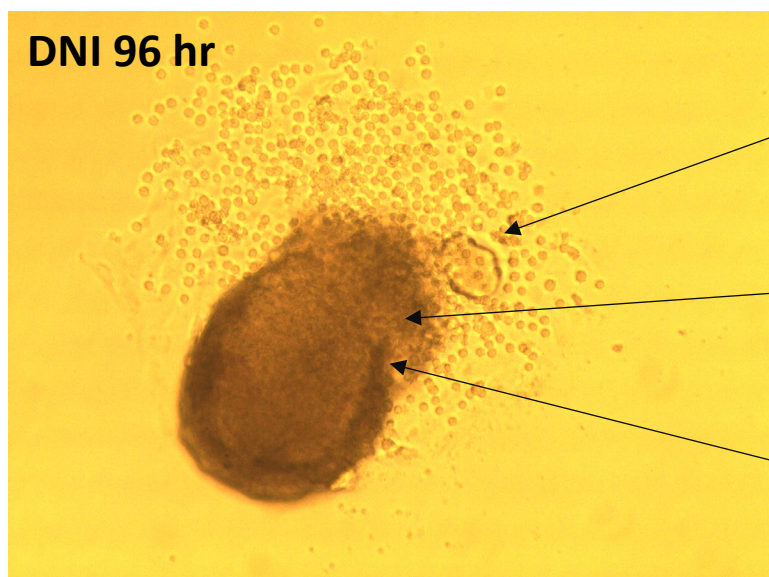
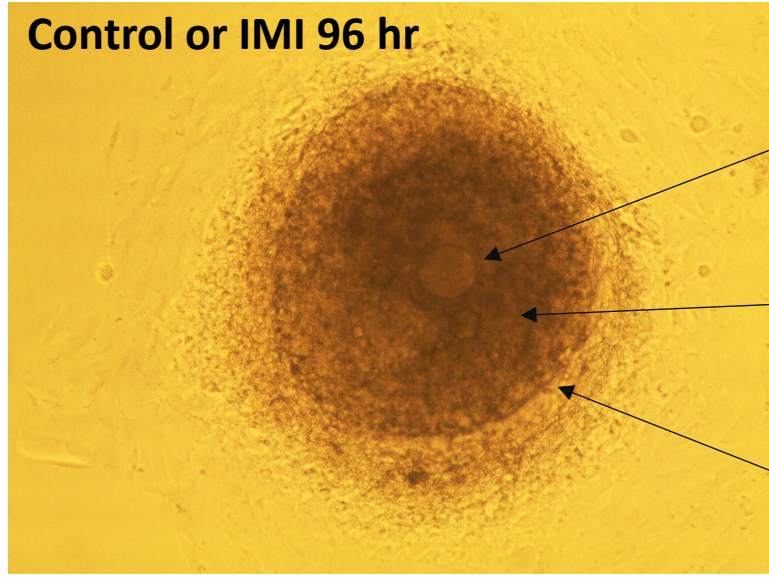
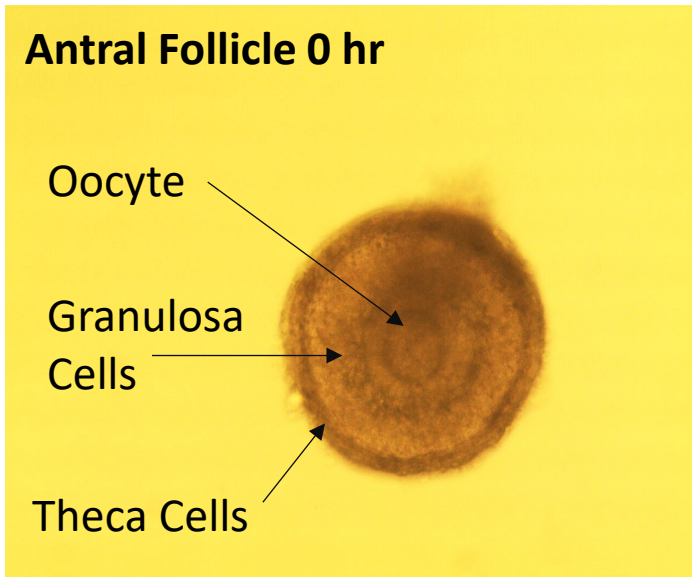
DNI



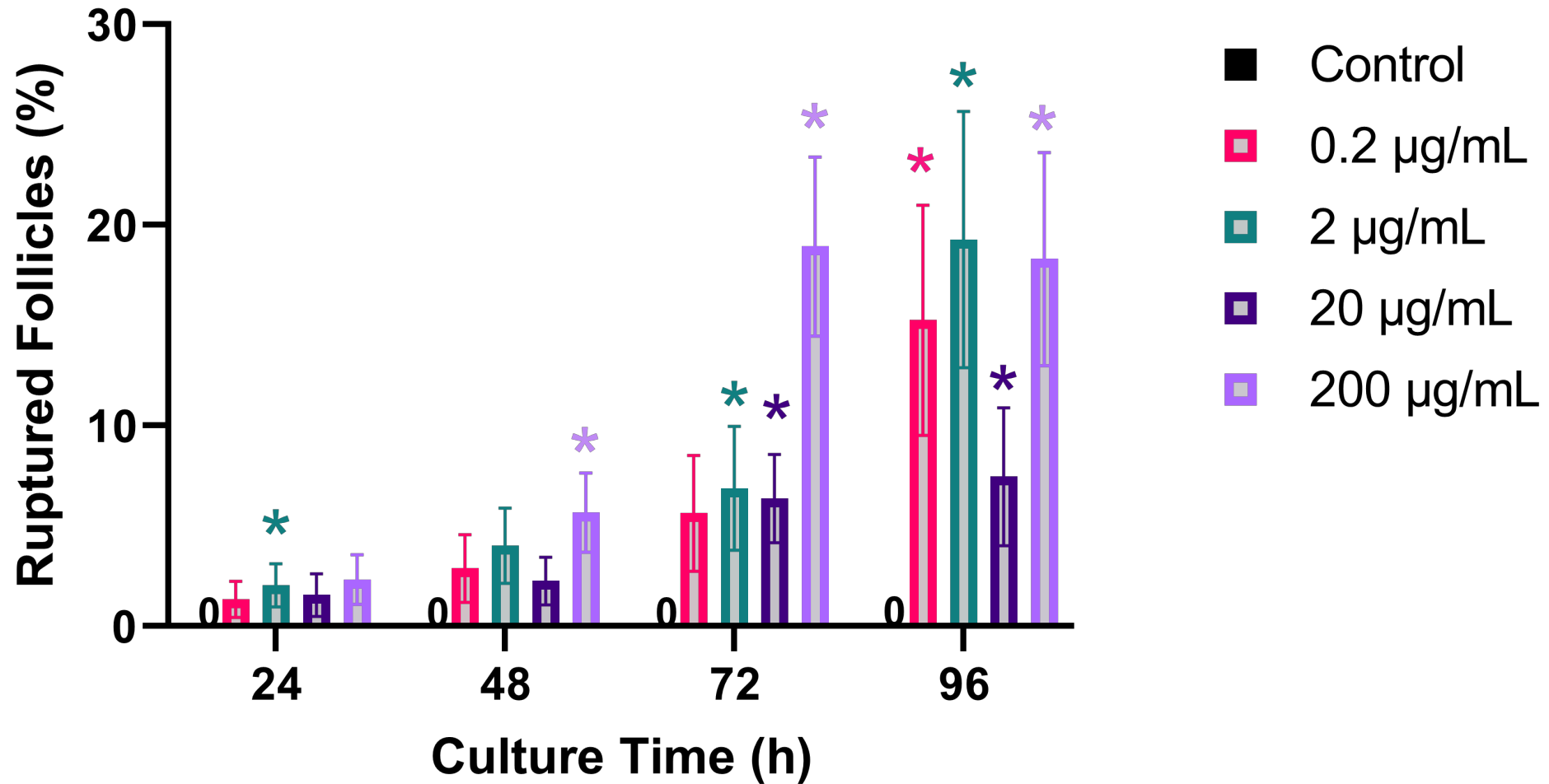
— Control — 0.2 µg/mL — 2 µg/mL — 20 µg/mL — 200 µg/mL

n= 3-4, *p ≤ 0.05, ^ 0.05 < p ≤ 0.1

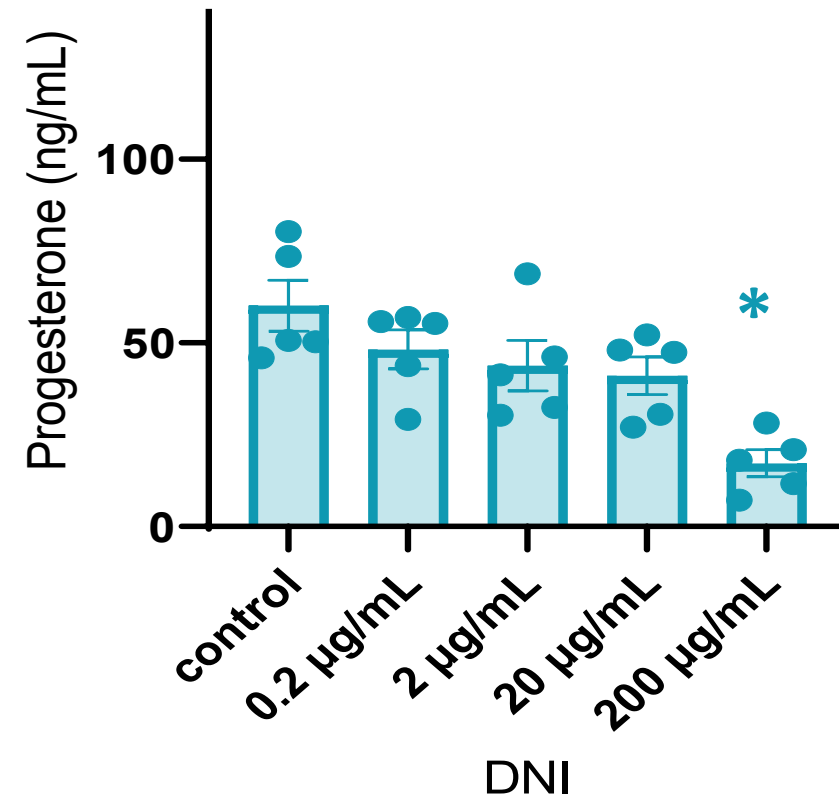
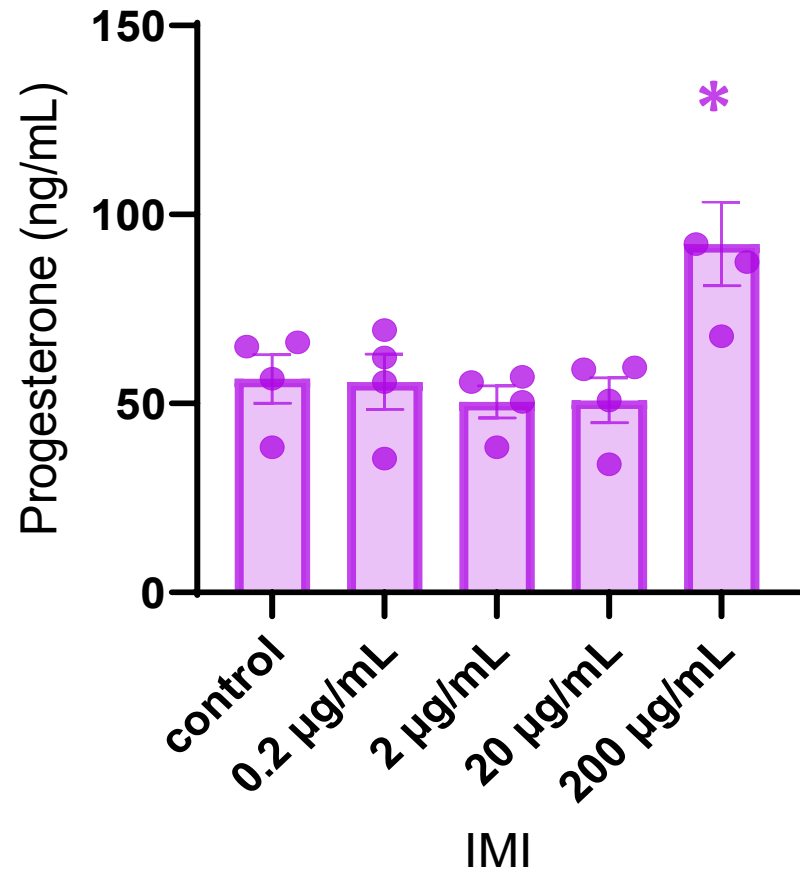
Effects of DNI on Follicle Rupture



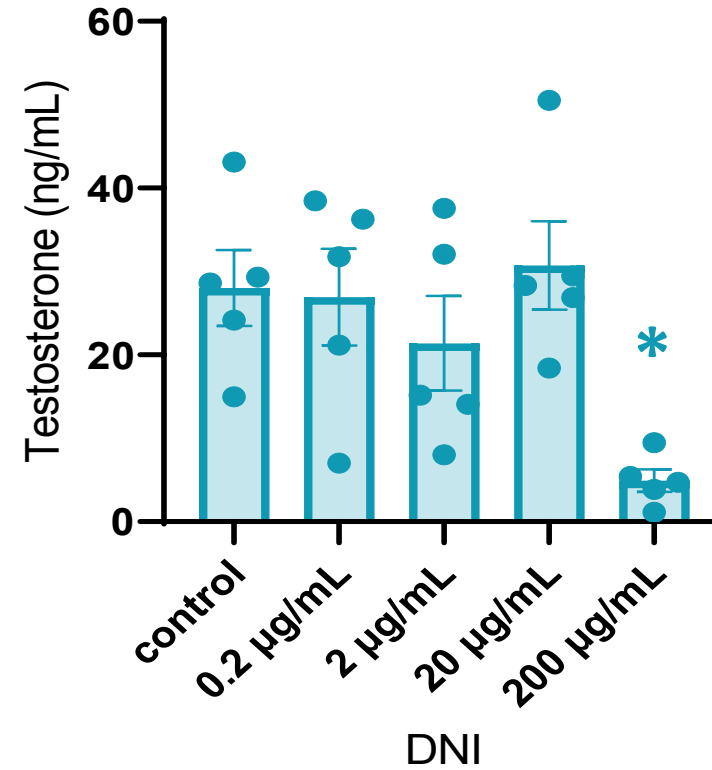
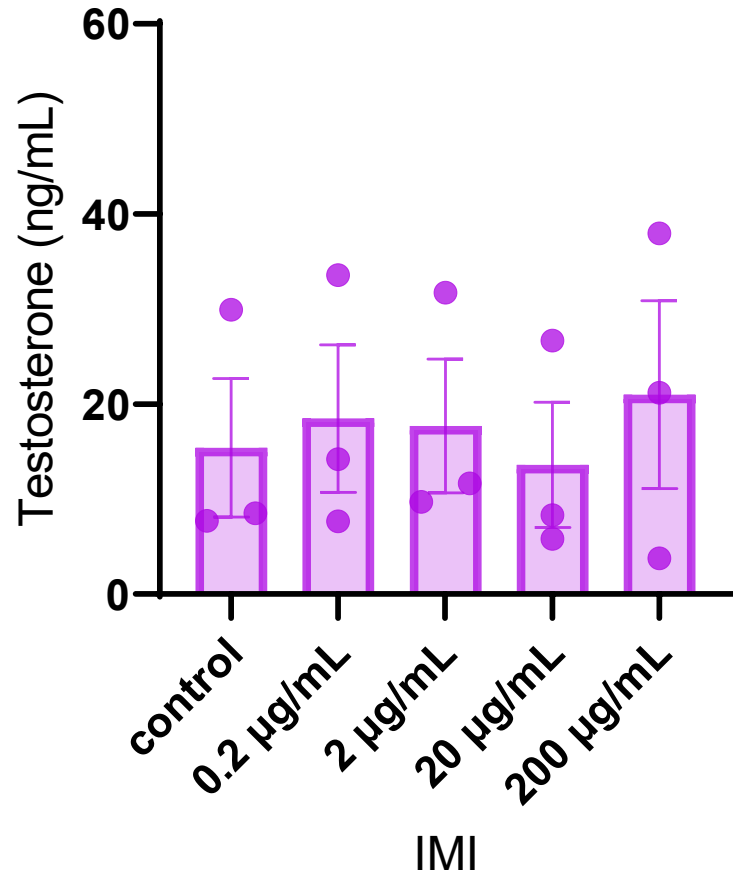
Effects of DNI on Follicle Rupture



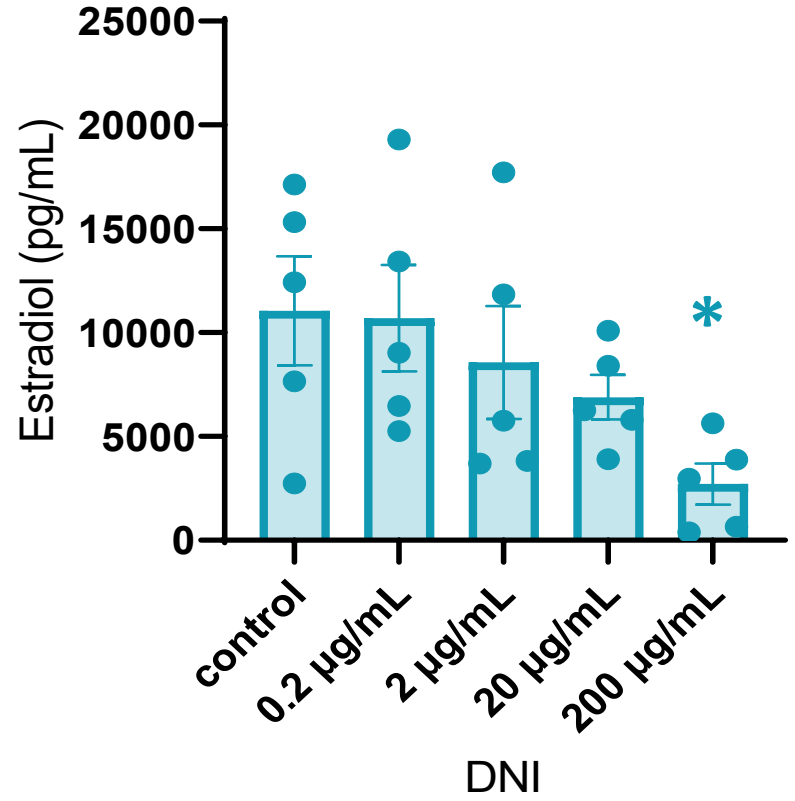
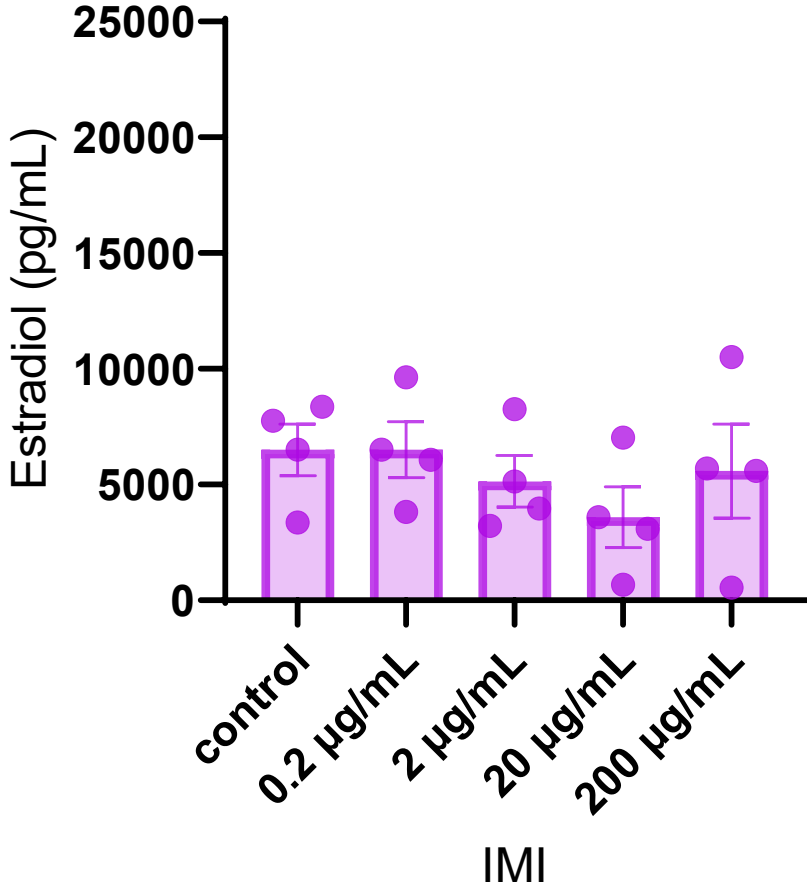
Effects of IMI and DNI on Progesterone



Effects of IMI and DNI on Testosterone

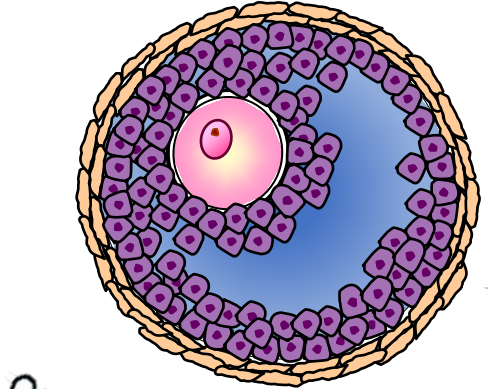
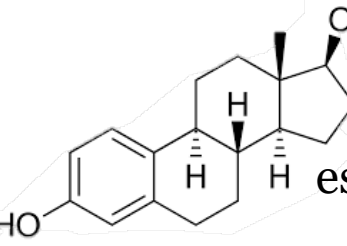
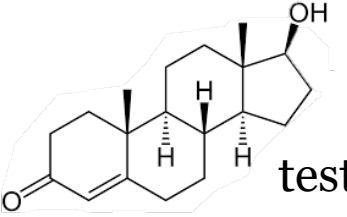
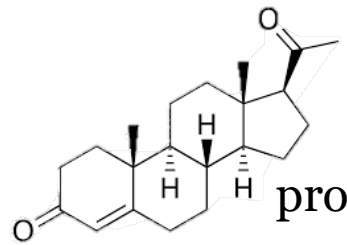
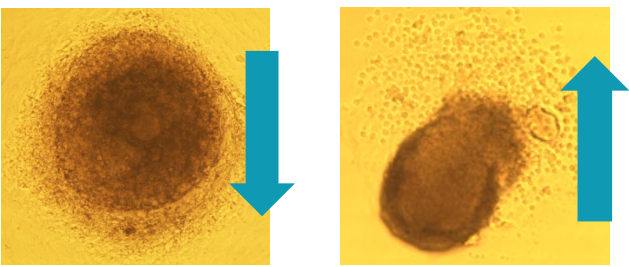
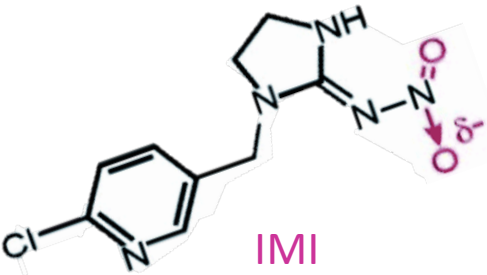


Effects of IMI and DNI on Estradiol



n = 4, *p ≤ 0.05, ^ 0.05 < p ≤ 0.1

Summary: Follicle Toxicity (In Vitro)



IMI
DNI

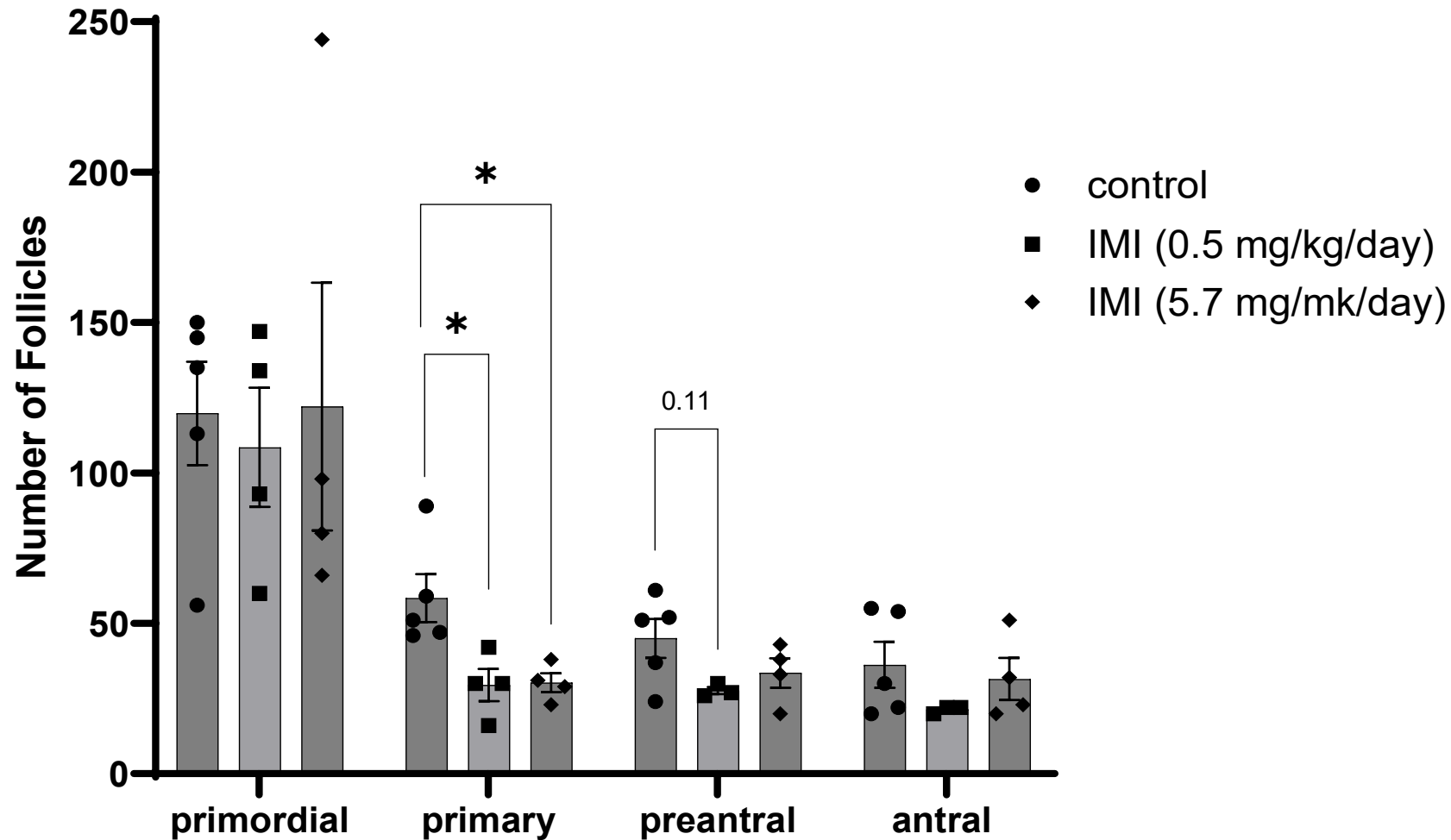
Does the IMI affect the ovary in vivo?



Hypothesis

**IMI adversely affects the ovary in vivo
(follicle numbers)**

Effect of IMI on Ovarian Follicle Numbers



Summary

- Does the ovary have nAChR?
 - YES
- Can the ovary metabolize IMI?
 - YES
- Are IMI and its metabolites toxic to the ovary?
 - YES, but DNI is more toxic than IMI
- Does IMI affect the ovary in vivo?
 - MAYBE, but we need more experiments

Do other neonicotinoid pesticides affect the reproductive system?



Acetamiprid

- Used on fruits, vegetables, cotton, and ornamental plants
 - Maximum acceptable daily intake = 0.7 mg/kg/bw
 - LOAEL = 51 mg/kg/bw/day in male rats
 - LOAEL = 60.1 mg/kg/bw/day in female rats

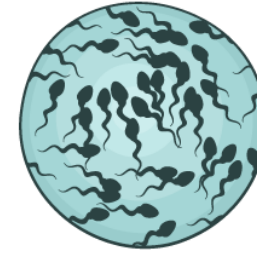


LOAEL

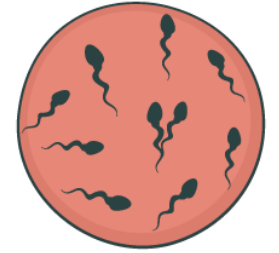


10 mg/kg/day or 20 mg/kg/day for 1 month

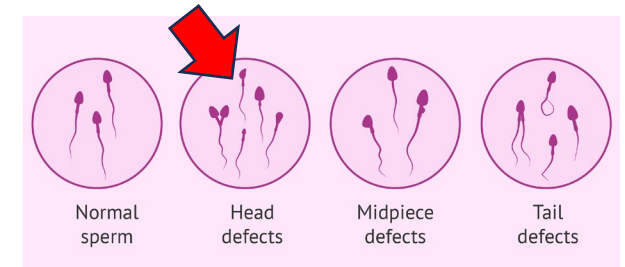
Normal sperm count



Low sperm count



25 mg/kg/day or 35 mg/kg/day for 3 months



35 mg/kg/day for 3 months



12.5-35 mg/kg/day for 3 months

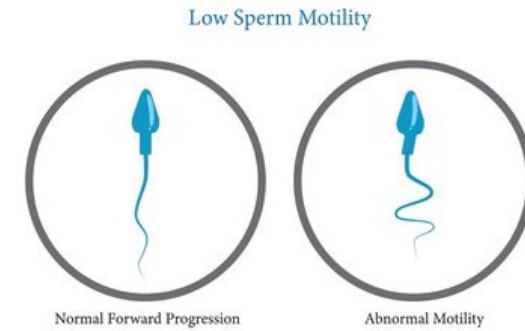
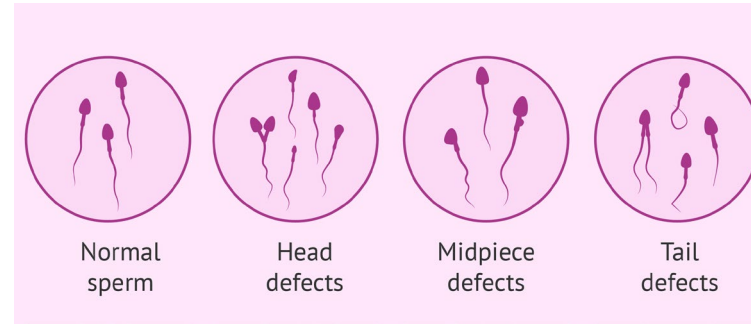
Clothianidin

- Seed treatment use on corn and canola in the US

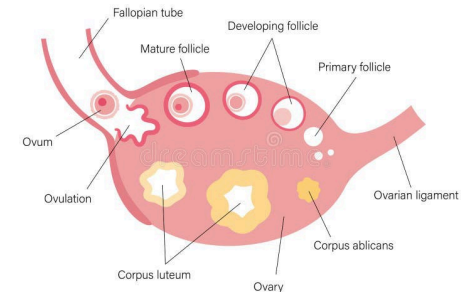
- Maximum acceptable daily intake = 0.1 mg/kg/bw
- LOAEL = 163.4 mg/kg/bw/day in male rats
- LOAEL = 188.3 mg/kg/bw/day in female rats



Transferred from mom to pups in milk
Increased stillborn pups
65 mg/kg/day



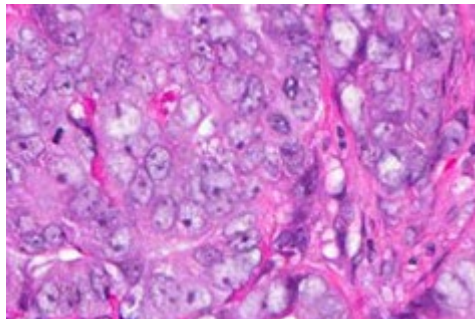
LOAEL



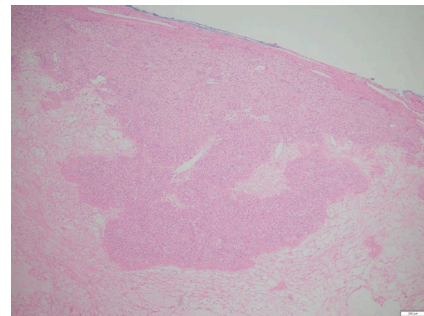
Progesterone
Cortisol

Thiacloprid

- Used on sucking insects on agricultural crops such as cotton and fruits
 - Maximum acceptable daily intake = 0.01 mg/kg/bw

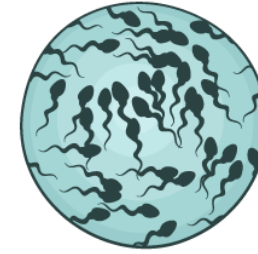


Uterine tumor



Ovarian luteoma

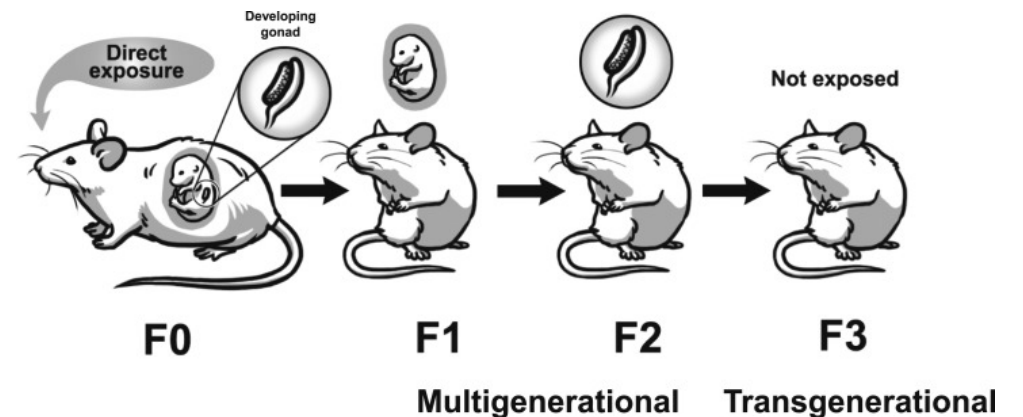
Normal sperm count



Low sperm count

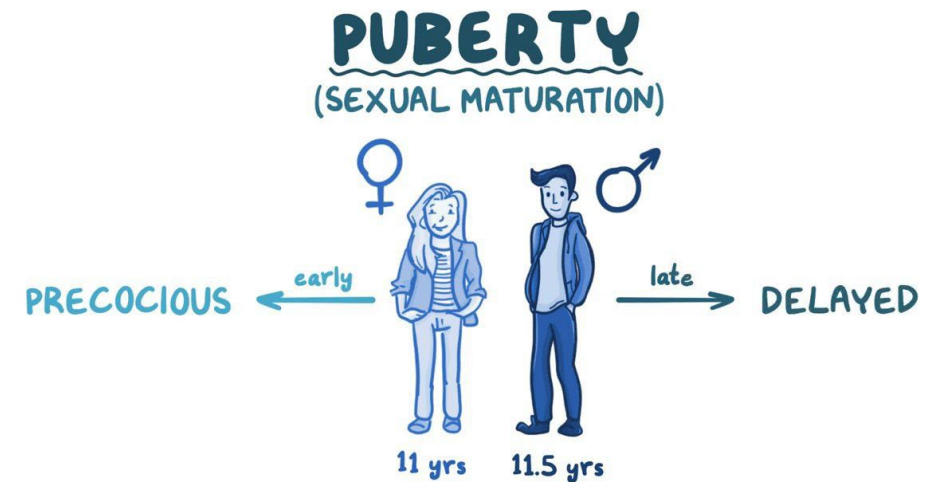
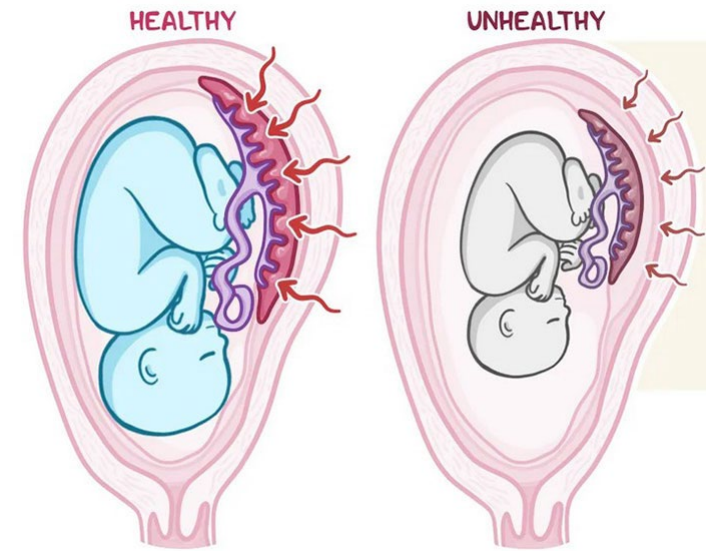


0.6, 6, and 100 mg/kg/bw



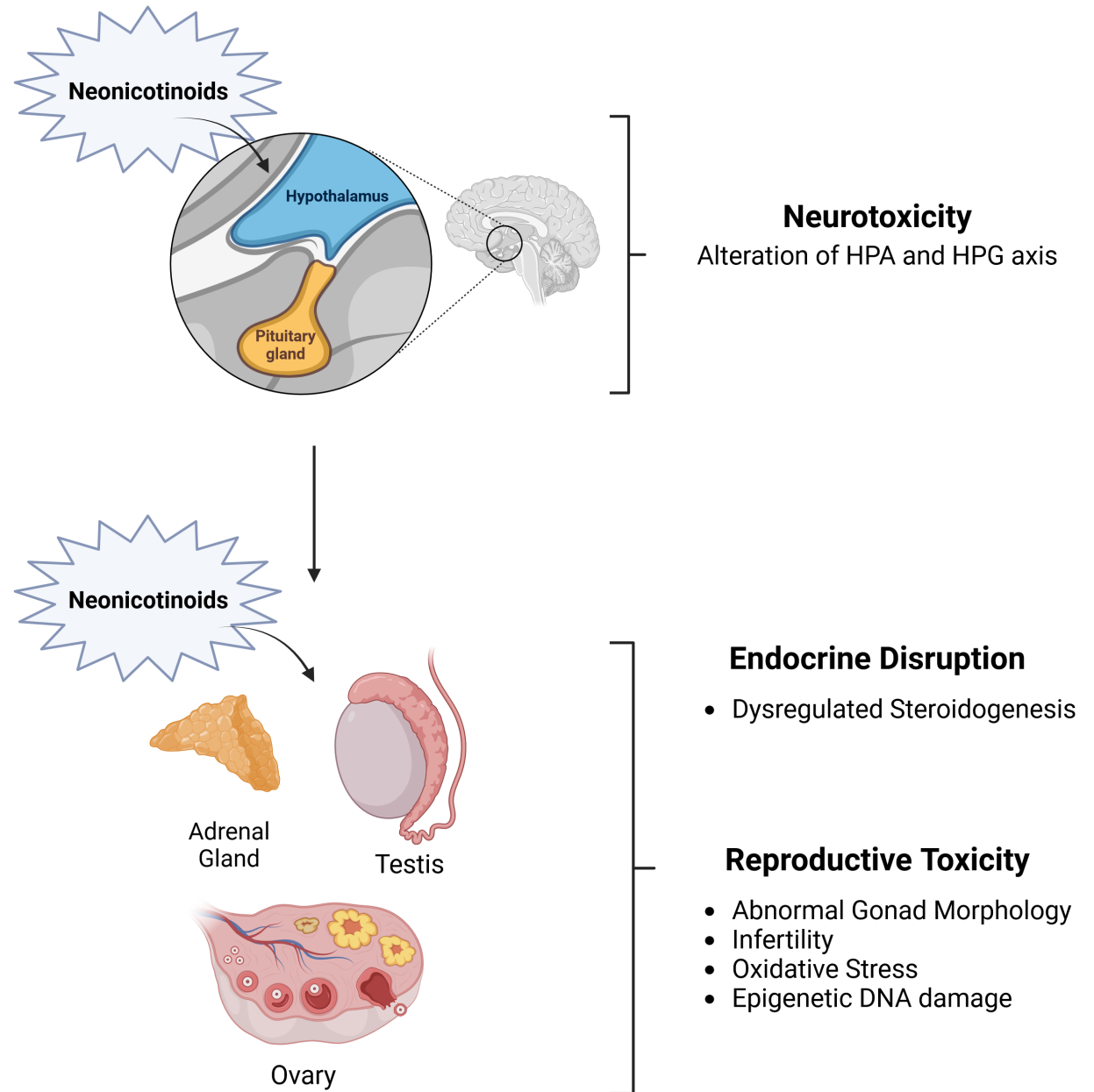
Mixtures – Human Studies

- Estimated daily intake varies
 - Acetaminid = 140.68 - 236.03 ng/kg/bw/day
 - Imidacloprid = 33.67-96.23 ng/kg/bw/day
 - Thiacloprid = 27.11 – 43.11 ng/kg/bw/day
 - Clothianidin = 6.29- 23.14 ng/kg/bw/day



Conclusions

- Neonicotinoids are reproductive toxicants
- Neonicotinoids are toxic to both the female and male reproductive system
- Neonicotinoids interfere with the hypothalamic-pituitary-gonadal axis
- More studies are needed on the effects of neonicotinoids on reproduction



Acknowledgments



Thank You!

Questions?