

Welcome collaboration

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**Discoveries of new receptors on bovine gonadotrophs:
possible new mechanisms to control reproductive functions.**

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Outline

Background



GnRH receptor on lipid raft



Membrane Estrogen receptor, GPR30



RNA-seq etc. to discover new receptors



Recent advance



Outline

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GnRH receptor on lipid raft



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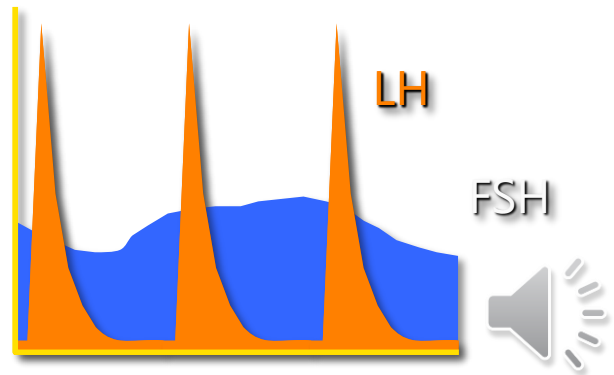
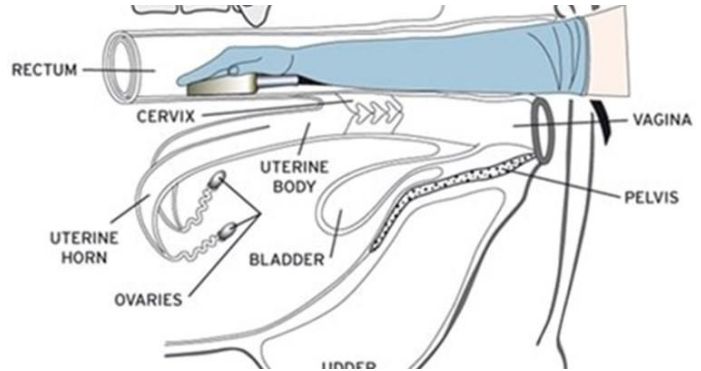
RNA-seq etc. to discover new receptors



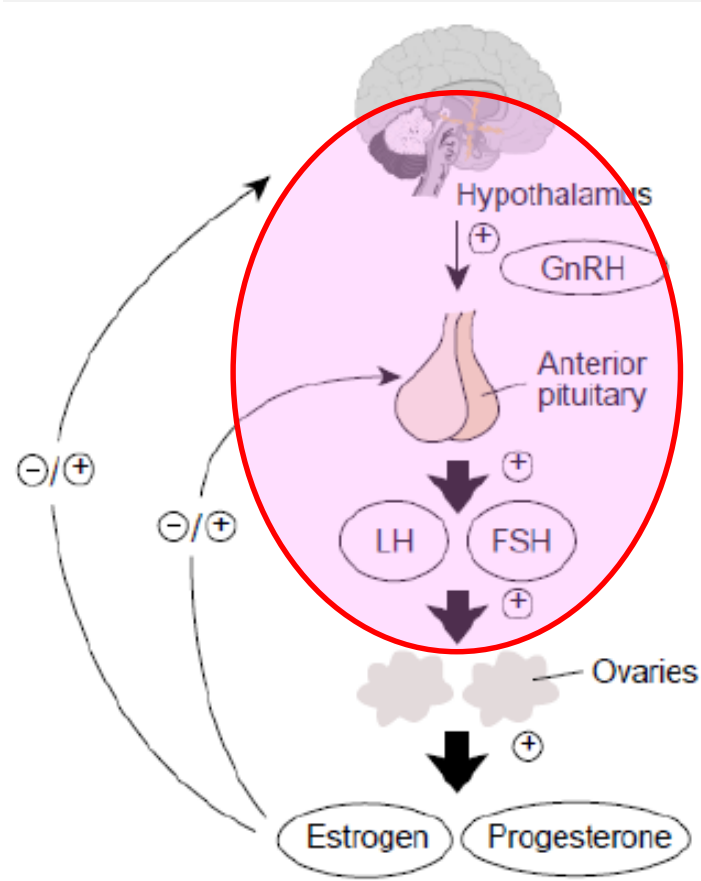
Recent advance



From 1987 to 2006 for Holsteins cows



Diseases caused by dysfunction of **hypothalamus-anterior pituitary**



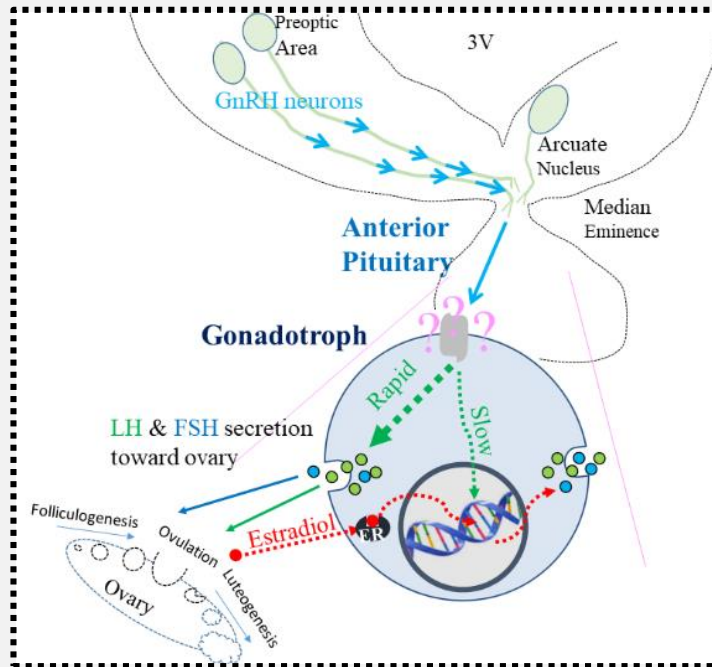
Delayed postpartum resumption
Ovulation failure
Delayed ovulation
Anovulation
Luteal hypoplasia
Ovarian hypoplasia
Ovarian quiescence
Ovarian atrophy
Follicular cyst
Anestrus
Silent heat
Low conception rate



Gonadotrophs secrete **LH** and **FSH** to control reproduction.

Classic mechanisms in females are:

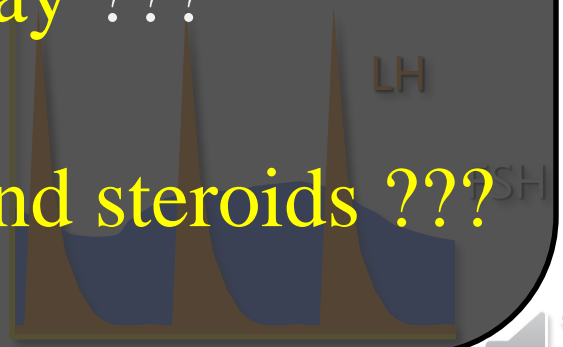
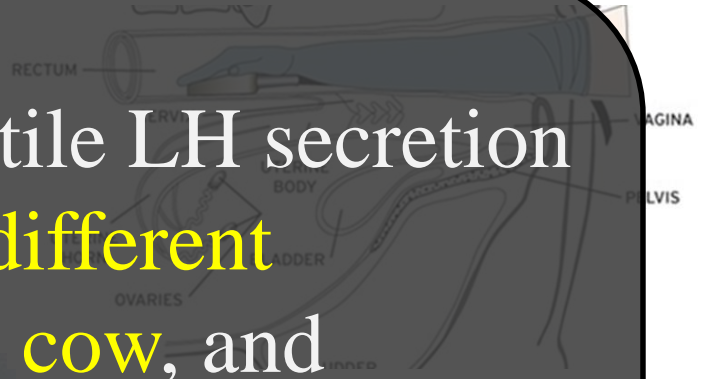
GnRH from hypothalamus stimulates gonadotrophs, while **estradiol** bound by nuclear estrogen receptors suppress.



For 20 years in Holsteins

Why the pulsatile LH secretion
is so different
cow by cow, and
day by day ???

Only by GnRH and steroids ???



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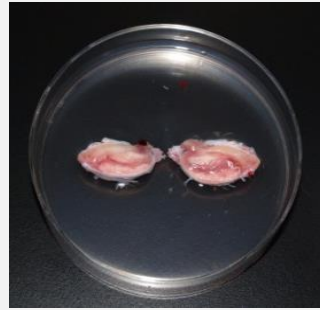
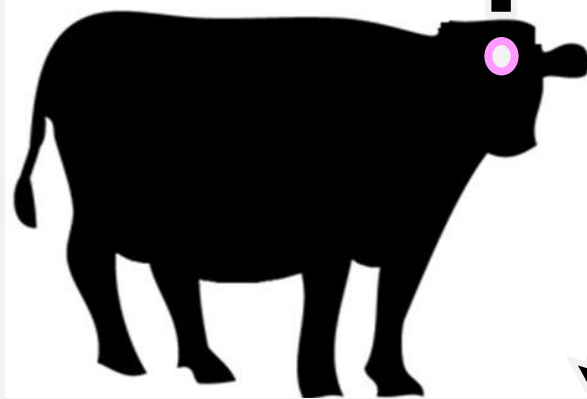
RNA-seq etc. to discover new receptors



Recent advance







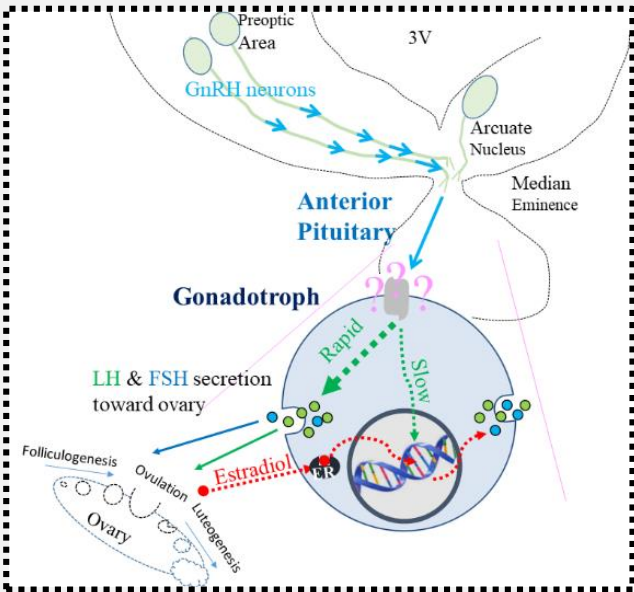
RT-PCR, WB,
immunohistochemistry,
and RNAseq *etc.*

Cell culture

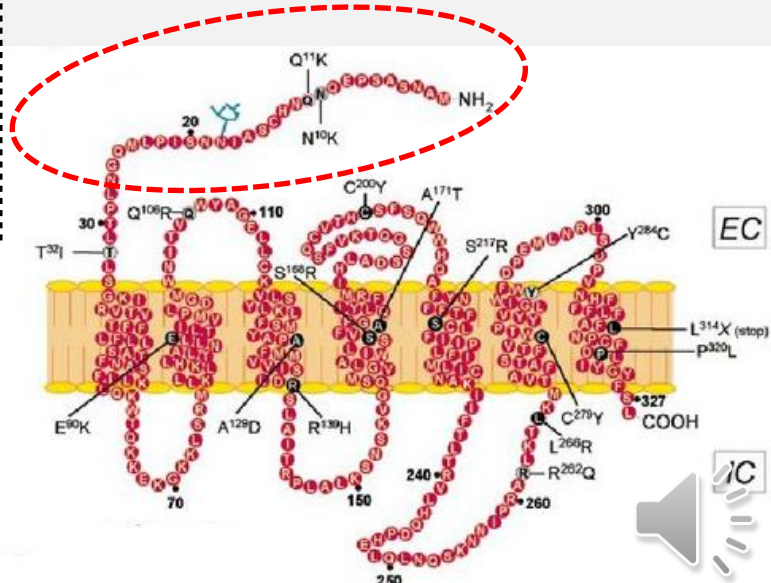
Evaluate effects of
various molecules on
LH & FSH secretion



We aimed to look GnRH receptor.



Then, we developed an original antibody to extracellular region of bovine GnRH receptor

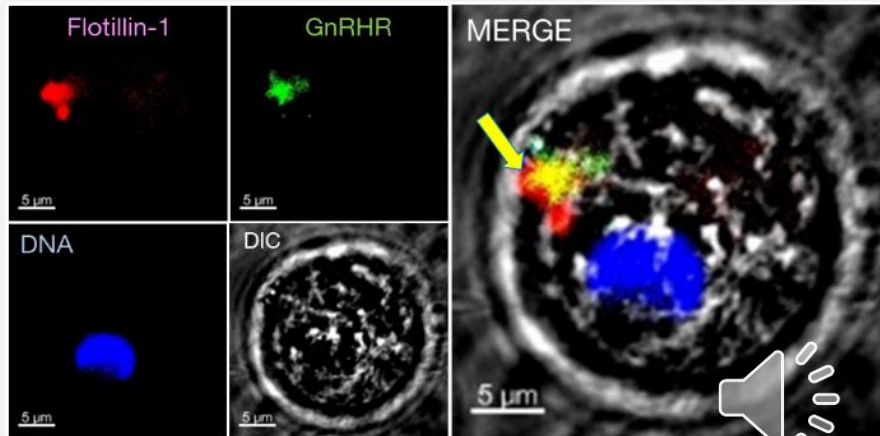
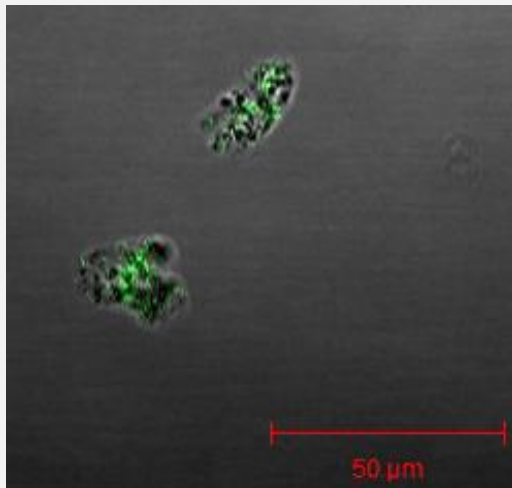


We developed a method to obtain **100% pure gonadotrophs** from bovine pituitary with the anti-GnRHR and FACS.

(Kadokawa *et al.* 2014. *Anim. Reprod. Sci.*)

GnRH receptor locates in lipid raft on plasma membrane of gonadotroph

Colocalization of **Lipid raft marker** and **GnRH receptor** on cell surface of gonadotrophs



(Kadokawa 2020. *J. Reprod. Dev.*)

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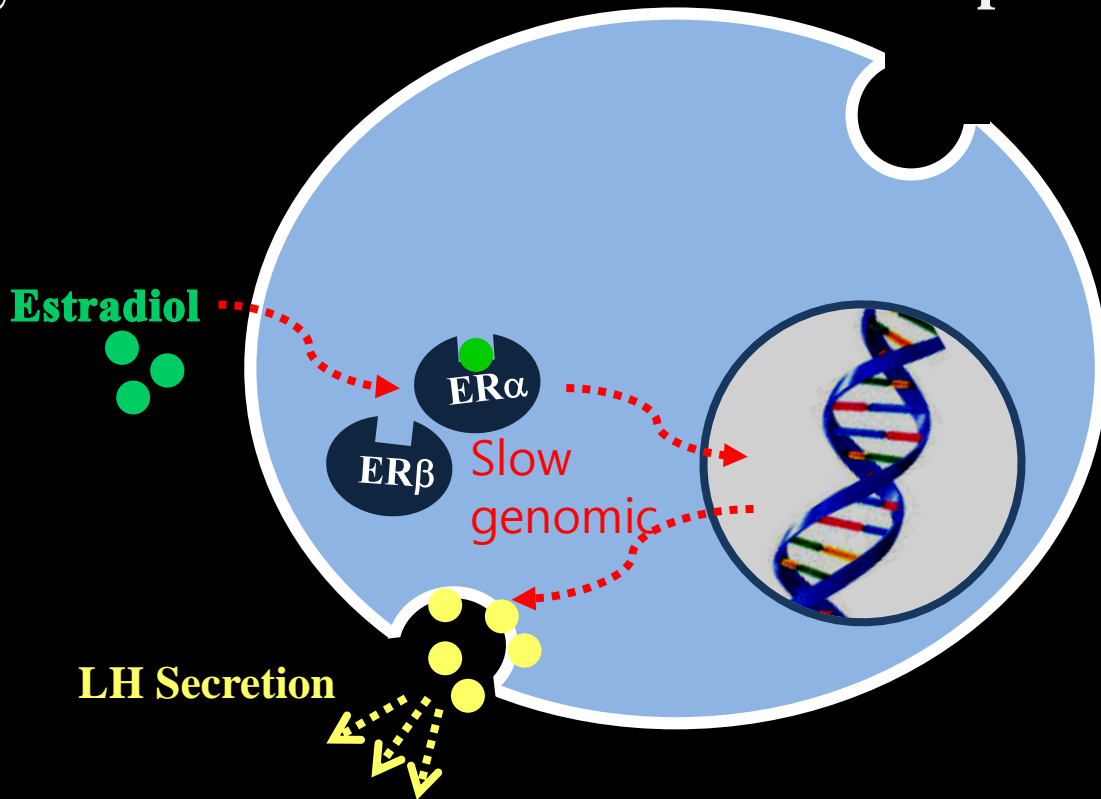


The pioneering papers report

Arreguin-Arevalo & Nett 2005, 2006; Iqbal *et al.* 2009; Davis *et al.* 2011

Estradiol can suppress LH secretion

only within 5 to 15 min in rats and sheep



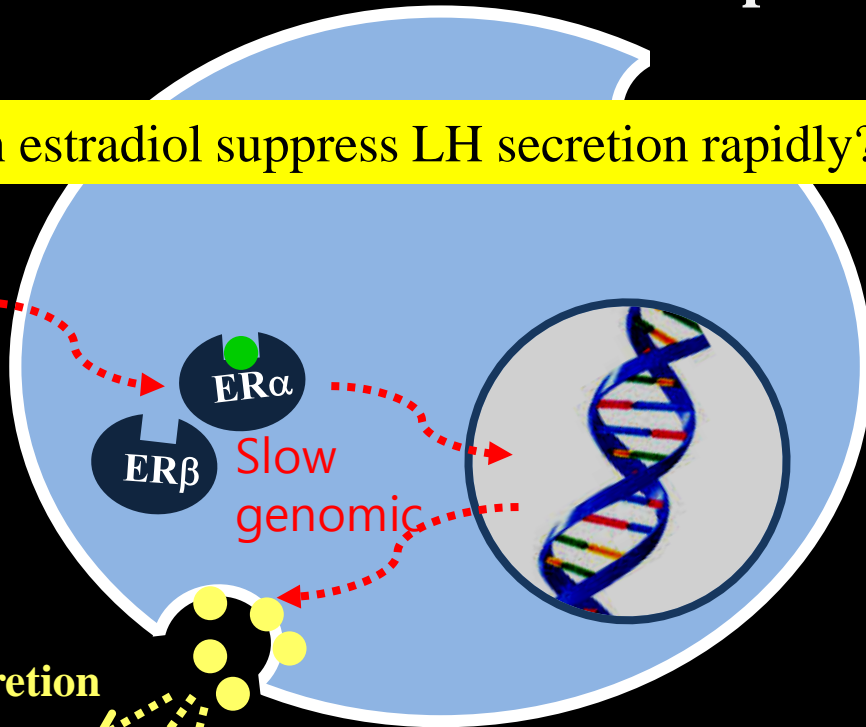
The pioneering papers report

Arreguin-Arevalo & Nett 2005, 2006; Iqbal *et al.* 2009; Davis *et al.* 2011

Estradiol can suppress LH secretion only within 5 to 15 min in rats and sheep

Why can estradiol suppress LH secretion rapidly?

Estradiol

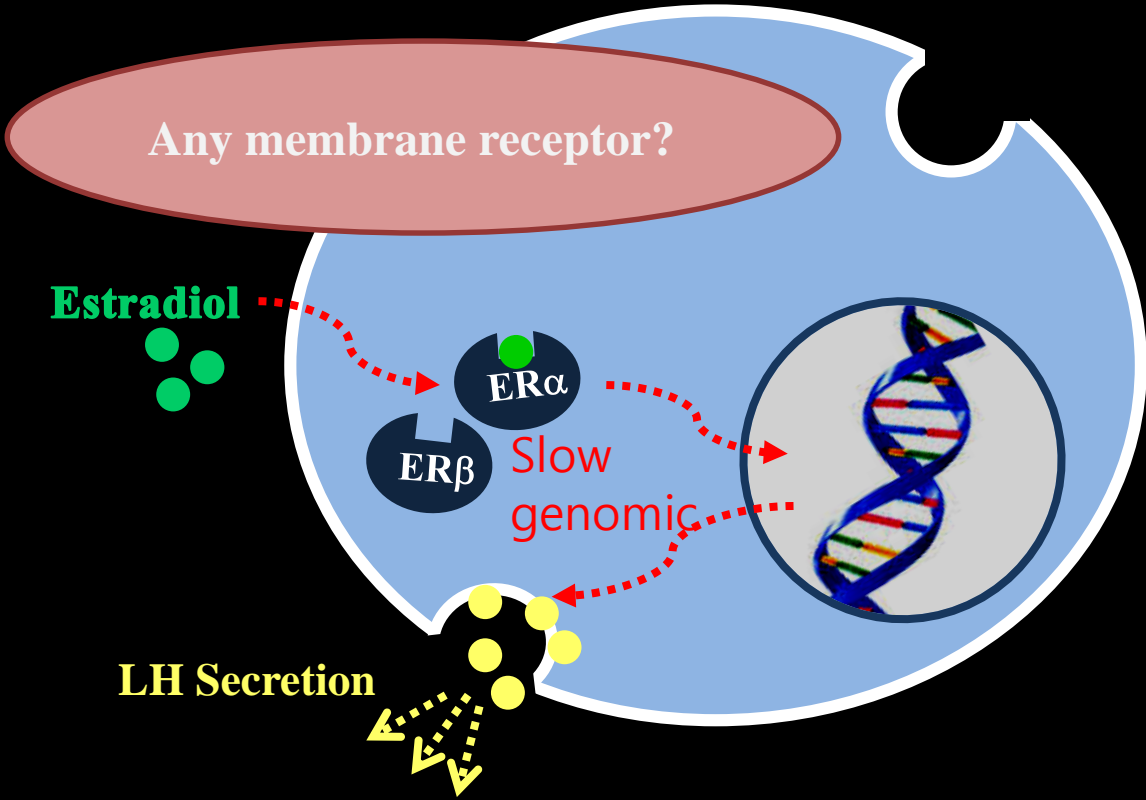


LH Secretion



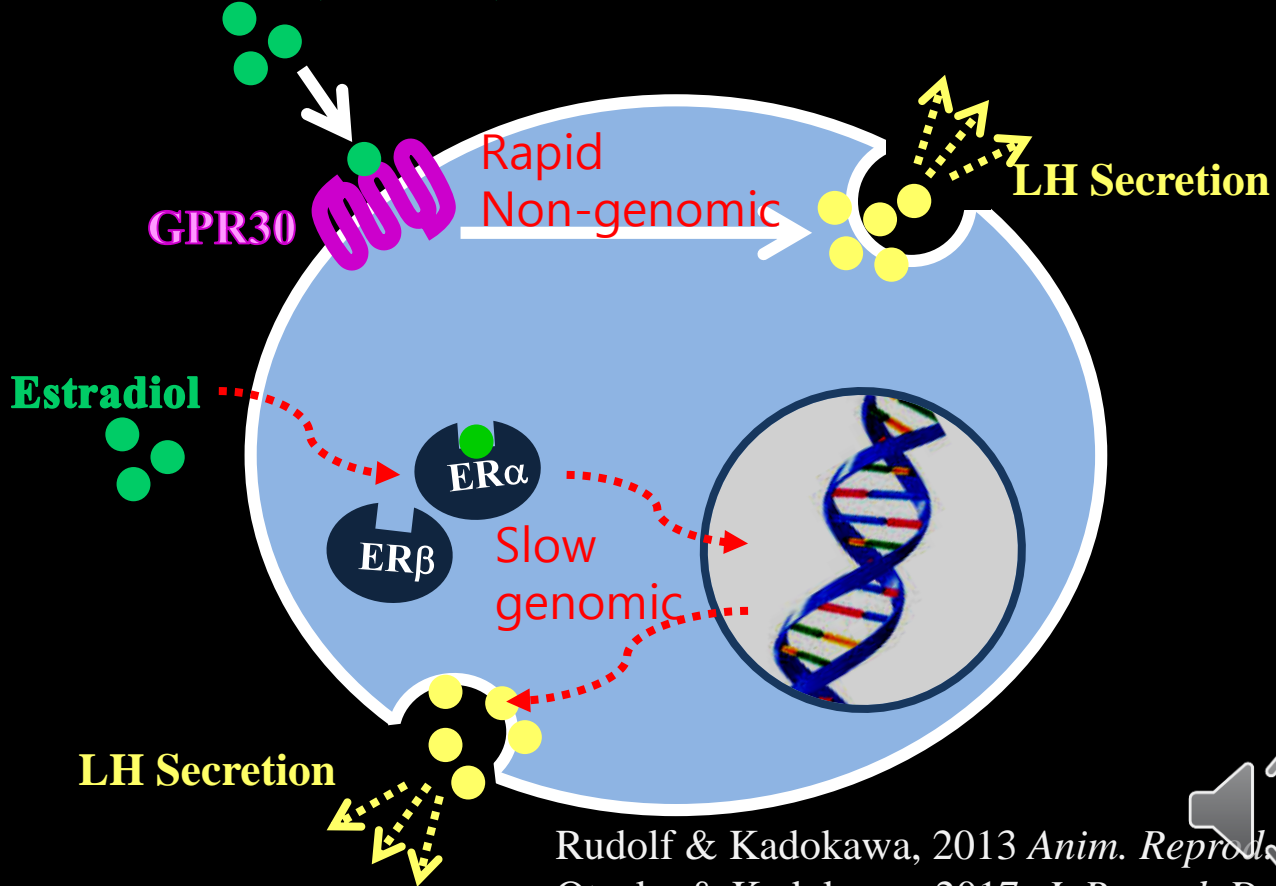
Why can estradiol suppress LH secretion rapidly? Only **within 5 to 15 min** in rats and sheep

(Arreguin-Arevalo & Nett 2005, 2006; Iqbal et al. 2009; Davis et al. 2011)



Discovery of new estrogen receptor, GPR30

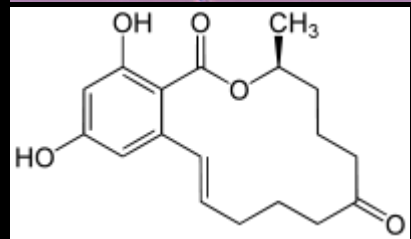
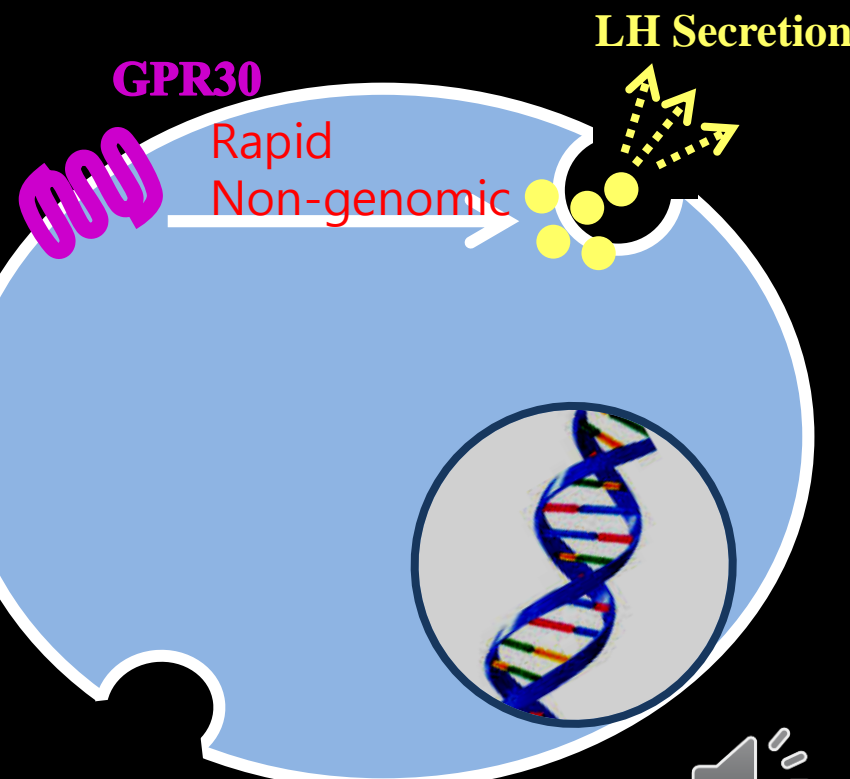
Estradiol, Estriol, and Estrone



Rudolf & Kadokawa, 2013 *Anim. Reprod. Sci.*
Otsuka & Kadokawa, 2017 *J. Reprod. Dev.*



Mycoestrogen (Zearalenone) suppress LH secretion via GPR30



Nakamura & Kadokawa, 2015 *Theriogenology*.
Nakamura et al., 2015 *Anim. Reprod. Sci.*



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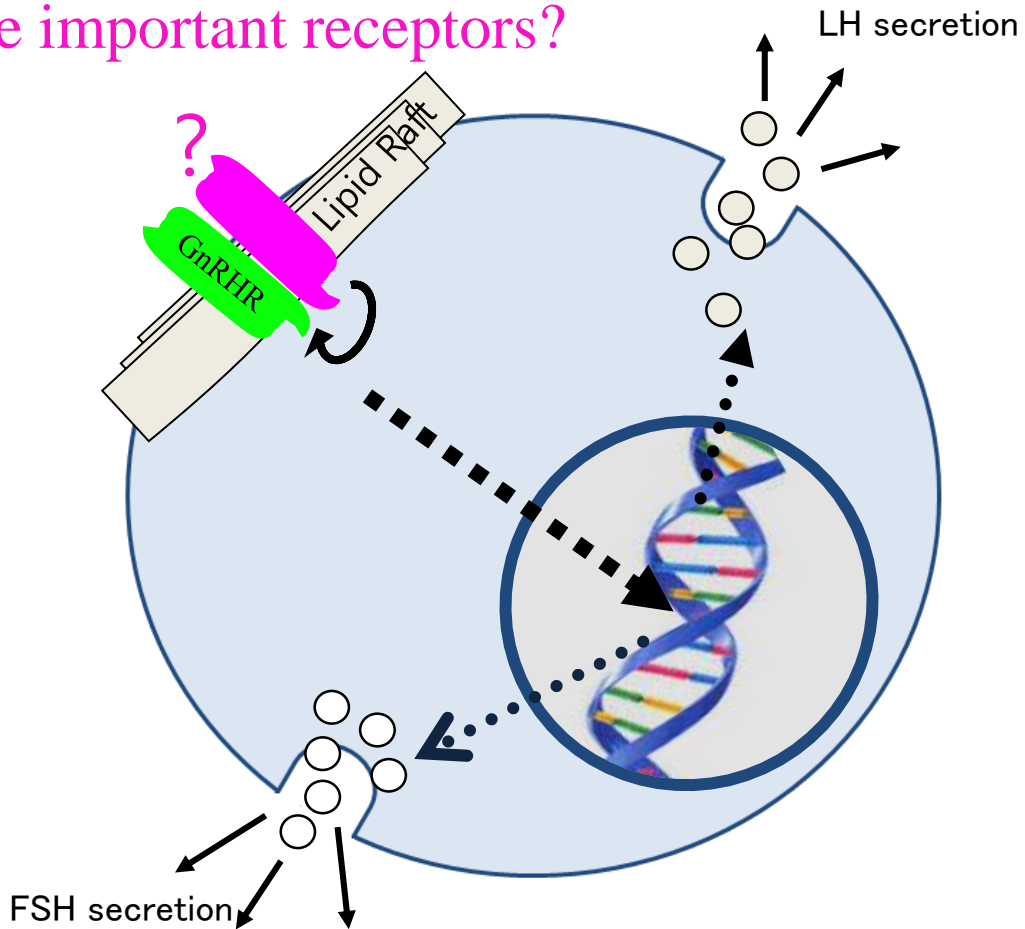
RNA-seq etc. to discover further receptors



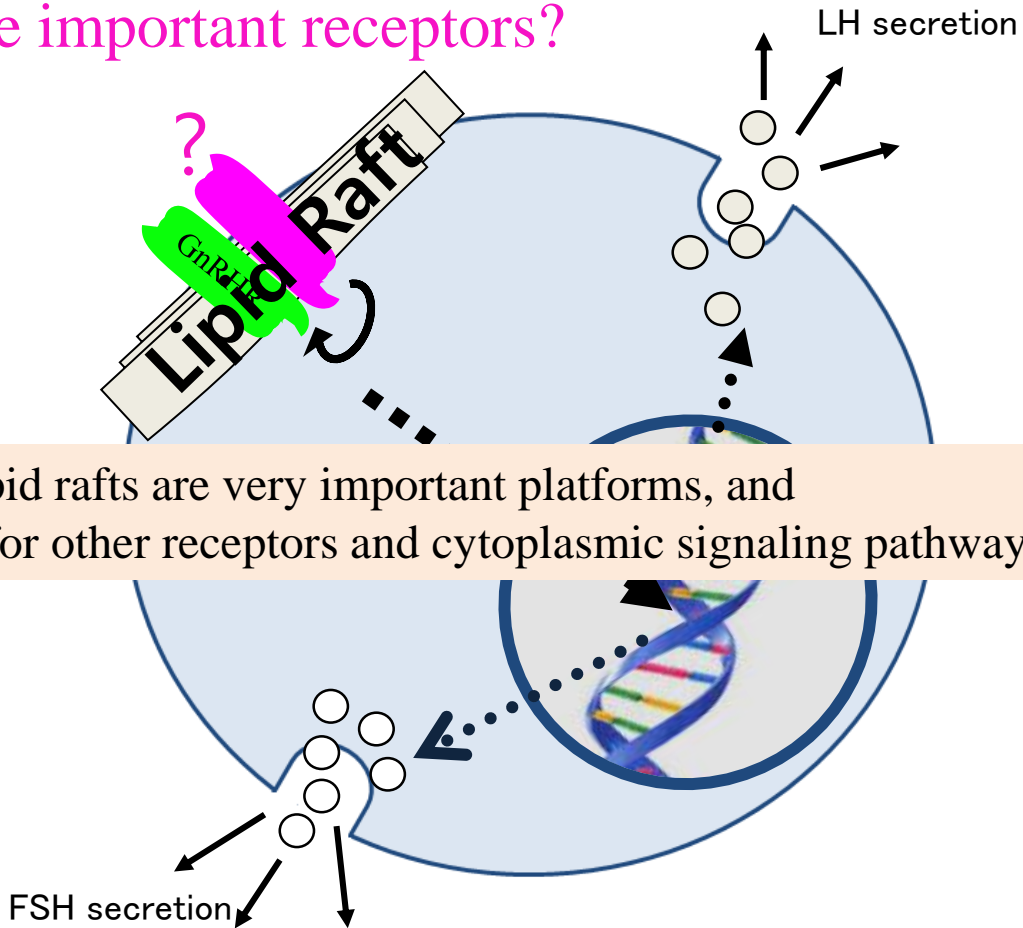
Recent advance



More important receptors?



More important receptors?



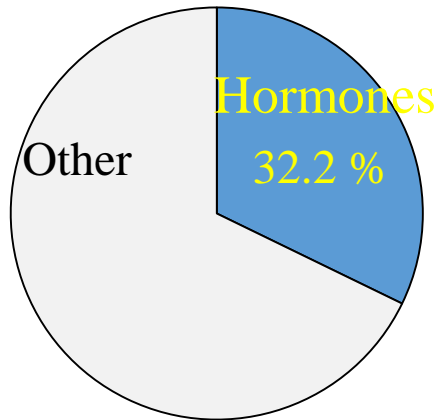
The lipid rafts are very important platforms, and hubs for other receptors and cytoplasmic signaling pathways.

Results of RNA-seq in bovine AP glands

30 – 40 % of transcriptomes are AP hormones

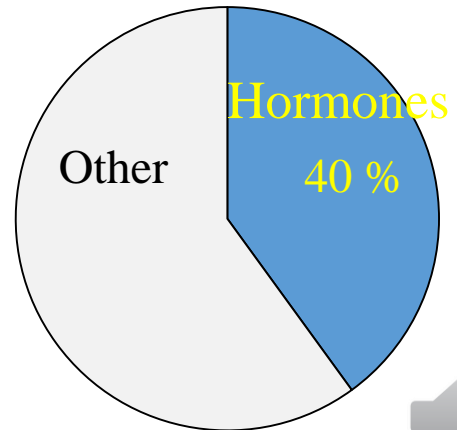
**Pre-ovulation
(LH surge)**

Total RPKM 599,676



**Post-ovulation
(active LH pulse)**

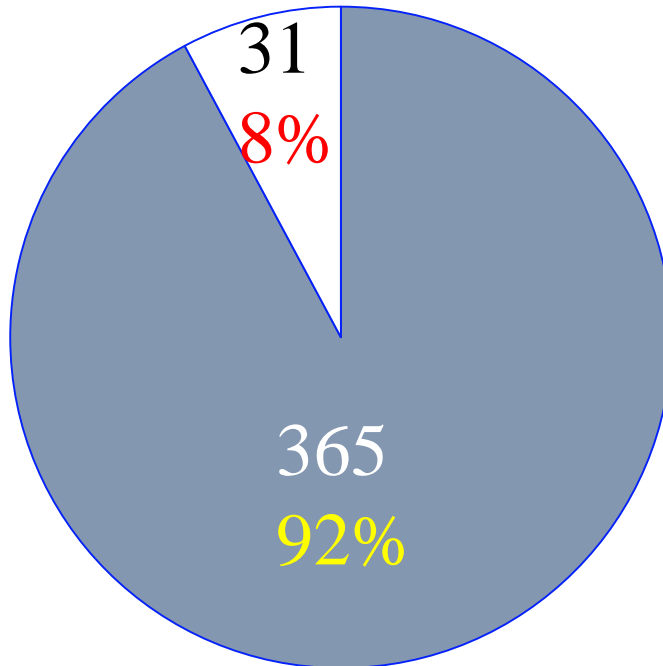
Total RPKM 668,209



396 differentially expressed genes

365 genes (92%): Pre-ov > Post-ov

31 genes (8%): Pre-ov < Post-ov



259 receptors in the AP glands



Nine differentially expressed receptors with previously identified ligands.

Two differentially expressed **orphan GPCRs** between pre- & post-ovulation:
GPR61 and GPR153

One receptor is not differentially expressed between the two periods, but the receptor has a reported relationship with fertility via an unknown mechanism:
AMH type 2 receptor (AMHR2)

(Pandey *et al.* 2016. *J. Vet. Med. Sci.*)



Using RT-PCR, western blots, and IHC etc., we found

GPR61, GPR153, and AMHR2 are **colocalized with GnRH receptor** on the cell surface of bovine gonadotrophs.



IHC with Three Antibodies

Anti-GnRHR
(guinea pig IgG)

Antibody to
GPR61, GPR153, or AMHR2
(rabbit or chicken IgG)

Extracellular

Membrane

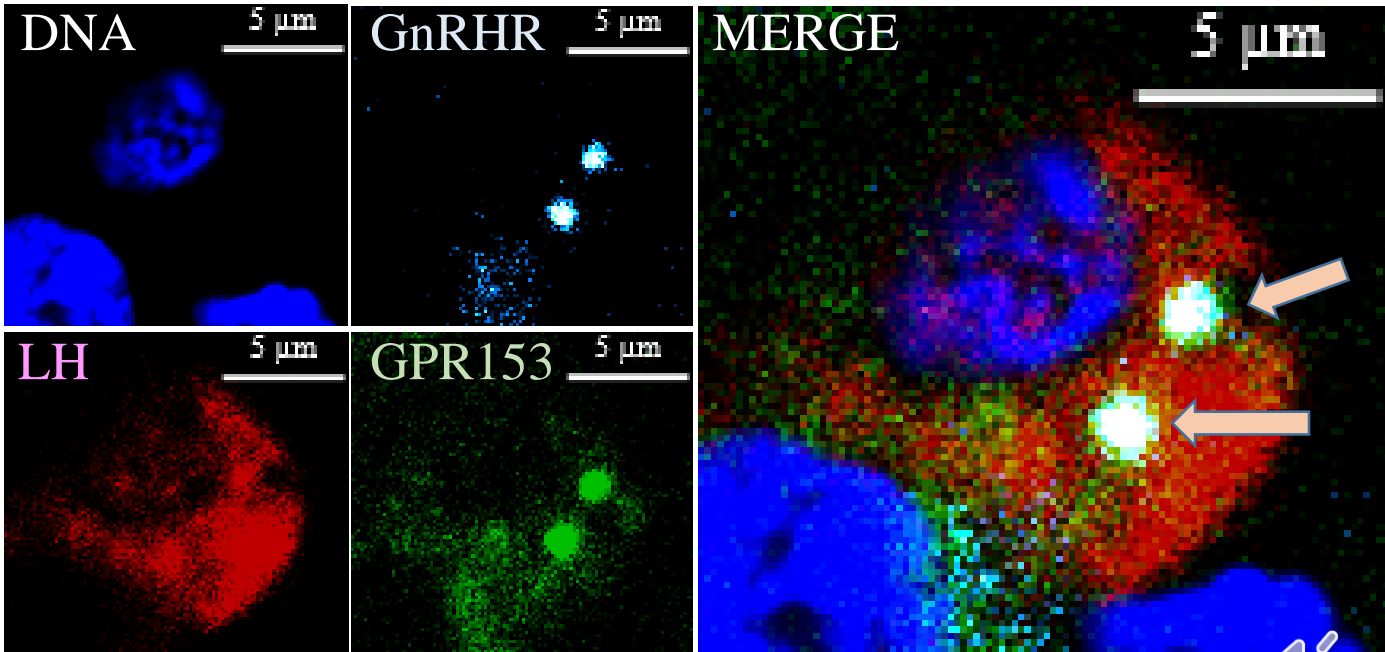
Cytoplasmic

Antibody to LH or FSH
(mouse IgG)

LH or
FSH



IHC to stain both surface and cytoplasm



Culture and stain AP cells in a chamber-slide

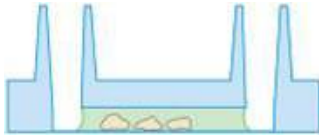


Immunofluorescence

Fixation



Staining

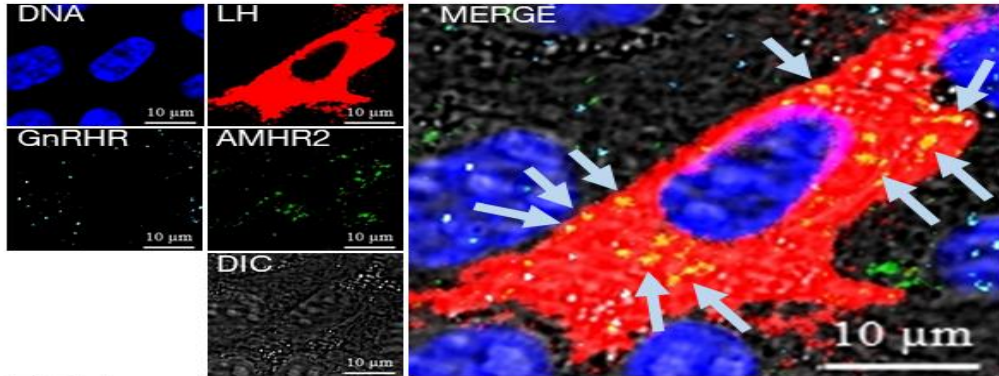


Observation
by confocal microscope

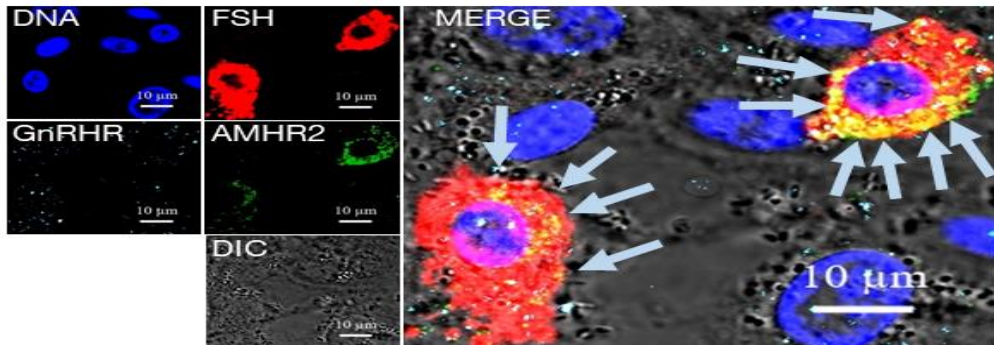


AMHR2 colocalize with GnRHR on cultured bovine gonadotrophs

(A) LH

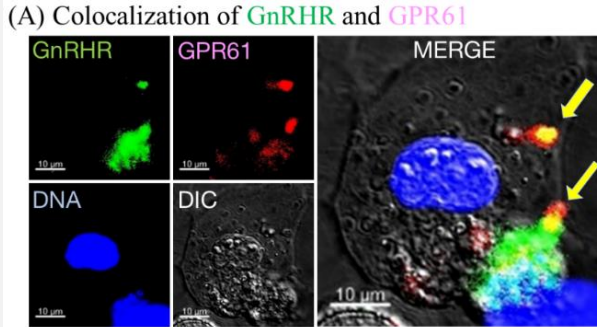


(B) FSH



(Kereilwe *et al.* 2018. *Repro. Fertil. Dev.*)

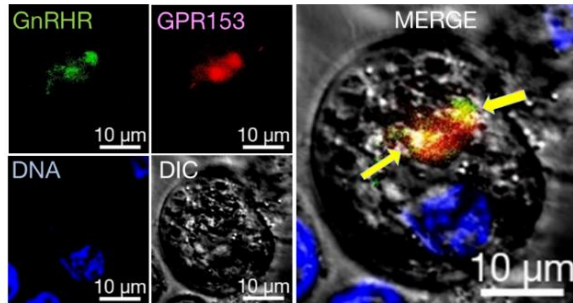




Ligand of GPR61 is
Ethanolamine plasmalogen

(Pandey *et al.* 2017.
Anim. Reprod. Sci.)

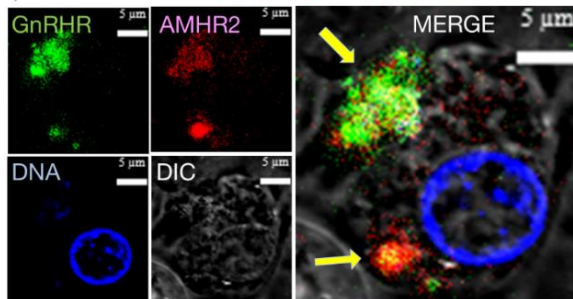
(B) Colocalization of **GnRHR** and **GPR153**



GPR153 is orphan

(Pandey *et al.* 2018.
Anim. Sci. J.)

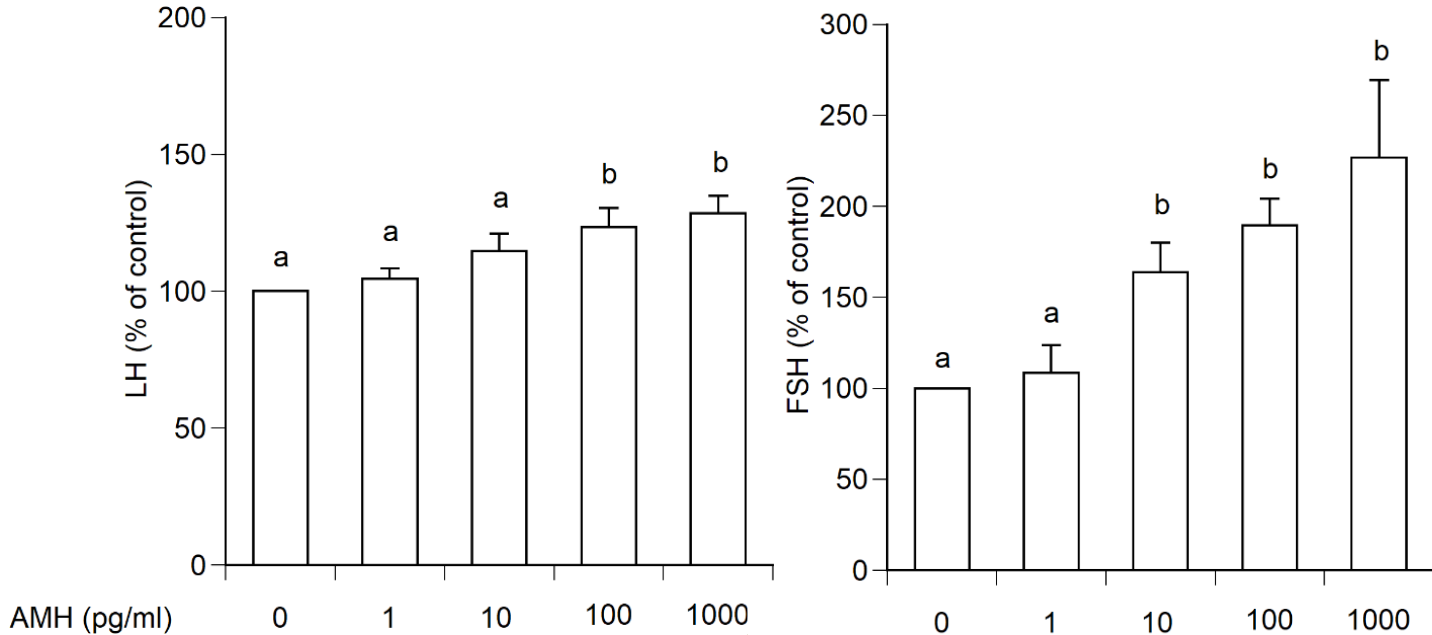
(C) Colocalization of **GnRHR** and **AMHR2**



Ligand of AMHR2 is
AMH

(Kereilwe *et al.* 2018
Repro. Fertil. Dev.)

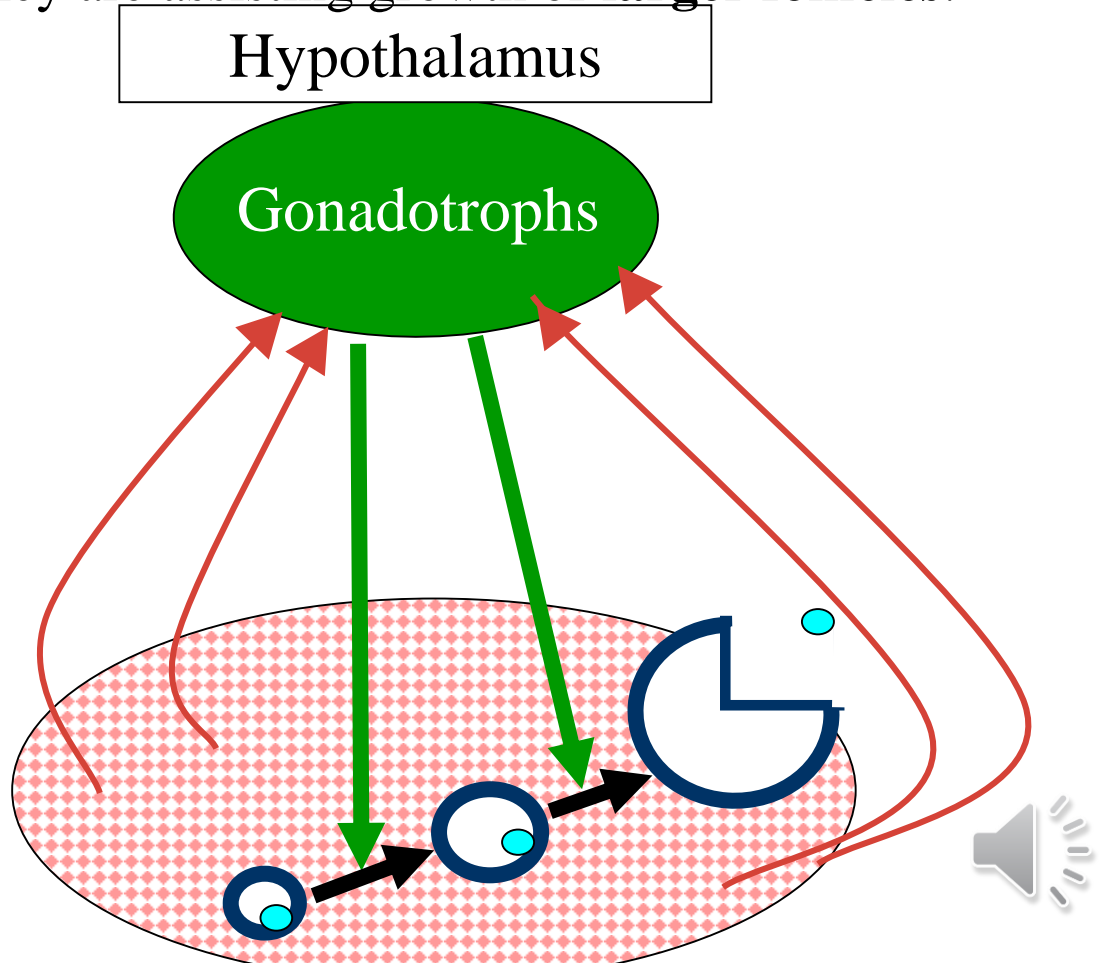
AMH stimulates LH and FSH secretion from cultured bovine gonadotrophs via AMHR2



(Kereilwe *et al.* 2018. *Repro. Fertil. Dev.*)



Small follicles secrete AMH to stimulate gonadotroph,
Thus, they are assisting growth of larger follicles.

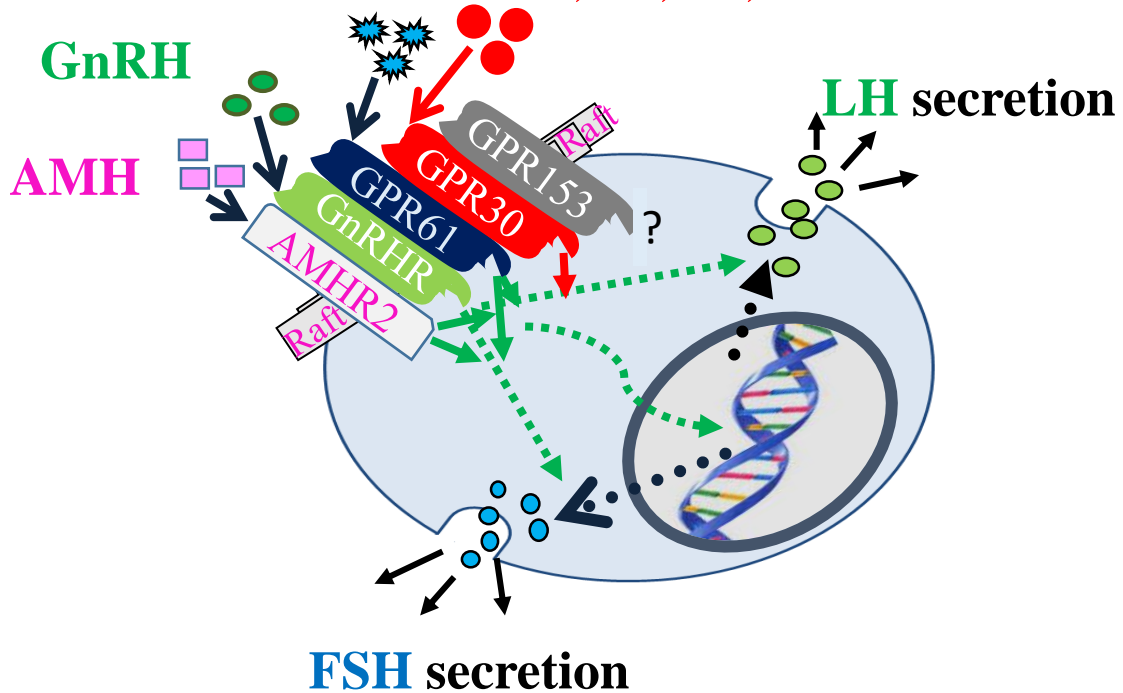


Conclusion in 2020

Ethanolamine

Plasmalogen

E1, E2, E3, ZEN



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RNA-seq etc. to discover new receptors



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AMHR2 and AMH

GPR61 and Ethanolamine plasmalogen



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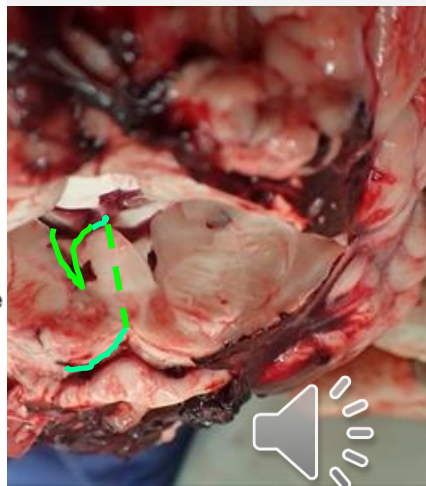
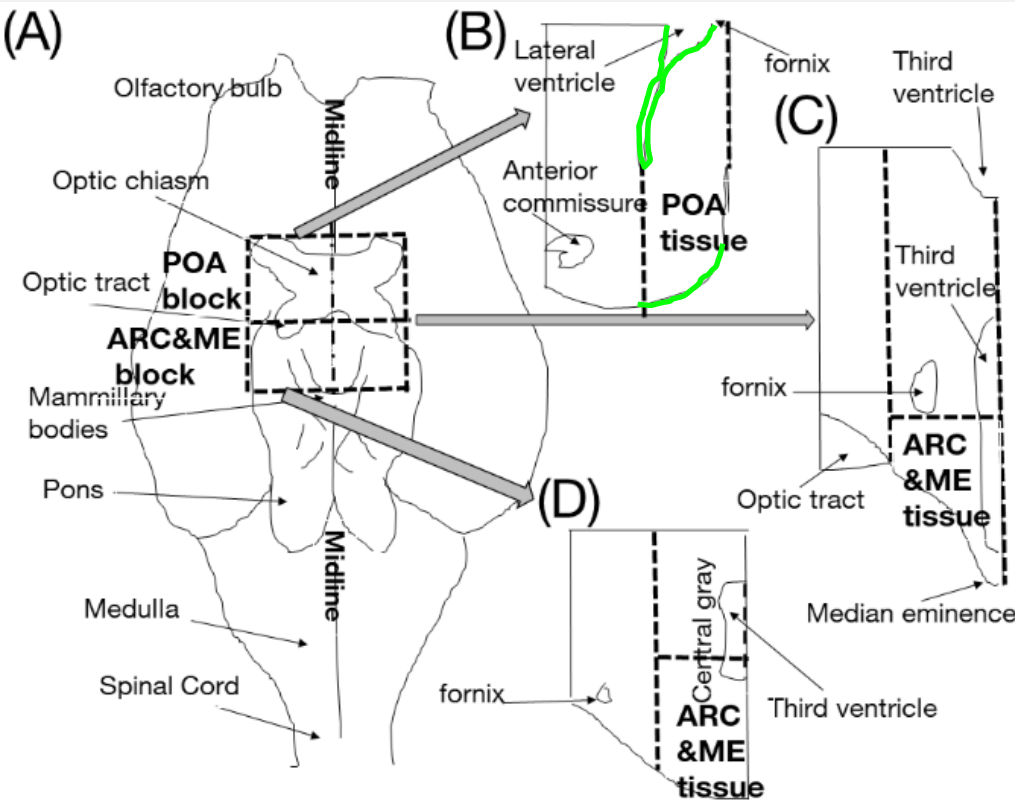
Recent advance

AMHR2 and AMH

GPR61 and Ethanolamine plasmalogen



Bovine hypothalamus sampling



4 %PFA fixation

20% sucrose

30% sucrose

OCT compound



Cryostat

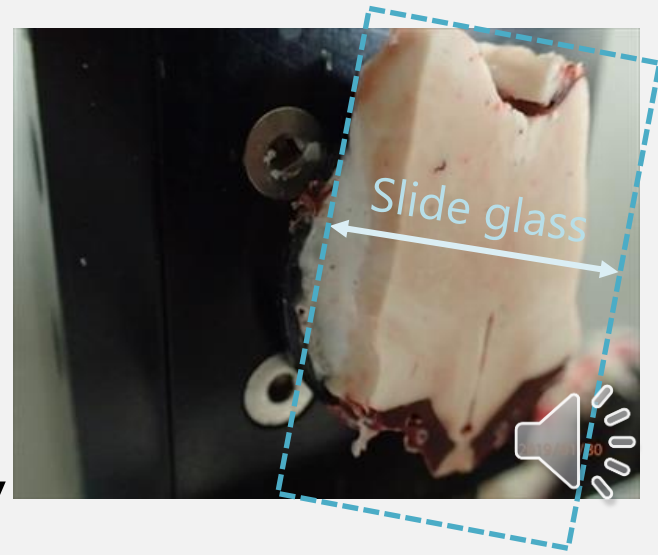


Original quenching



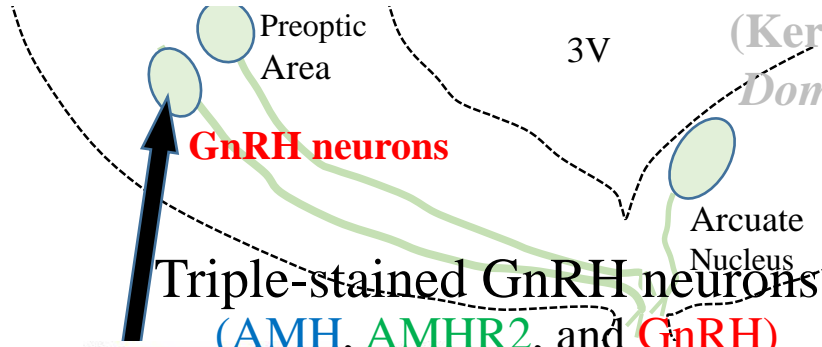
IHC

Confocal microscopy

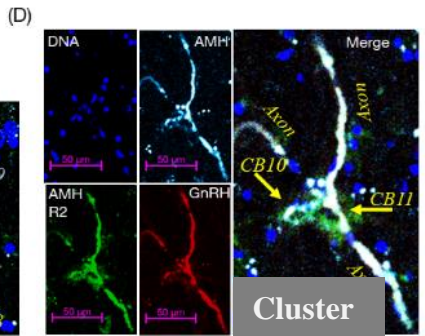
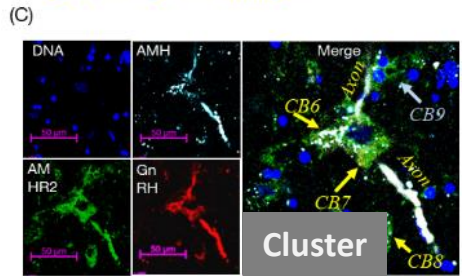
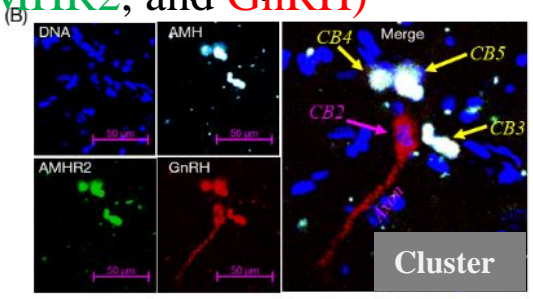
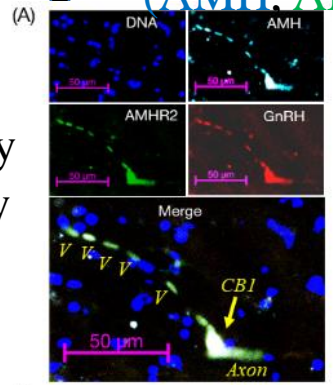


GnRH neurons are synthesizing GnRH, AMH, and AMHR2.

(Kereilwe *et al.* 2020. *Domest. Anim. Endoc.*)

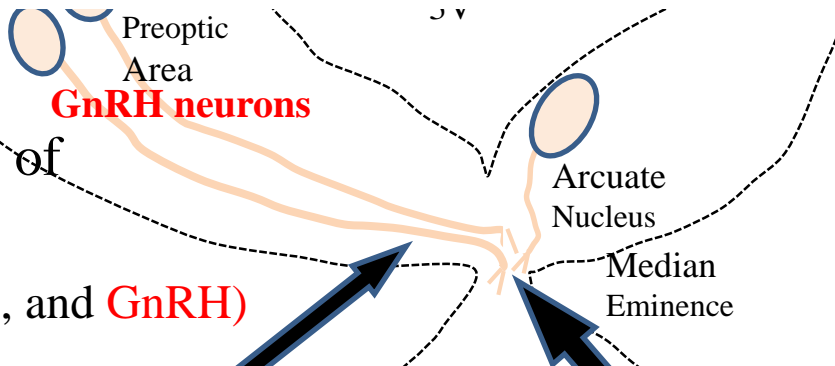


CB is cell body
V is varicosity

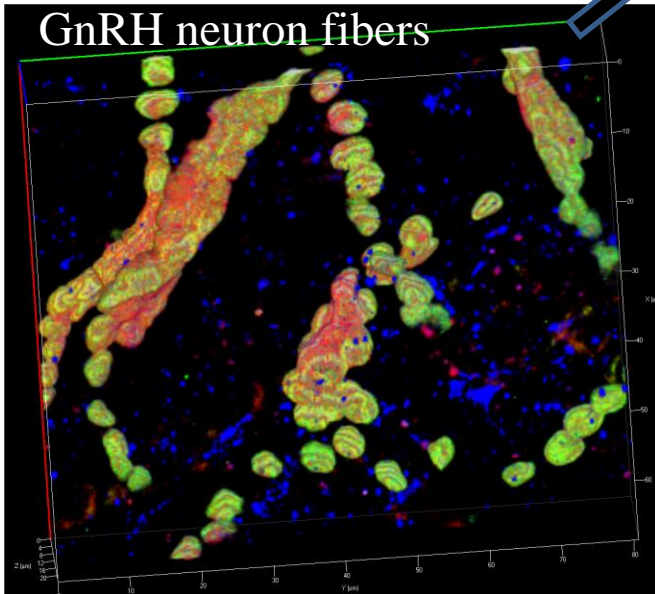


GnRH neurons are secreting both **GnRH** and **AMH**.

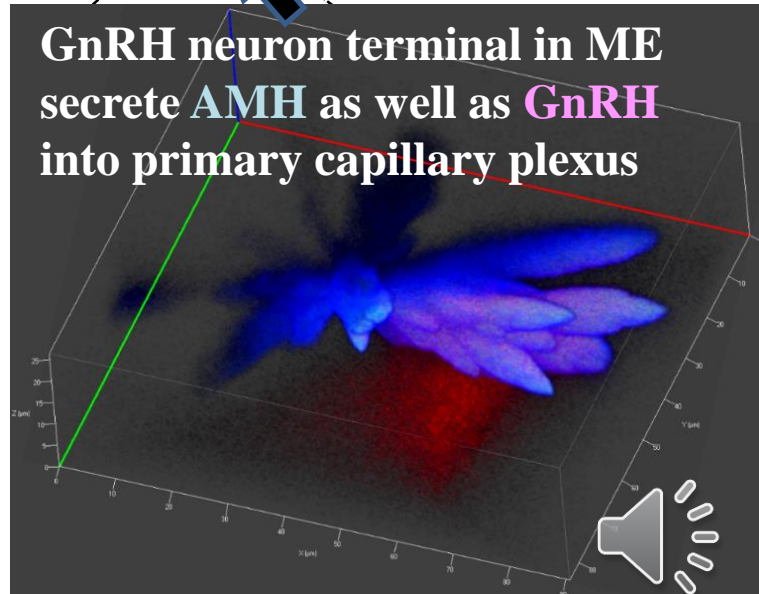
z-stack images of
triple-stained
(AMH, AMHR2, and GnRH)



GnRH neuron fibers

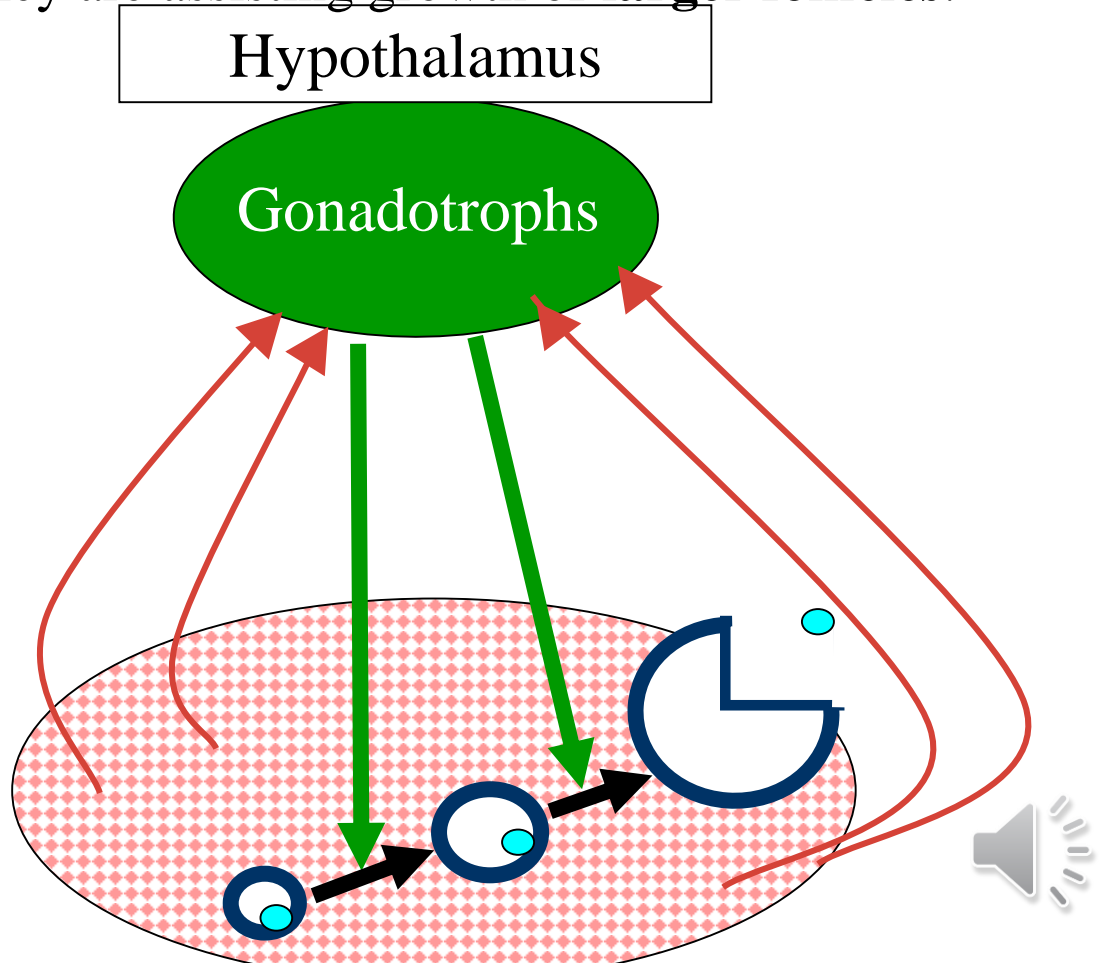


GnRH neuron terminal in ME
secrete **AMH** as well as **GnRH**
into primary capillary plexus

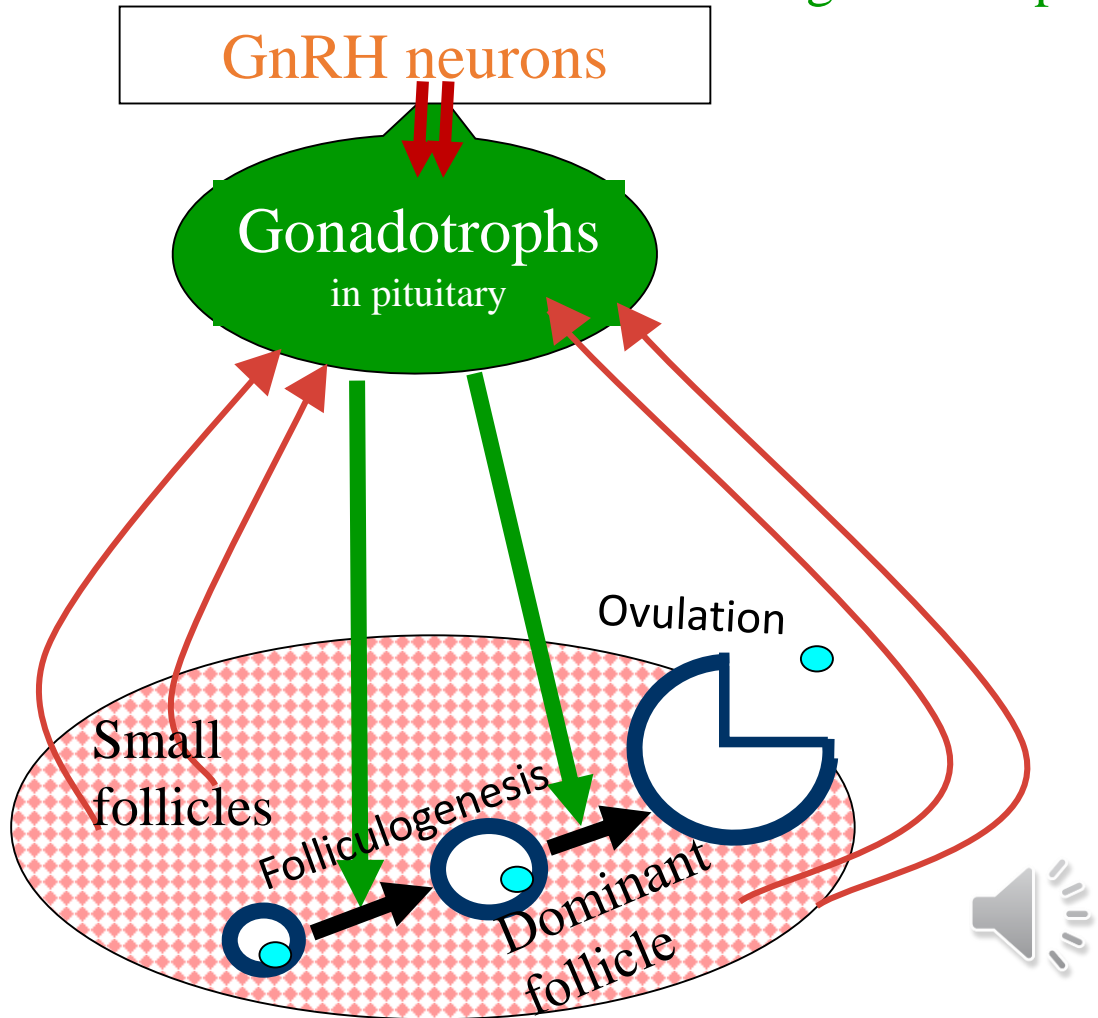


(Kereilwe *et al.* 2020. *Domest. Anim. Endoc.*)

Small follicles secrete AMH to stimulate gonadotroph, Thus, they are assisting growth of larger follicles.



Also **GnRH neurons** secrete **AMH** to stimulate **gonadotroph**.



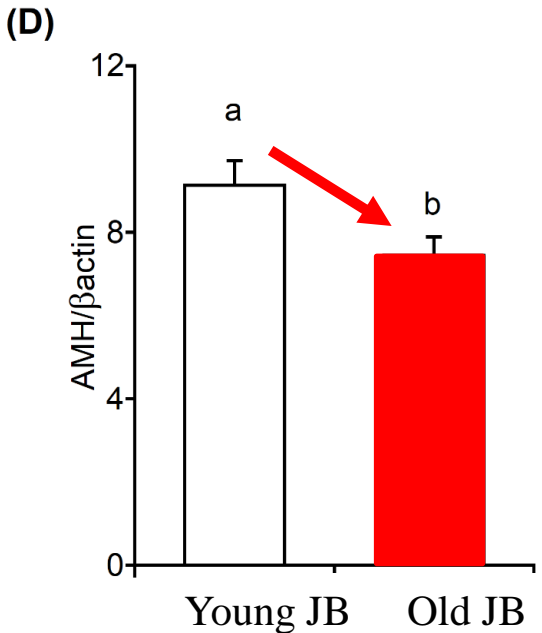
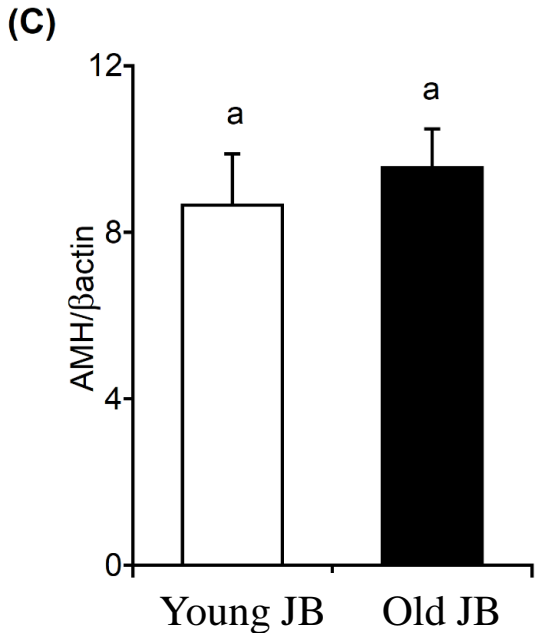
AMH in ARC&ME decrease after aging

AMH in POA

AMH in ARC & ME

(A) Young JB (26 months) Old JB (90 months)

(B) Young JB (26 months) Old JB (90 months)



AMH in ARC & ME decrease after aging

AMH in POA

AMH in ARC & ME

(A)

Young JB Old JB

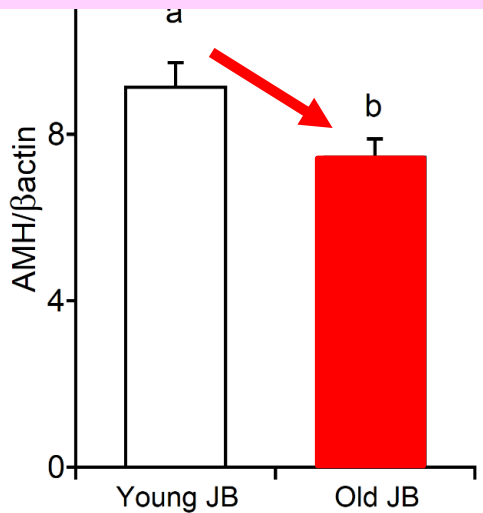
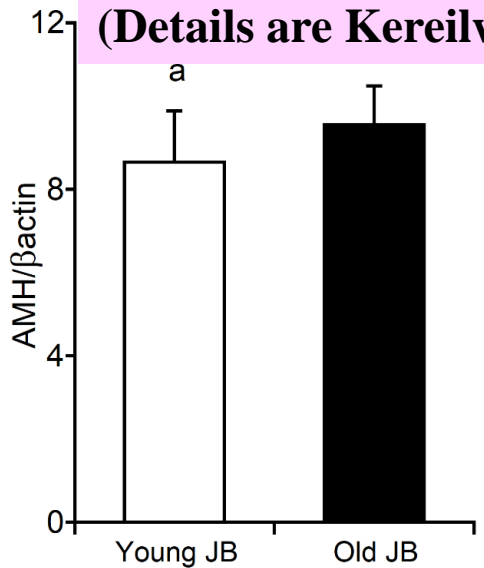
(B)

Young JB Old JB

AMH and AMHR2 may have important roles in age-related infertility.

(C)

(Details are Kereilwe *et al.* 2020. *J. Vet. Med. Sci.*)



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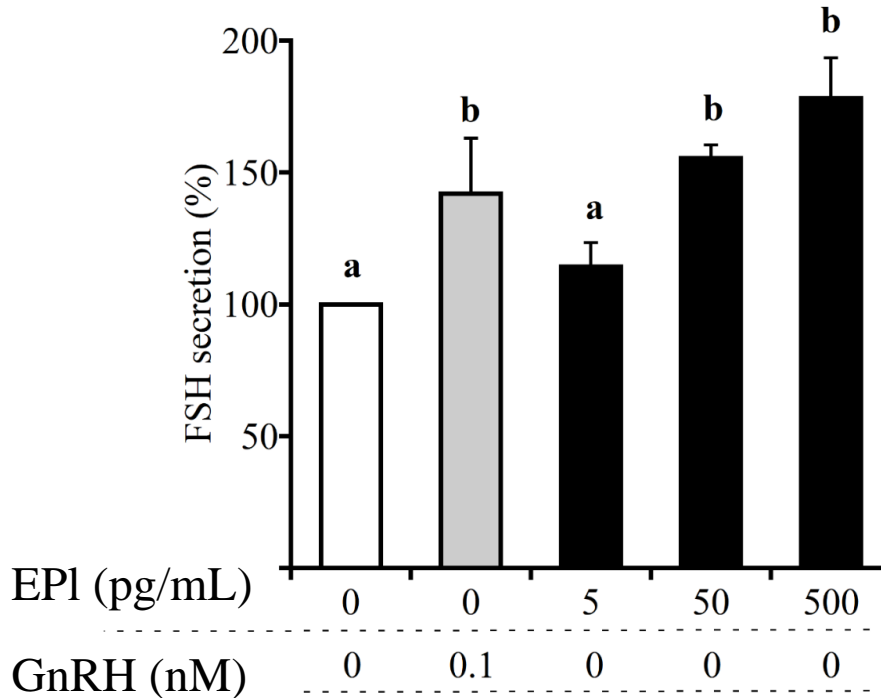
Recent advance

AMHR2 and AMH

GPR61 and Ethanolamine plasmalogen



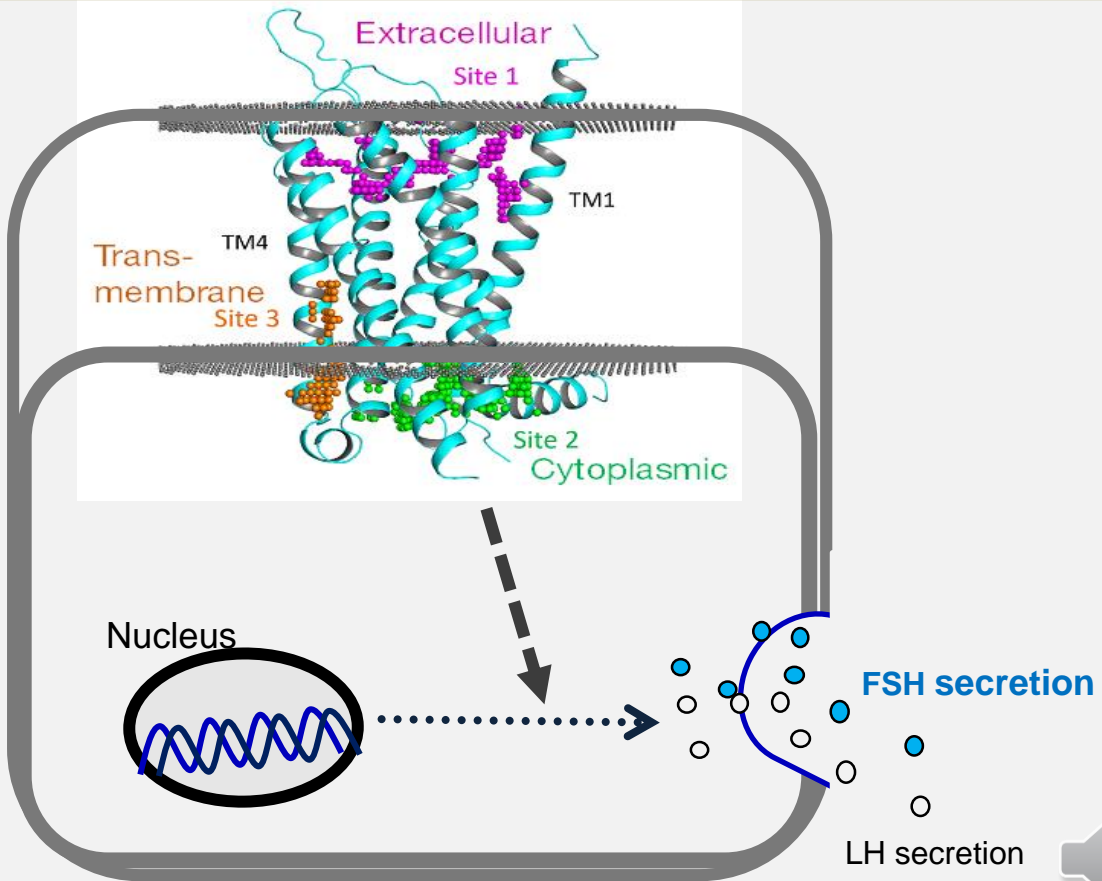
Brain **ethanolamine plasmalogens (EPI)** is the ligand of GPR61 to stimulate FSH secretion from cultured bovine gonadotrophs.



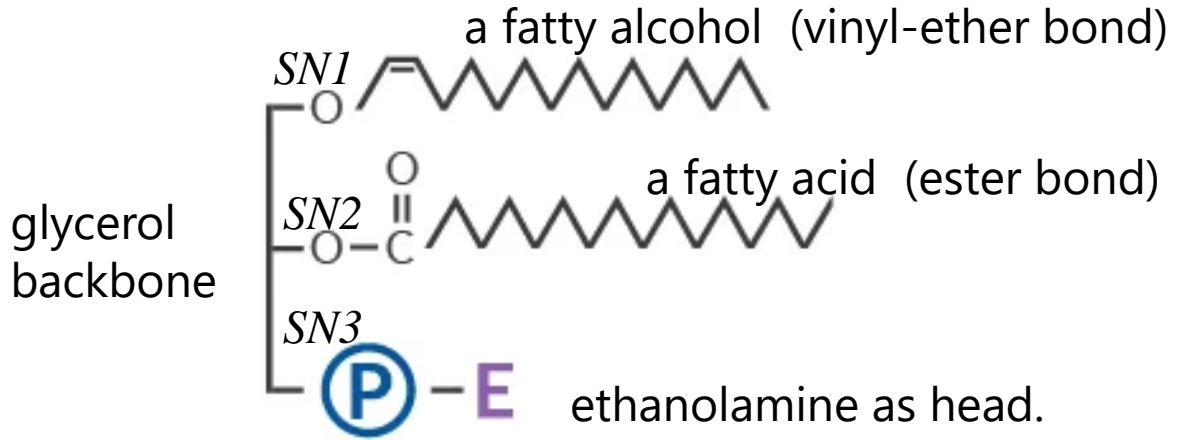
(Kereilwe *et al.* 2018. *Domestic Animal Endocrinology*)



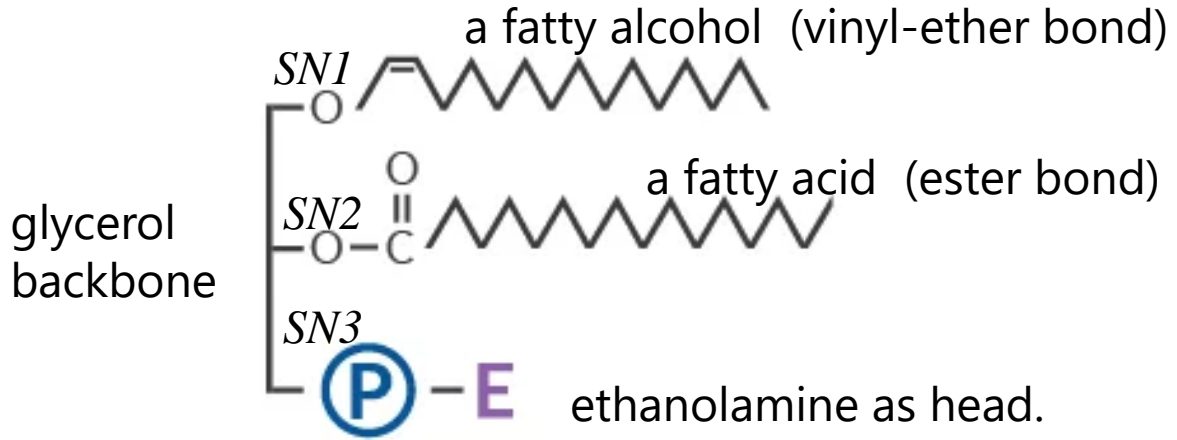
Binding sites in three dimensional model of GPR61 for EPI (created by deep-learning algorithm, AlphaFold2)



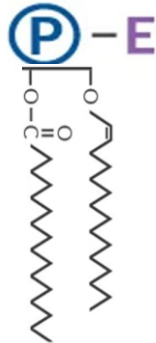
Common structure of **ethanolamine plasmalogens** (EPIs)



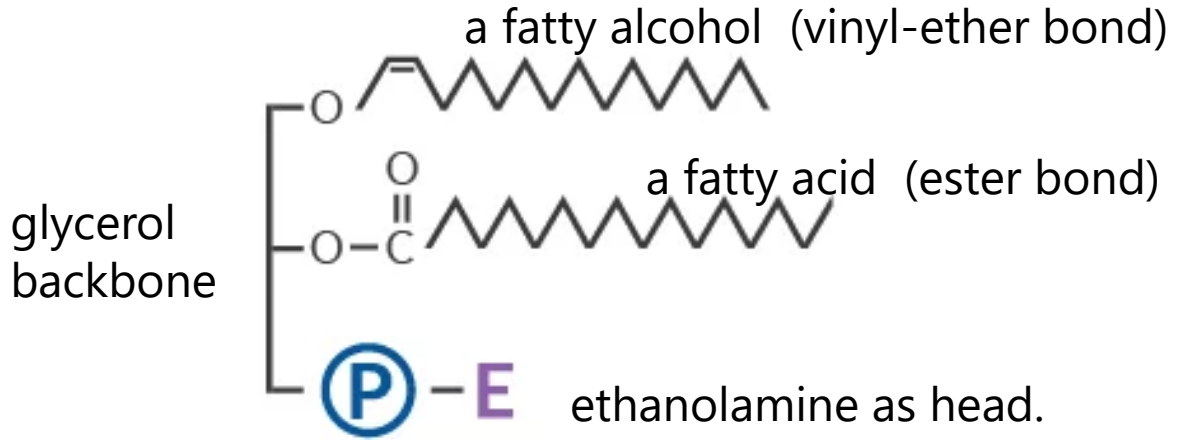
Common structure of **ethanolamine plasmalogens** (EPIs)



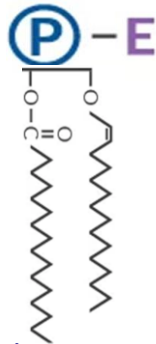
Two-legs
octopus



Common structure of **ethanolamine plasmalogens (EPIs)**



Two-legs
octopus

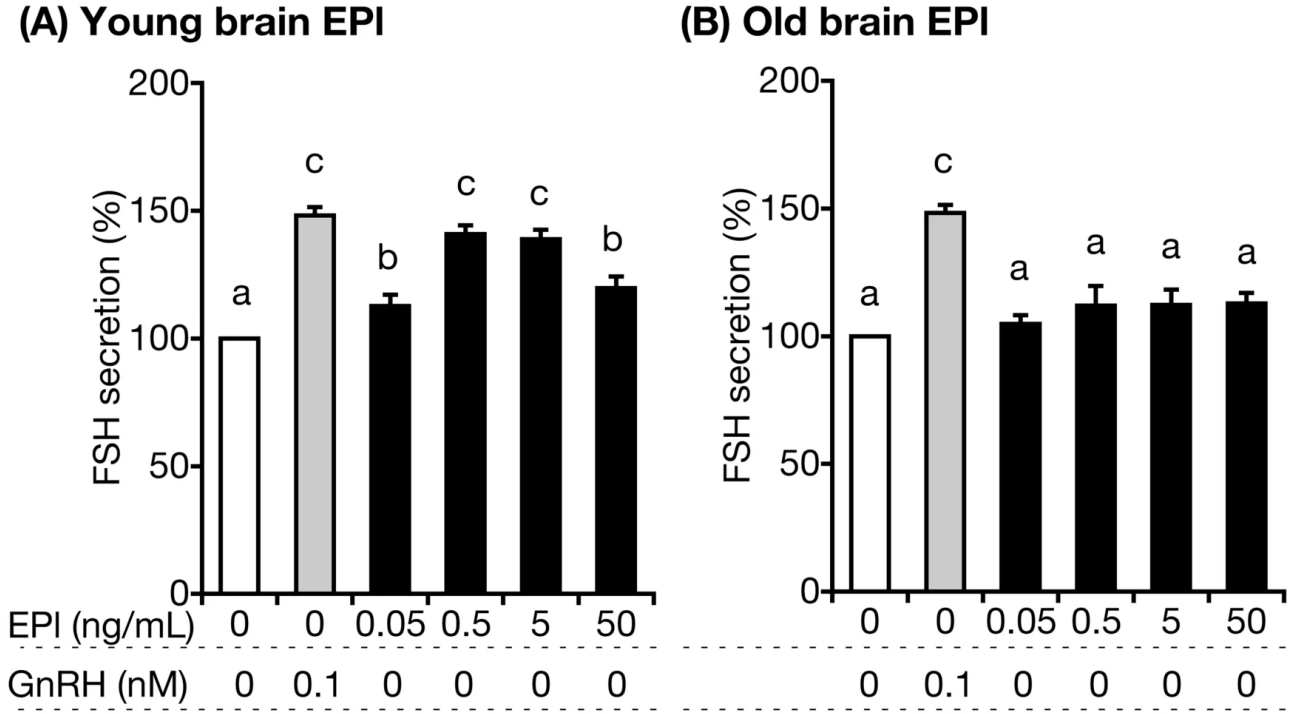


Good octopus?
Bad octopus?

Based on the various possible combinations of fatty alcohols and acids, the bovine hypothalamus contains **at least 20 EPI** molecular species.



Only **young brain EPI** (26 months old heifer),
but **not old brain EPI** (91 months old multiparous cows),
can **stimulate FSH secretion** from cultured young gonadotrophs!

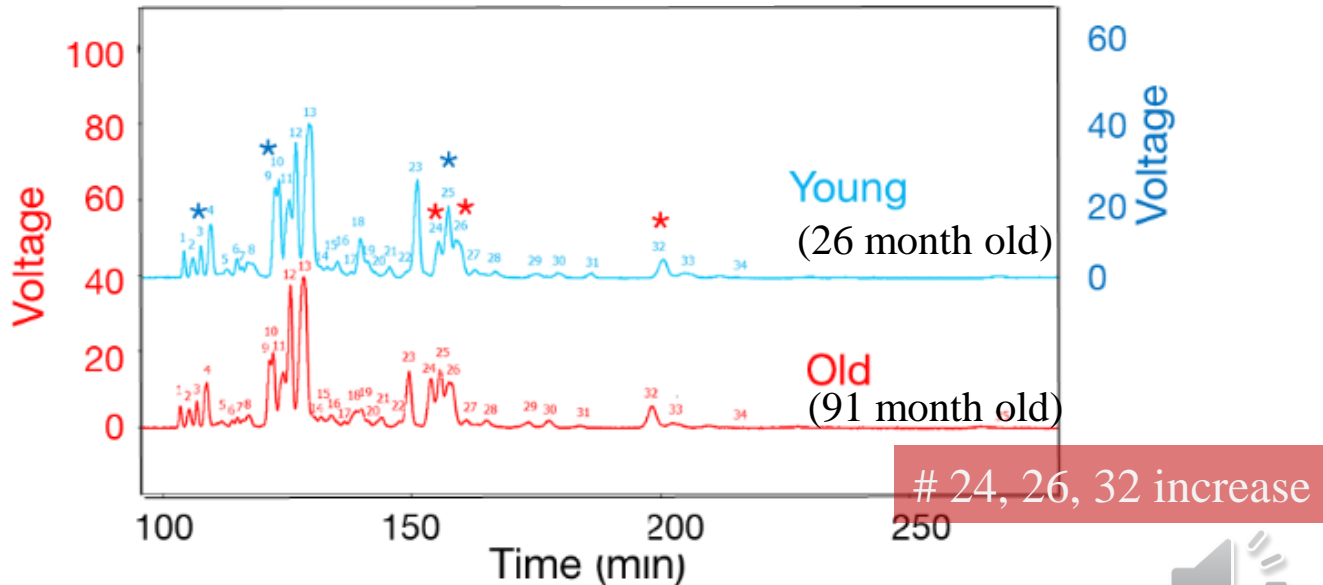


(Kadokawa et al. 2021. Scientific Reports)

Quality of hypothalamic EPIs becomes worse after aging (2D-LCMS)

After aging

#3, 9, 25 decrease

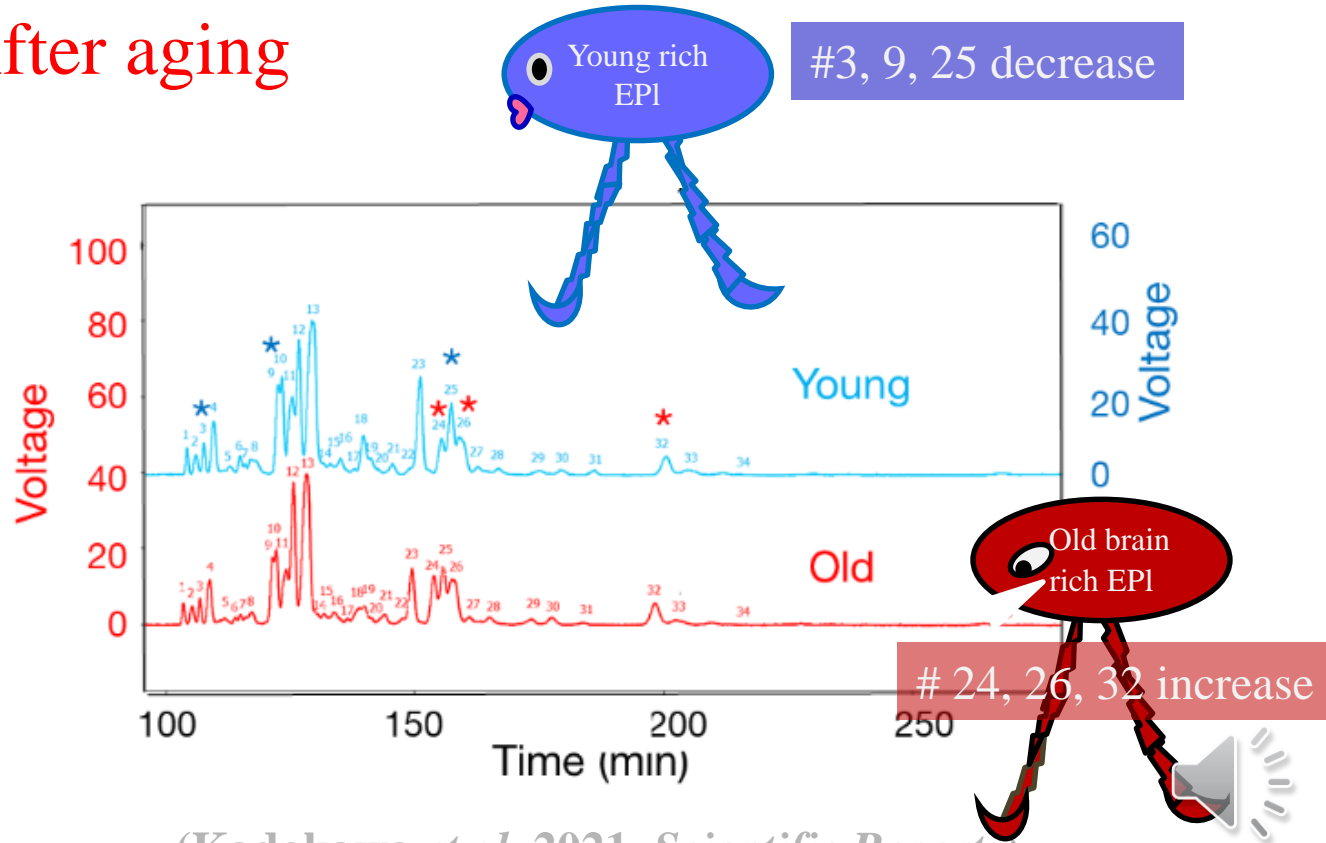


(Kadokawa *et al.* 2021. *Scientific Reports*)



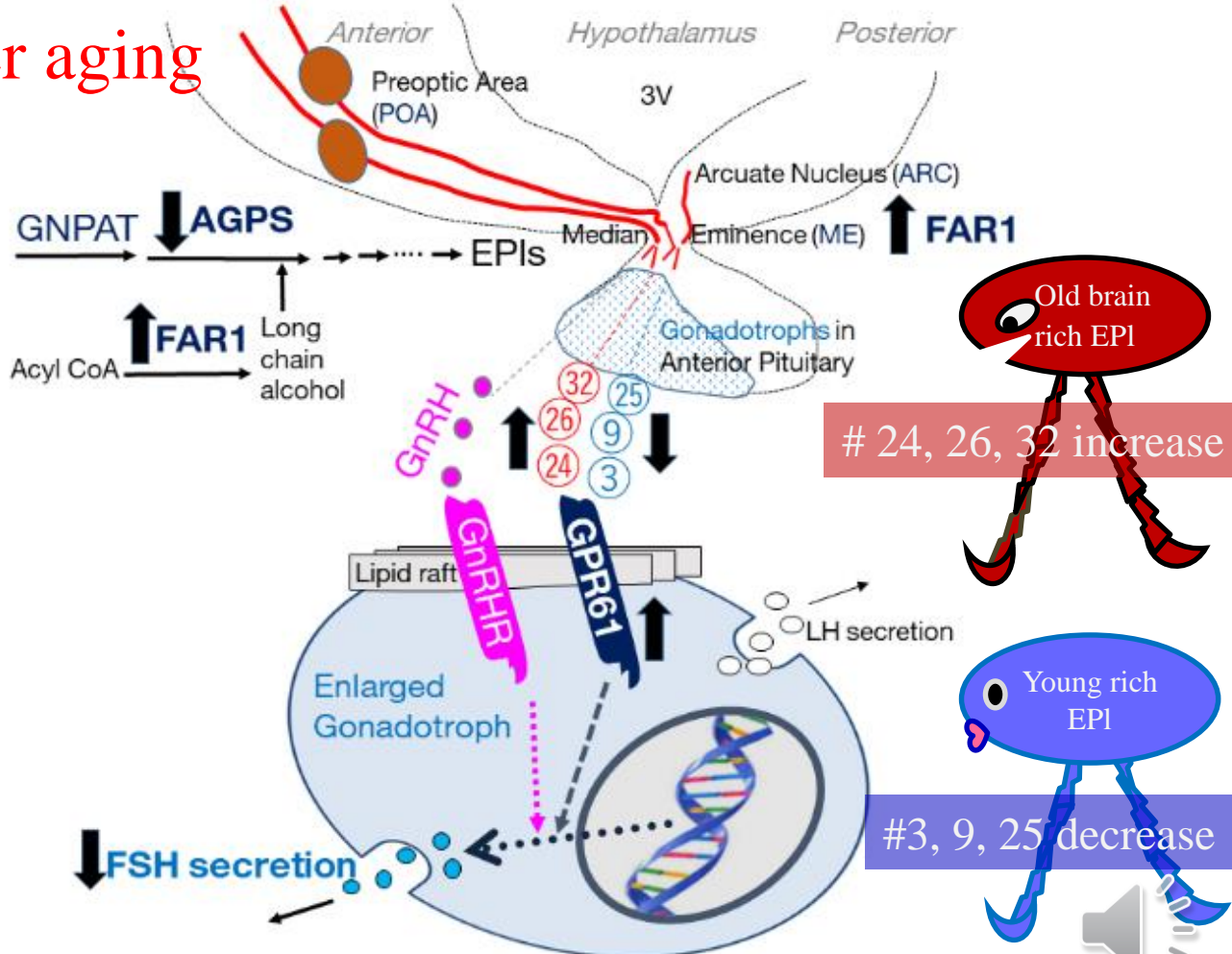
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After aging



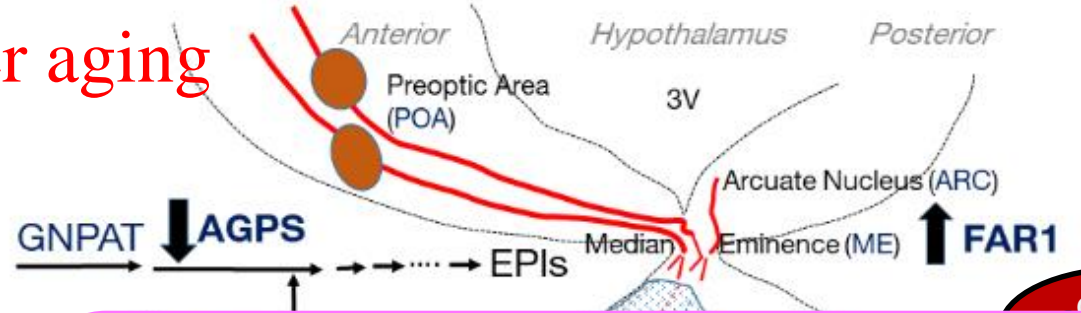
(Kadokawa *et al.* 2021. *Scientific Reports*)

After aging



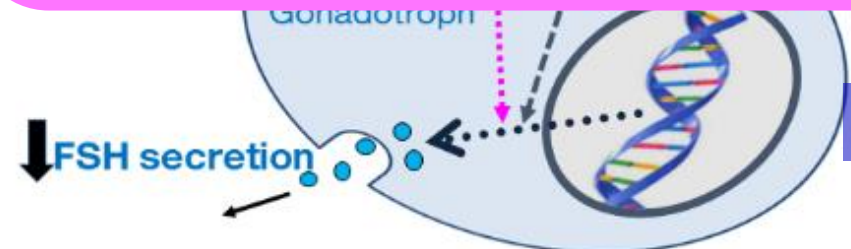
(Kadokawa et al. 2021. Scientific Reports)

After aging



Ethanolamine plasmalogen and GPR61 may have important roles in age-related infertility.

Details are in Poster W92



Old brain EPI

decrease

rich EPI

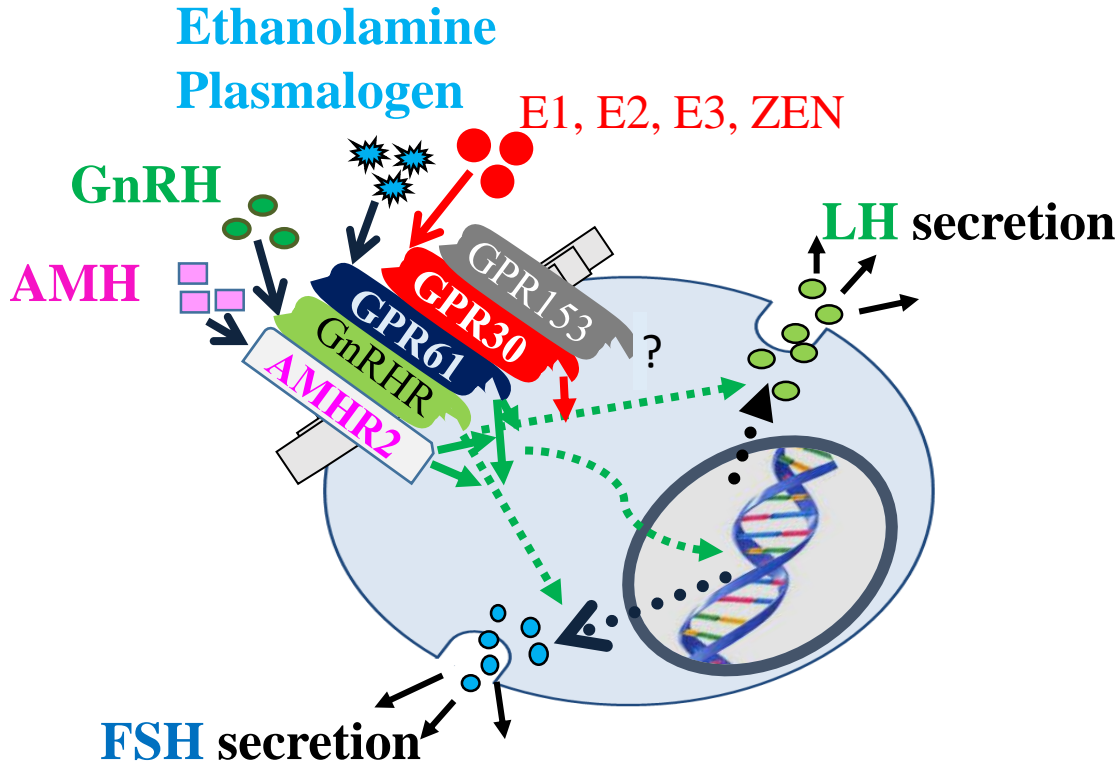
#3, 9, 25 decrease

(Kadokawa et al. 2021. Scientific Reports)

Conclusion in ICAR2020+2



These discoveries of new receptors on gonadotrophs open a new paradigm of the mechanisms controlling reproduction in mammals.





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Thank you for your attention.

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Welcome
collaboration

