



Barn Biology: What Sheep and Mice Neurons Are Teaching Us About Our Own Reproductive Axis

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Carlson College of
Veterinary Medicine

Land Acknowledgement

The Portland Metro area rests on traditional village sites of the Multnomah, Wasco, Cowlitz, Kathlamet, Clackamas, Bands of Chinook, Tualatin, Kalapuya, Molalla, and many other tribes who made their homes along the Columbia River. Indigenous people have created communities and summer encampments to harvest and enjoy the plentiful natural resources of the area for the last 11,000 years.

We want to recognize that Portland today is a community of many diverse Native peoples who continue to live and work here. We respectfully acknowledge and honor all Indigenous communities—past, present, future—and are grateful for their ongoing and vibrant presence.

We also acknowledge the systemic policies of genocide, relocation, and assimilation that still impact many Indigenous/Native American families today. As settlers and guests on these lands, we respect the work of Indigenous leaders and families, and pledge to make ongoing efforts recognize their knowledge, creativity, and resilience.

- Adopted 06/12/23 from the Portland Parks Foundation

Roxanne

Year: Fourth

Major: Bioengineering

Interests: Creative

writing, hiking,

photography, and

reproductive research.



Blake

Year: Fourth

Major: Biology

Minor: Spanish and Chemistry

Option: Pre-med

Interests: Piano, running,

surfing, and brains!

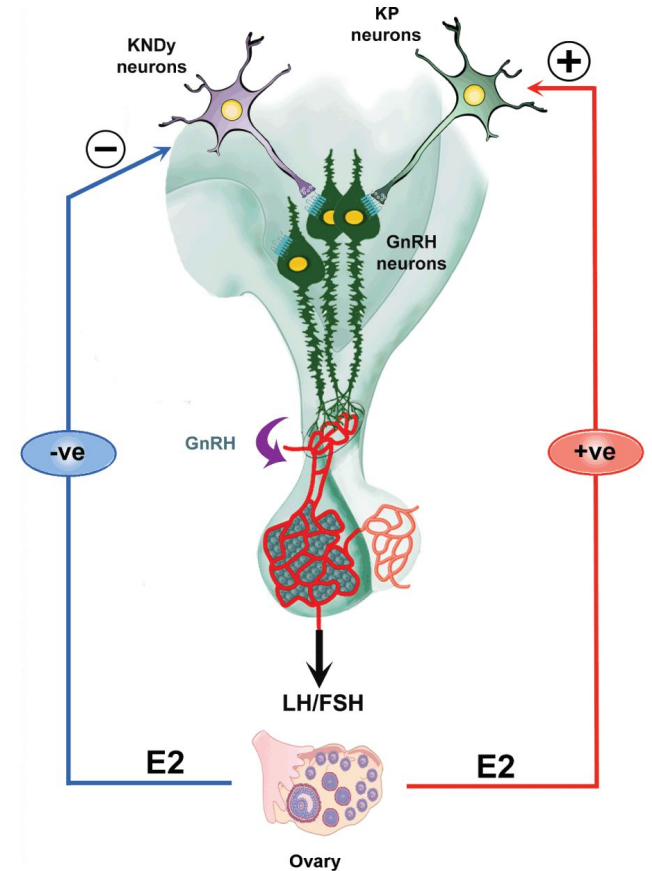
The Chappell Lab

Located on the Corvallis campus of Oregon State University, the Chappell laboratory is an animal physiology lab in the Carlson College of Veterinary Medicine led by Dr. Patrick Chappell. We study the reproductive axis and hormone pathways within it.

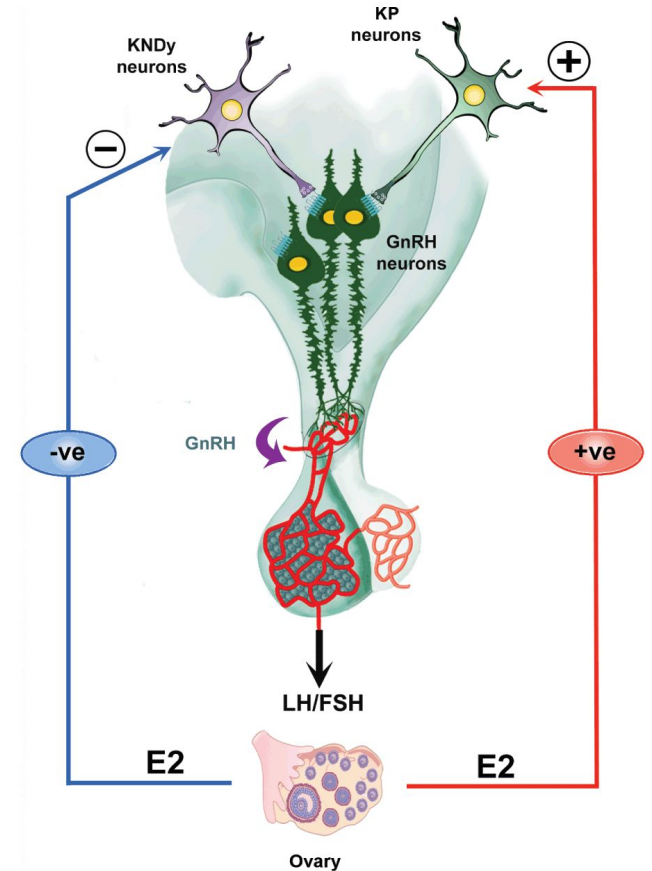


HPG? What's that?

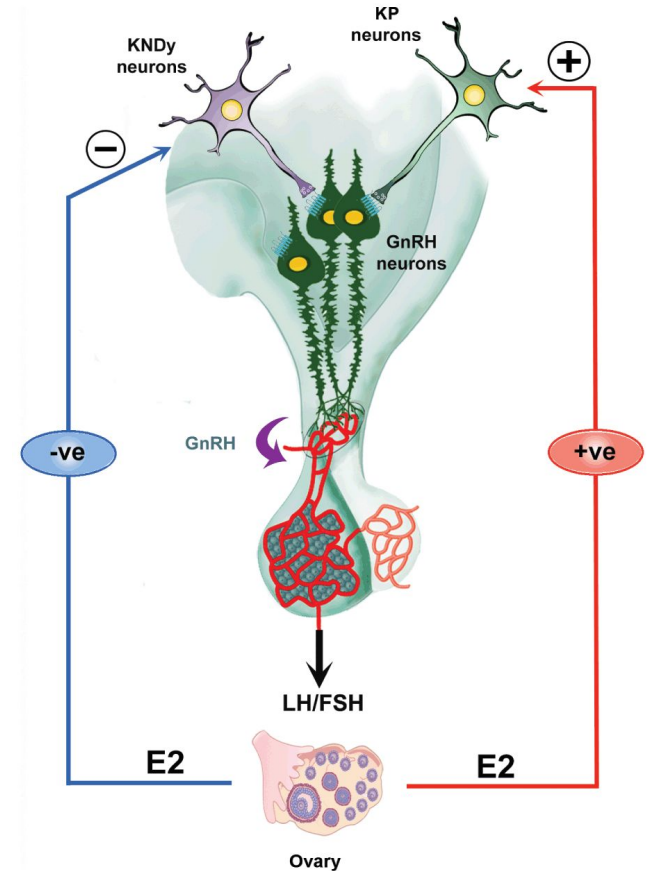
The term “HPG axis” stands for Hypothalamic–pituitary–gonadal axis. We will sometimes refer to it as “the reproductive axis” or just “the axis”.



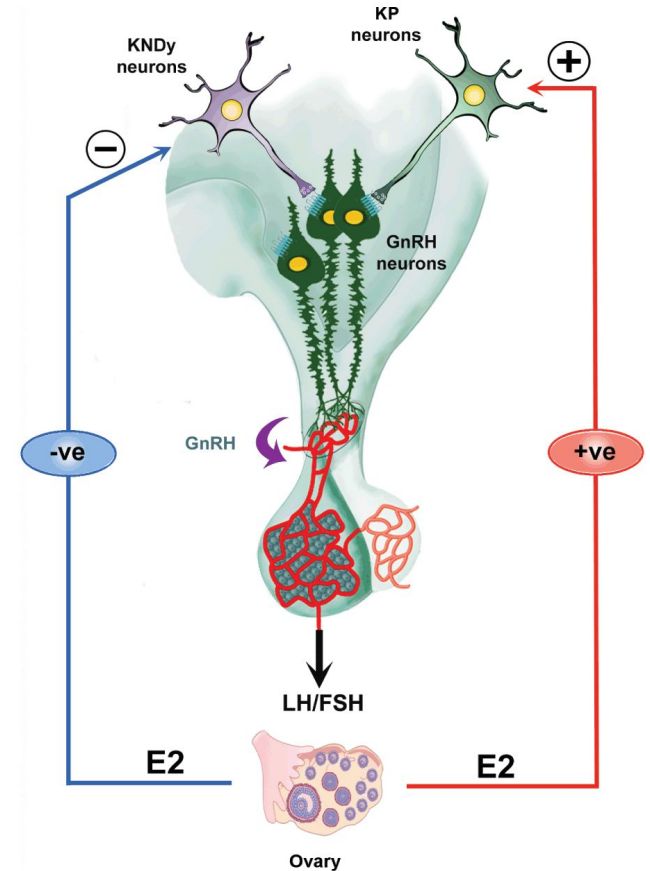
The hypothalamus is a region of the brain responsible for hormone regulation.



The pituitary gland secretes hormones (LH/FSH) that stimulate the gonads. It is a point of interest in hormone regulation.



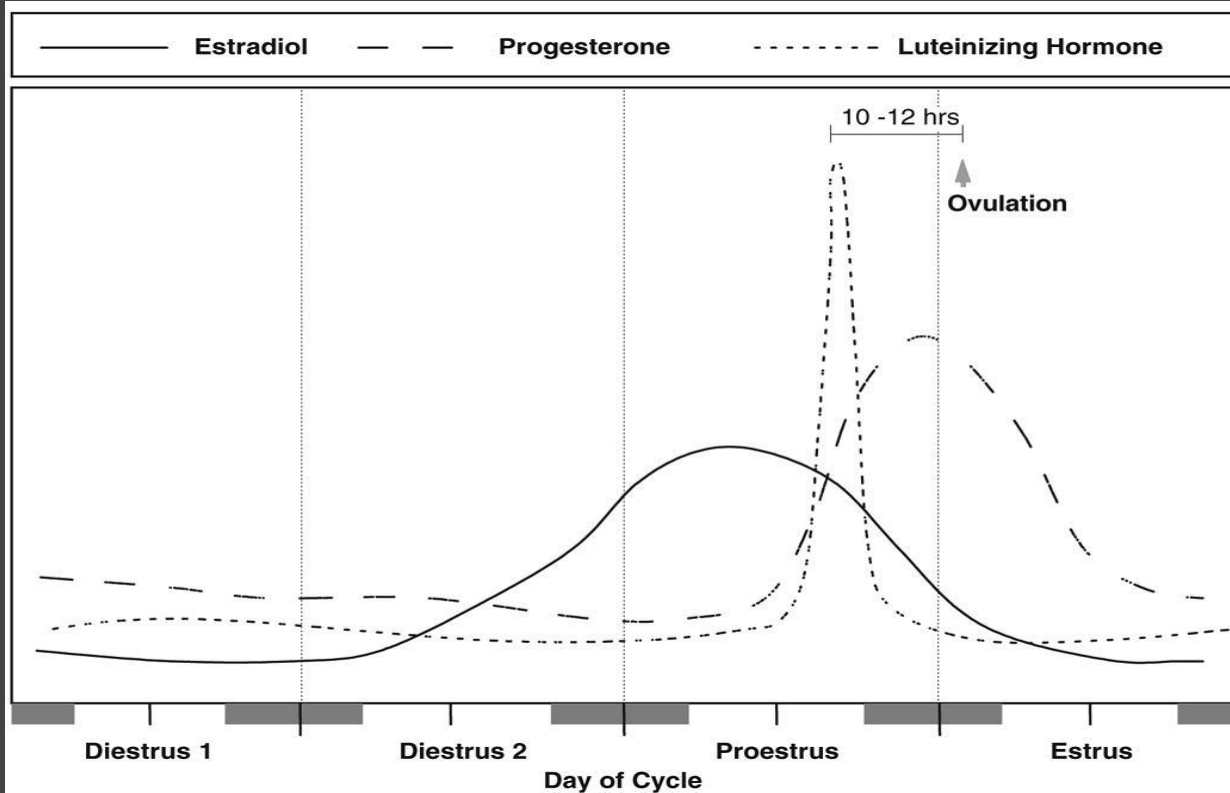
The gonads are primary sex organs (testes and ovaries), which receive signals from the pituitary gland to secrete sex hormones. We specifically care about estrogen.





Why do we care about estrogen?

(Its kinda weird that's why)



**Are sheep or mice a “better”
representation of our (human)
HPG?**

We're asking you!



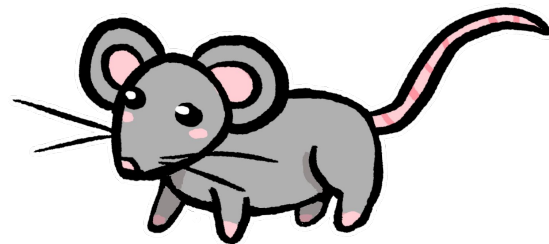
Neither!

What makes these cell lines distinct?



Similarities

- Mammalian/Vertebrates
- Full HPG
- Have estrous cycle
 - Same phases
- Two-parent sexual reproduction

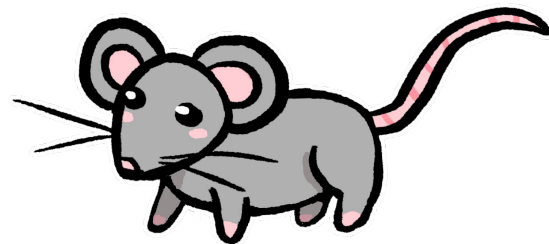


What makes these cell lines distinct?



Differences

- Different length estrous cycles
 - Sheep > Mice
- Sheep are short day breeders
- Mice are opportunistic breeders
- Lifespan

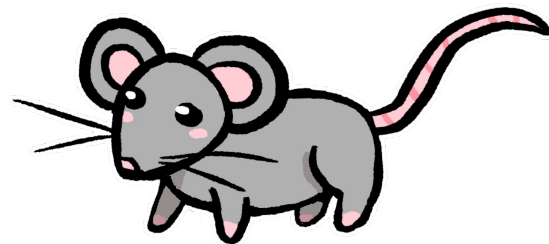


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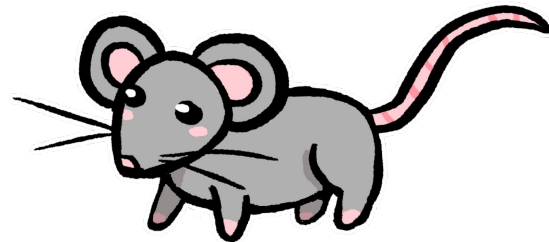
Humans are opportunistic breeders like mice, but have longer estrous cycles, similar to sheep.

Humans	~1 month
Sheep	~2.5 weeks
Mice	~5 days

What makes these cell lines distinct?



Mice models have a history of significance in reproductive/endocrine research. While the mechanisms underlying the interaction of circannual timing with the HPG axis in seasonal breeders such as sheep remain unclear.



TIMELINE of Cell Culture

Human Cervical
Cells (HeLa)

1951

1963

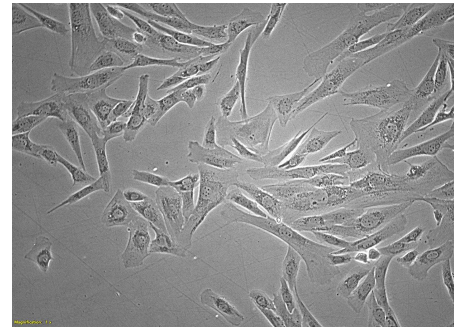
Murine
Fibroblast (NIH
3T3)

2019

Ovine Epithelial
(oAECs)

2023

Noggin Fest!



Mice and Sheep cells:

- Our laboratory previously generated immortalized neuronal models of murine kisspeptin (Kiss-1) neurons in vitro.
- The KTaR-1 cell line was derived from Arcuate KNDy neurons, and the KTaV-3 line from the AVPV of a female kiss1-GFP mouse.
- These *in vitro* models possess many characteristics of Kiss-1 neurons of female mice in vivo.
- We have continued this work by generating immortalized ovine kisspeptin/neurokinin/dynorphin (KNDy) neurons.
- Our oMBH and oPit cell lines were immortalized from fetal sheep brain using similar T antigen lentiviral infection strategies as before.



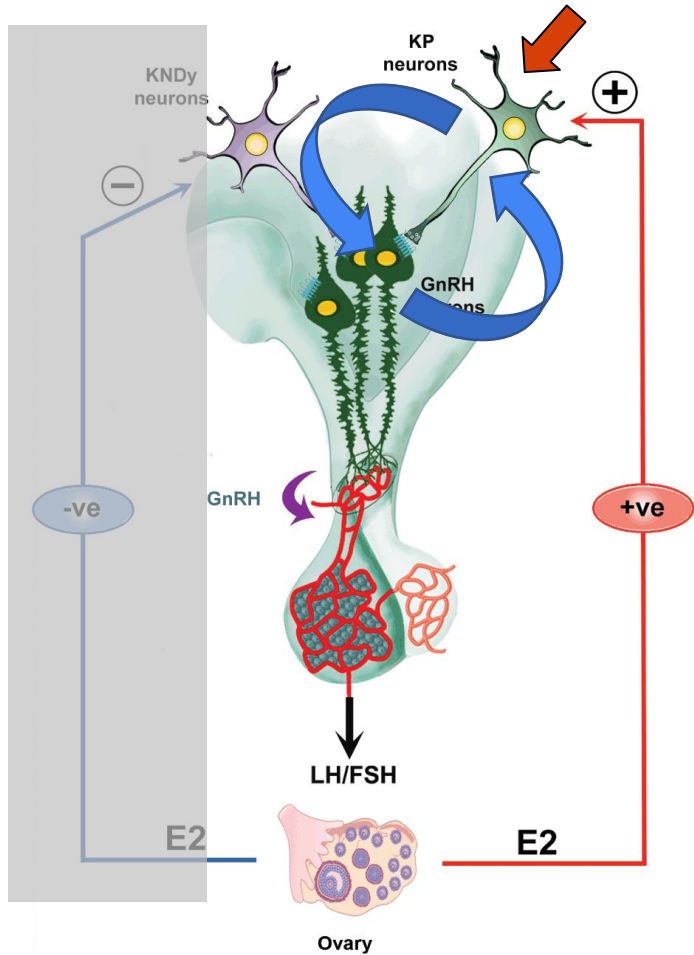
Mice and Sheep cells:

- The genes: Levels of *kiss1*, *tac3*, and *pdyn*, as well as the receptors *tacr3* and *opkr1*, and steroid hormone receptors *esr1* (estrogen receptor α), *esr2* (estrogen receptor β), and *pgr* (progesterone receptor) were all probed under baseline conditions and varying concentrations of sex steroid hormone exposure.
- The presence of these genes, which are also present and commonly evaluated in mice models, supports these cells as a valid ovine model of KNDy neurons.

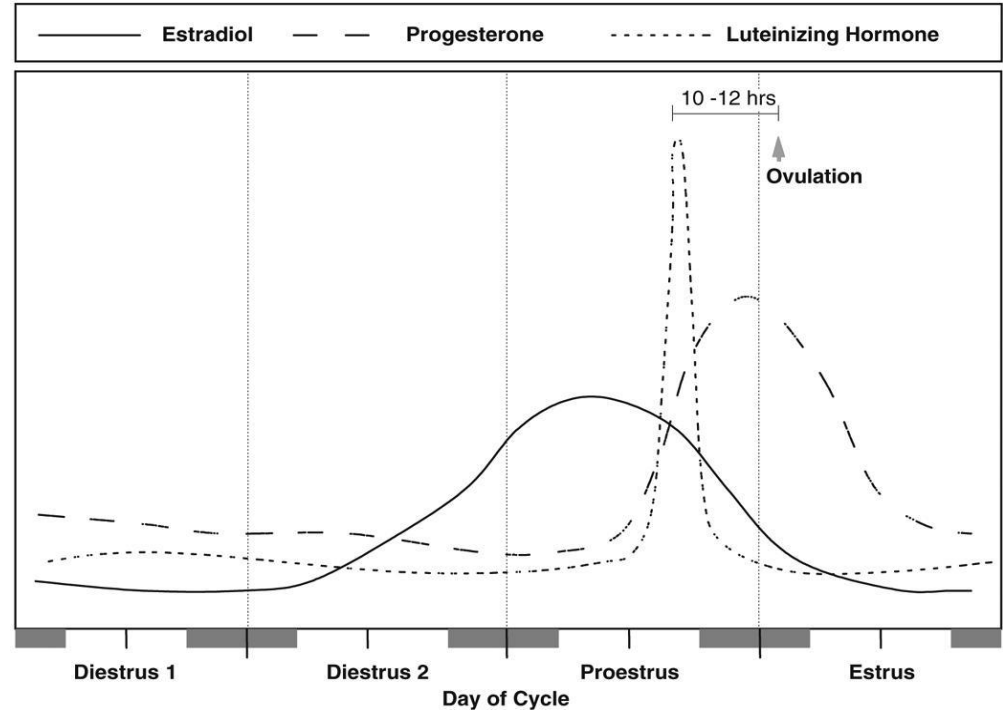




What is the Chappell lab
researching and why?
(Just part of what we're doing)

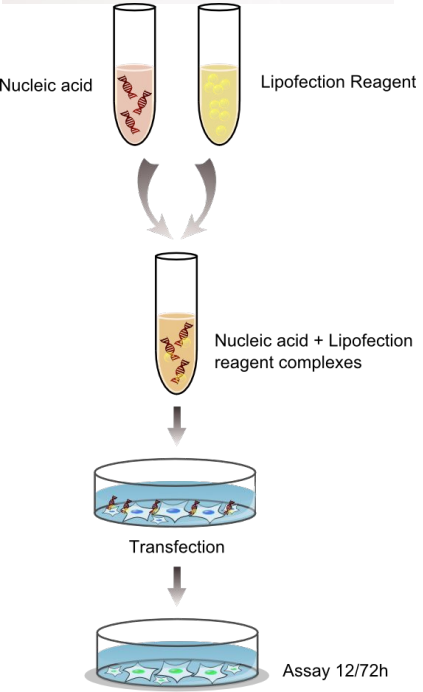
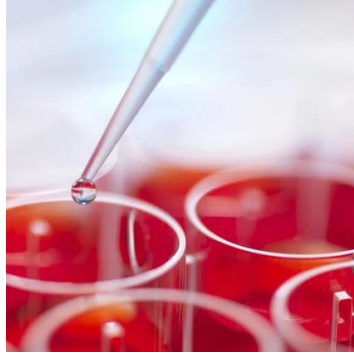


Do Kiss-1 neurons express GnRH receptor in immortalized mouse cell lines under the appropriate conditions? What regulates this expression?



Methods

RT - PCR



Isolate total RNA or mRNA



Anneal anchored oligo (dT) primers,
random primers or specific primer



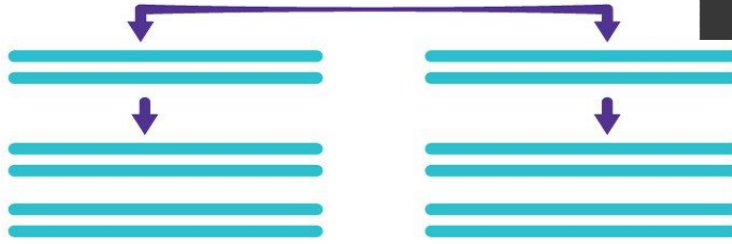
First strand synthesis



First cycle PCR



Amplify by PCR



Marker

samples

A B C

1,200 bp

1,000 bp

900 bp

800 bp

700 bp

600 bp

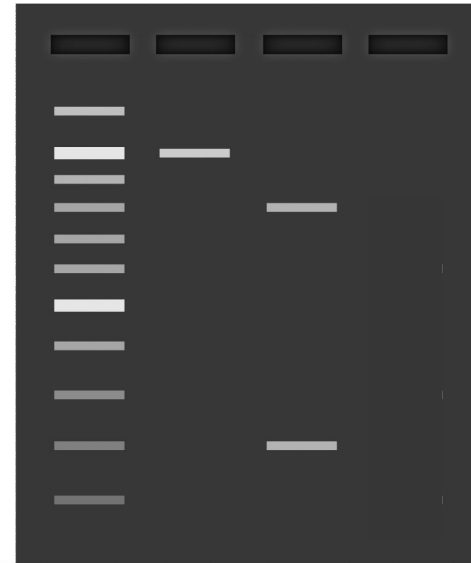
500 bp

400 bp

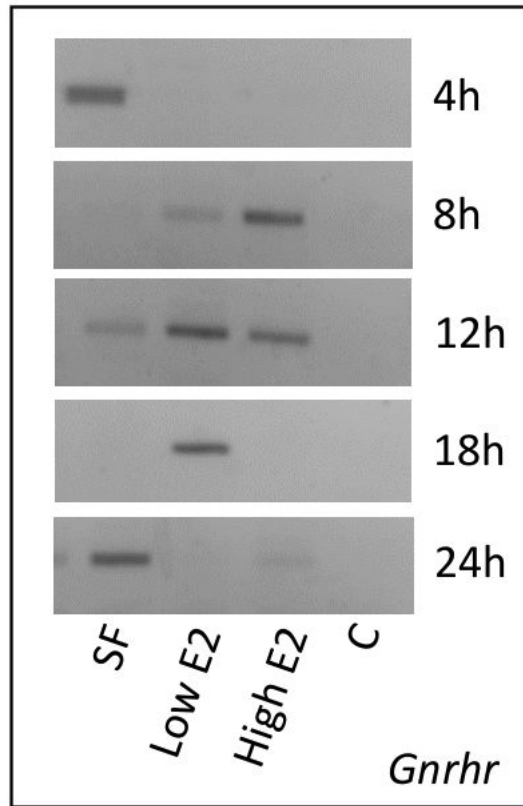
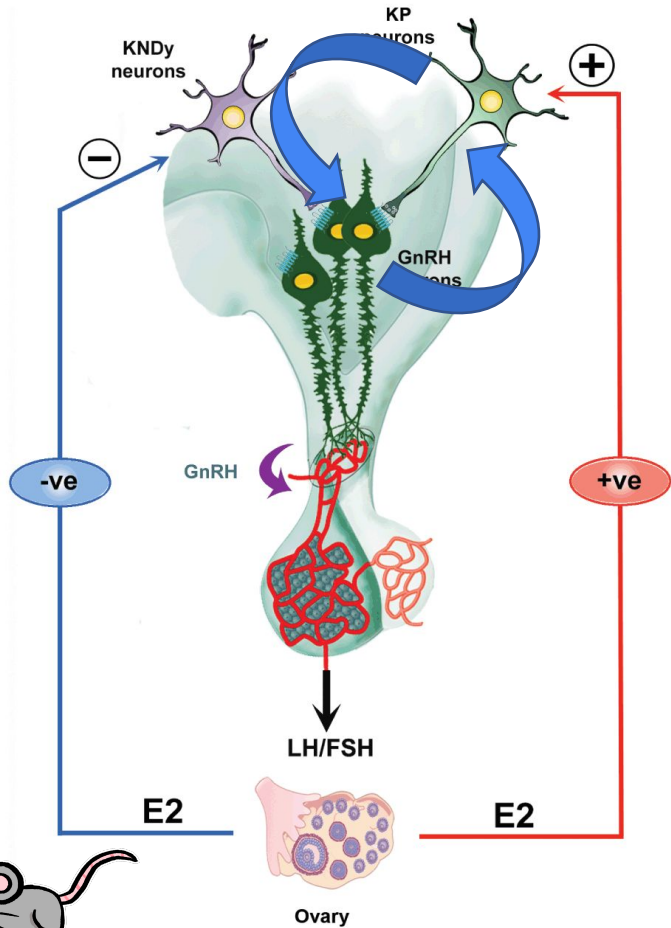
300 bp

200 bp

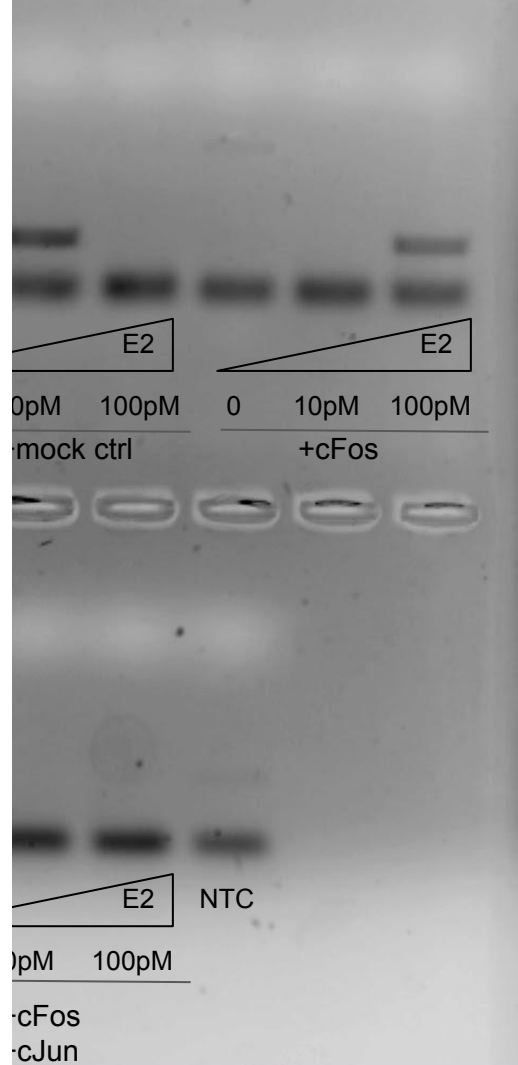
100 bp



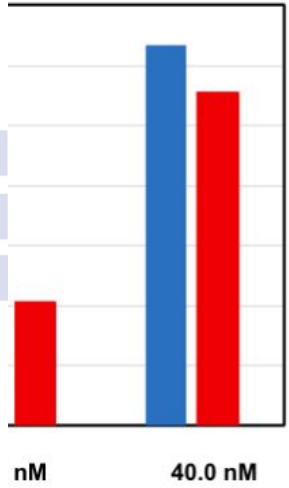
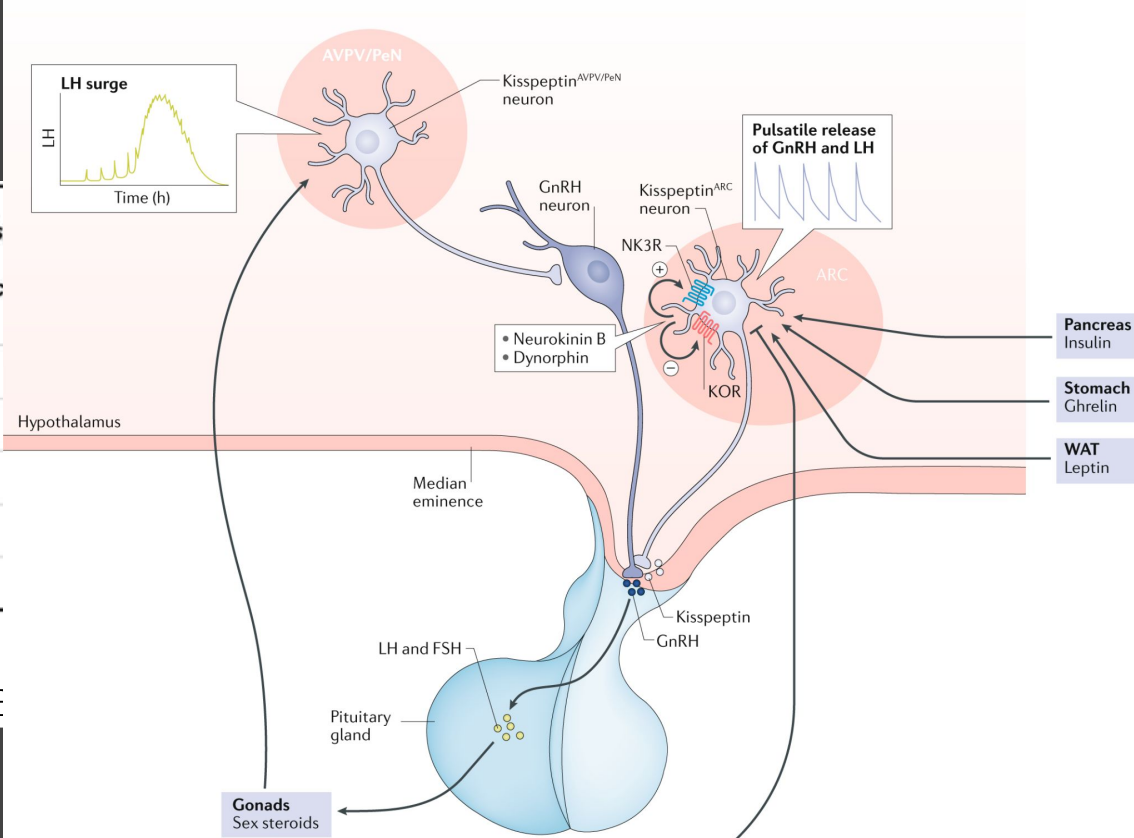
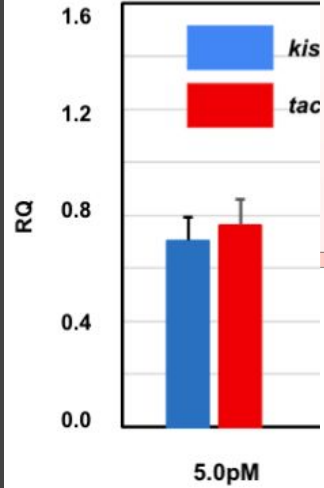
KTaV-3



Low E2: 10.0 pM
High E2: 100.0 pM



Do E2 and P4 Change Expression of Kiss1 and Tac3?



E

D

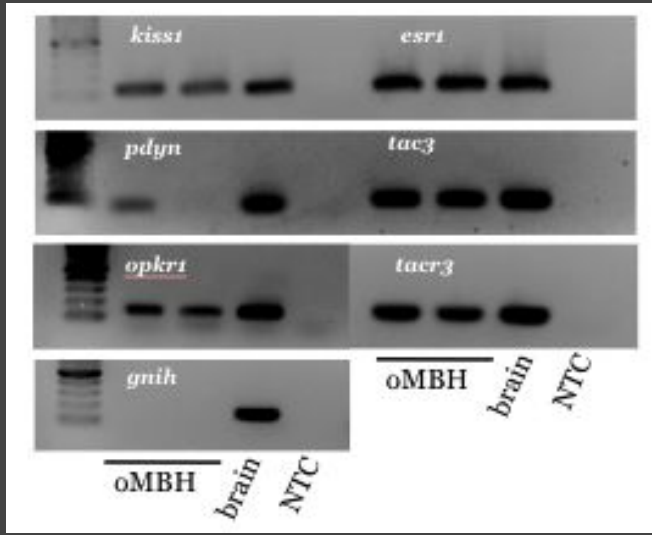


FIGURE 5.

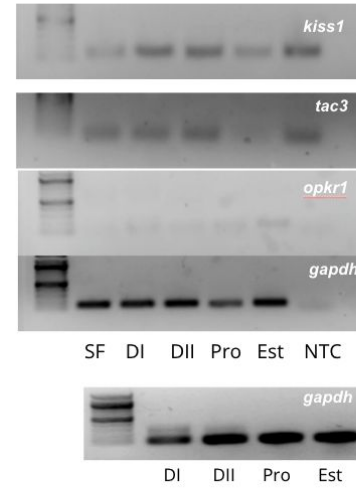


FIGURE 6.

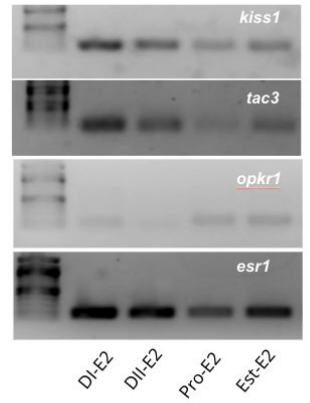


FIGURE 8. Estrous cycle *Kiss1* expression

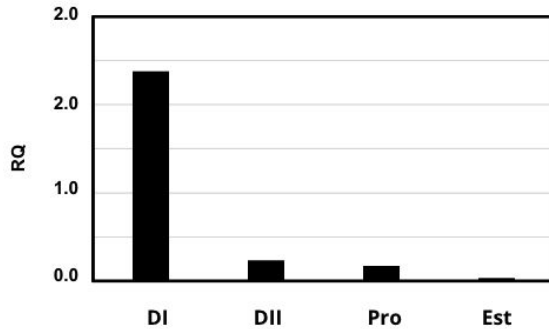
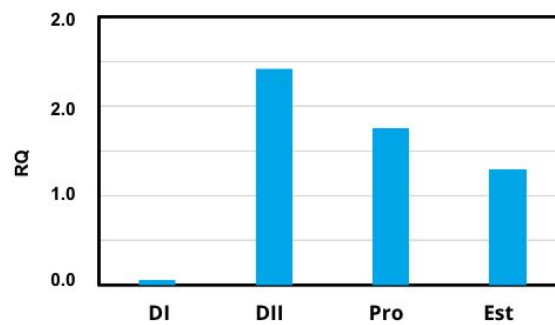
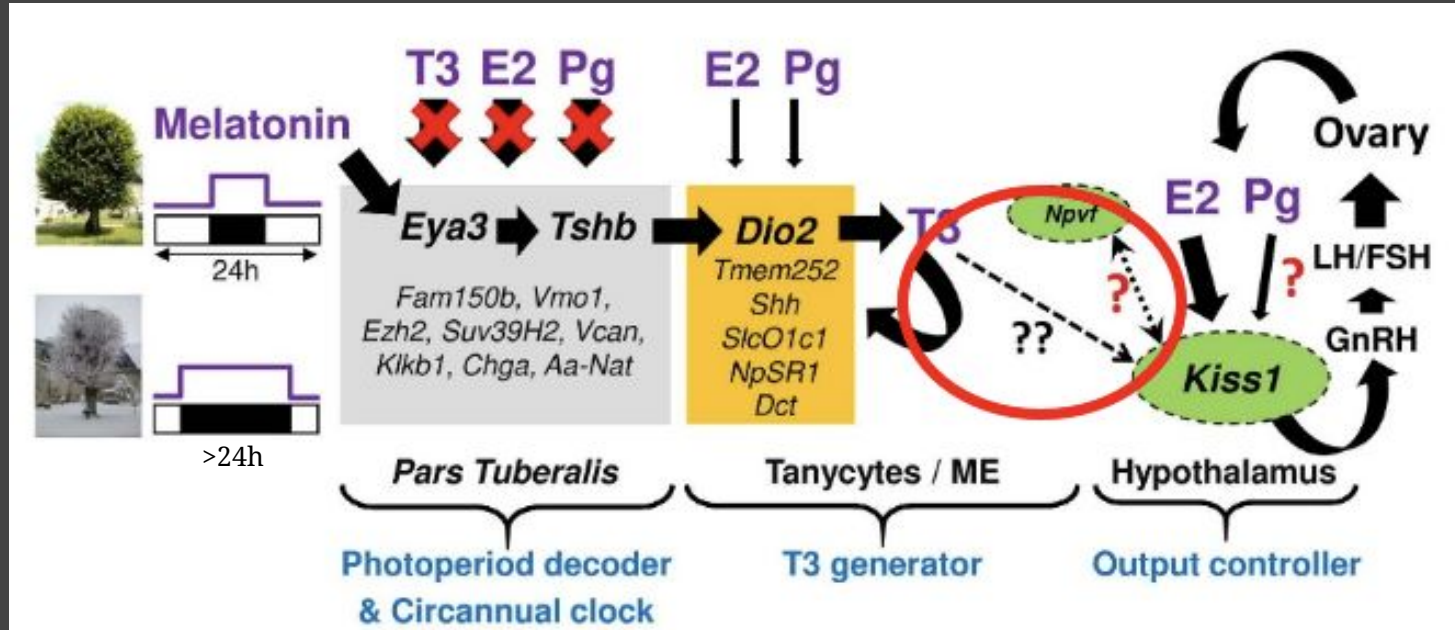


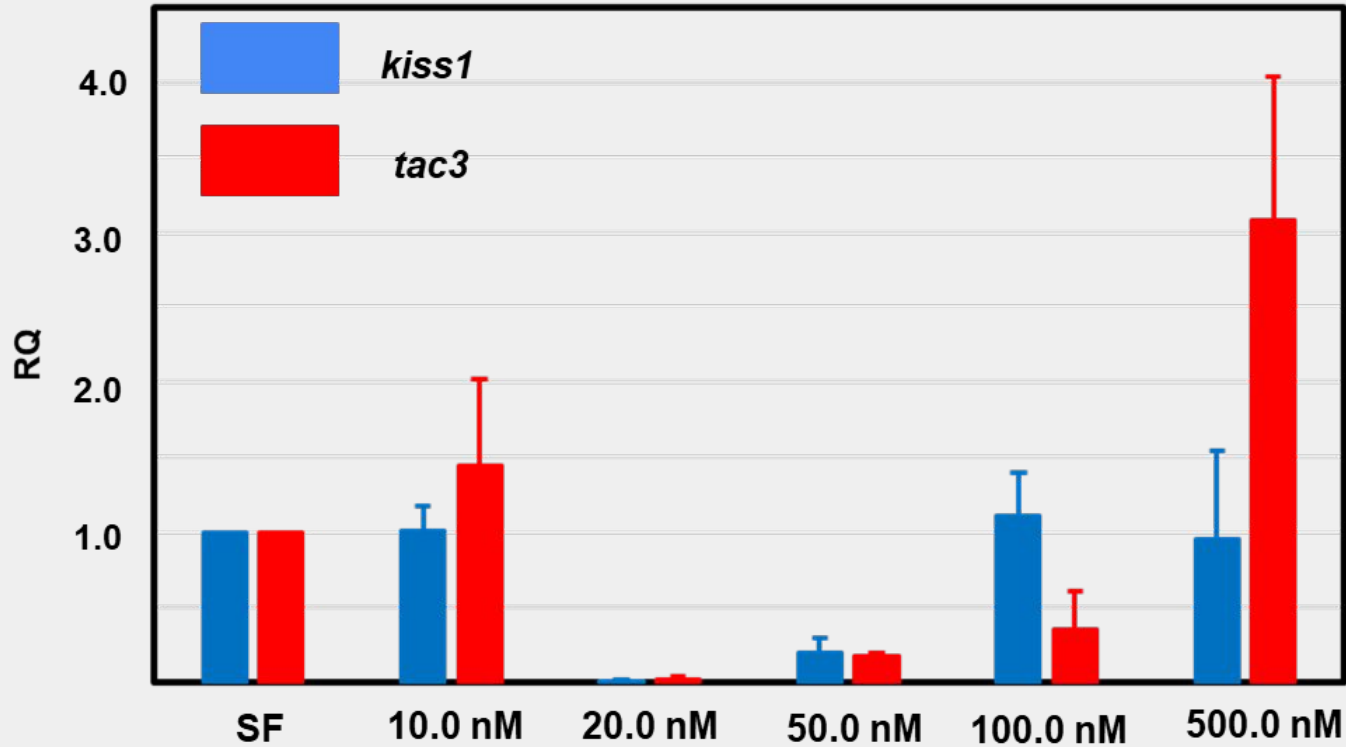
FIGURE 9. Estrous cycle *Tac3* expression



Could thyroid hormone be modulating Tac3 and Kiss1?

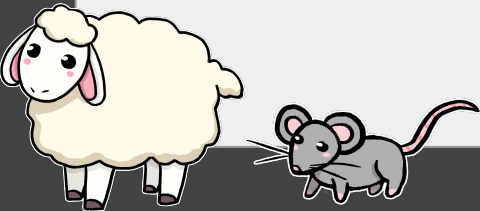


Thyroid Hormone as seasonal signal to KNDy neurons



What Can We Take Away From The Chappell Lab?

- Expression of other genes (Fos and Jun) could be the reason behind how the Kisspeptin neurons create the LH/FSH surge
 - E2 and P4 strongly regulate kisspeptin function and gene expression
 - In seasonal breeders, thyroid hormone could be the cue to the HPG axis that it's time to start reproducing!



**Now that you've learned about
sheep and mice models, what other
animals do you think would serve as
accurate representations to the
human HPG?**

We're asking you again!

Monkeys & Guinea Pigs

Monkeys & Guinea Pigs

Monkeys are primates; with longer life spans who menstruate.

Guinea pigs exhibit invasive implantation like primates, while the steps that follow implantation are more similar to rodents.

Thank you!

This research and presentation would not have been possible without the help and hard work of many people. Special thanks to:

- Dr. Patrick Chappell
- Dr. Charles Roselli (OHSU)
- Charles Estill (OSU Dept. of Clinical Studies)
- Rebekka Toyoizumi
- Alia Starman
- Emily Rodriguez
- Noa Rayzman
- Teagan James
- Jessica Ewton
- Varsha Karthikeyan

We will now take questions :)

What are Kiss and Tac?

Verify with Pat what you think this is...

Tac3 is a genetic precursor to tachykinin, which help control GnRH release, at least in part through actions on Kiss1 neurons. If the Kiss1 neurons are producing tachykinin, then they could be self-regulating their stimulation of GnRH. (include NKB and Dynorphin)

Kiss is the gene that encodes for the production of kisspeptin, which has many functions in the HPG axis, one of which is the inhibition of GnRH (when signal comes from the ARC kiss neurons)