NOVEL MECHANISM AND ACTIONS OF GONADOTROPHINS

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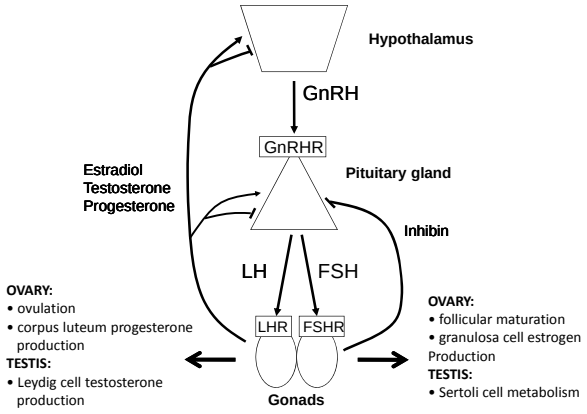
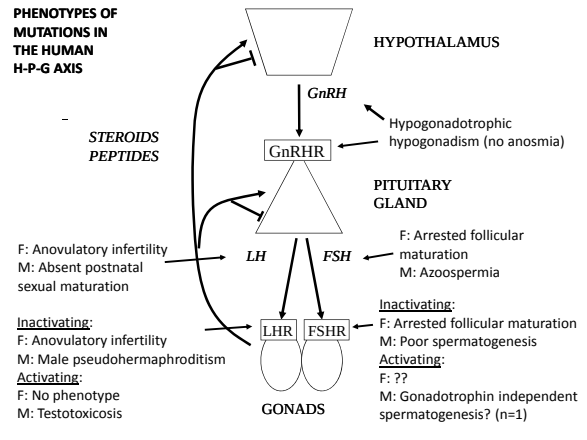
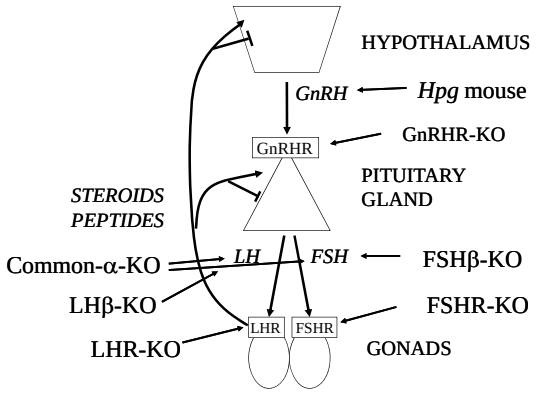
THE HYPOTHALAMIC-PITUITARY-GONADAL AXIS

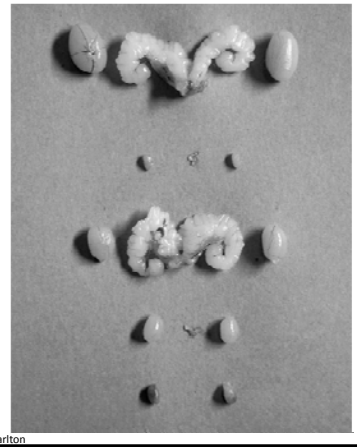


PHENOTYPES OF MUTATIONS IN THE HUMAN H-P-G AXIS



The HPG Axis: Existing Knockout Mouse Models





Normal

Hpg/GnRHR-KO
(GnRH deficient)

FSHR-KO

LHR-KO

Tfm
(androgen receptor deficient)

from H. Charlton

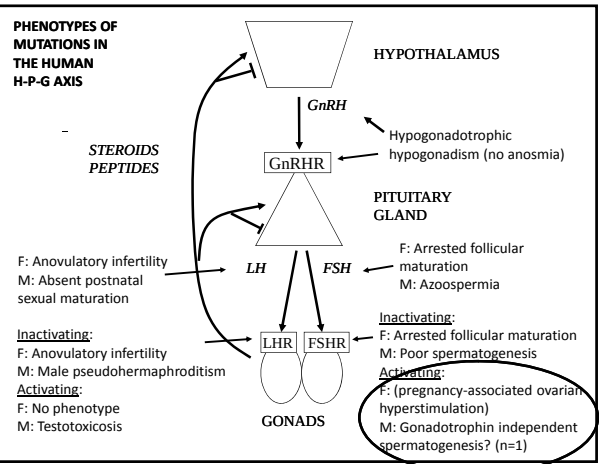
FSHβ/FSHR Inactivation

HUMAN	MOUSE
Male	Male
• normal sexual differentiation and maturation	• normal sexual differentiation and maturation
• reduces testis size and quality of sperm (FSHR)	• reduced testis size
• fertility possible (FSHR; n = 5)	• normal fertility
• azoospermia (FSHβ; n = 3)	
Female	Female
• normal sexual differentiation	• normal sexual differentiation
• sexual infantilism + infertility	• delayed vaginal opening
• primary/early 2nd amenorrhoea	• no estrous cycle
• ovaries full of immature follicles	• ovaries full of immature follicles
• lack of follicular maturation	• lack of follicular maturation

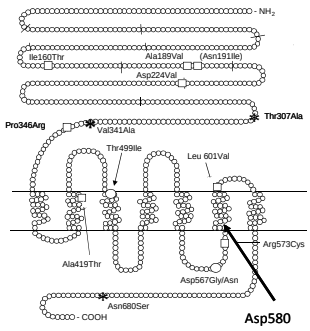
LH/LHR Inactivation

HUMAN	MOUSE
Male	Male
• pseudohermaphroditism (LHR)	• normal sexual differentiation
• normal sexual differentiation (LHβ)	• Leydig cell hypoplasia
• Leydig cell hypoplasia	• lack of pubertal maturation
• lack of pubertal maturation	• infertility
Female	Female
• normal sexual differentiation	• normal sexual differentiation
• delayed pubertal maturation	• delayed vaginal opening
• olgomenorrhea/amenorrhea	• no estrous cycle
• anovulatory infertility	• anovulatory infertility

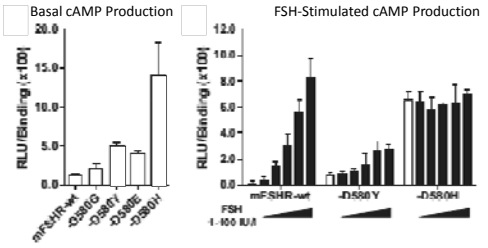
PHENOTYPES OF MUTATIONS IN THE HUMAN H-P-G AXIS



HUMAN FSH RECEPTOR MUTATIONS

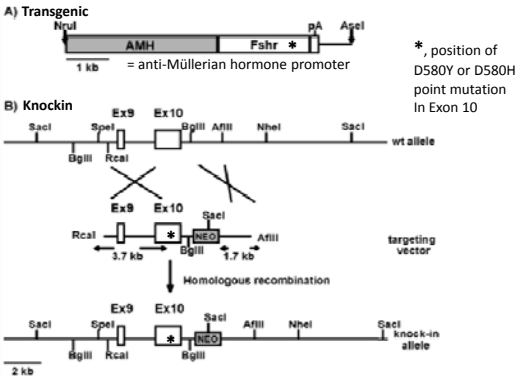


Mutations of D580 in mouse *FSHR* induce constitutive receptor activation in tranfected cells



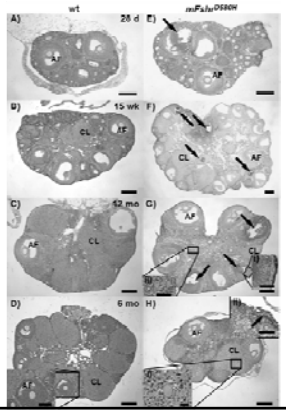
Peltoketo et al. Endo 2010.

Transgenic and Knockin Constructs to Produce Constitutively Activating Mutation (CAM) of *Fshr*



*, position of D580Y or D580H point mutation In Exon 10

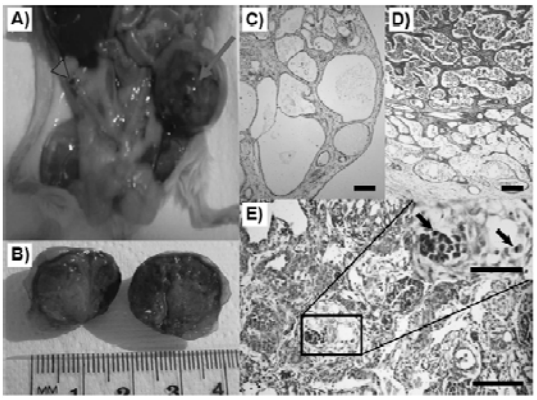
FEMALE PHENOTYPE OF CONSTITUTIVELY ACTIVATING *FSHR* MUTATION



- Haemorrhagic and squamous cell cysts
- Luteinised unruptured follicles
- Accelerated follicular maturation and depletion
- Accelerated ovarian ageing -> accumulation of of lipofuscin pigment (PAS staining) and collagen

Peltoketo et al. Endo 2010.

Ovarian Teratomas and Teratocarcinomas in *FSHR*-CAM Mice

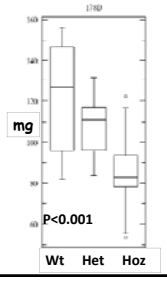


Peltoketo et al. Endo 2010.

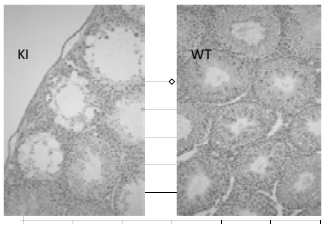
Mild phenotype in *Fshr*-CAM Males

- Variable reduction of testis size and occasional degradation of semiferous epithelium

Weight of right testis of *Fshr-D580Y* KI mice



Degeneration of seminiferous epithelium



Peltoketo et al., unpublieshed..

Human phenotypes of *FSHR*-CAM ?

FEMALE
- Haemorrhagic ovarian cysts
- Premature ovarian failure (POF)
- Luteinised unruptured follicles (LUF)
- Ovarian teratomas

MALE
- Marginal reduction of testis weight and mild disturbance of spermatogenesis

The luteinizing hormone/chorion gonadotropin receptor (LHCGR)

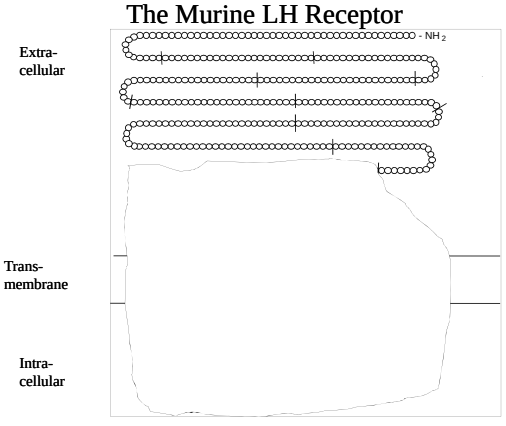
- G protein coupled receptor (GPCR)
- located on plasma membrane
- long extracellular ligand-binding domain (leucine rich repeats)
- transmembrane signaling domain
- intracellular tail
- ligands: luteinising hormone (LH) and choriongondotropin (CG)
- main 2nd messengers cAMP/PK-A and PL-C/Ca^{2+}/IP_3/PK-C
- LH/CG target cells:
  - testis: Leydig cells
  - ovary: theca, late granulosa and luteal cells

Normal Prenatal but Arrested Postnatal Sexual Development of Luteinizing Hormone Receptor Knockout (LuRKO) Mice

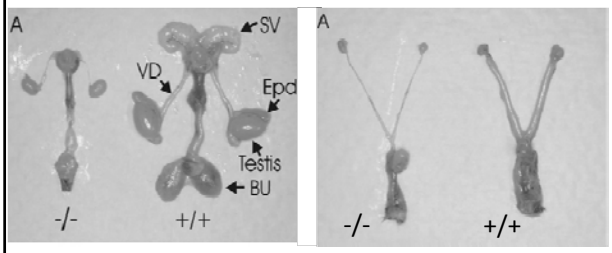
Fu-Ping Zhang, Matti Poutanen, Johannes Wilbertz, and Ilpo Huhtaniemi

Mol Endocrinol 2001;15: 172183.

The Murine LH Receptor



Phenotype of LHR knockout mice



Males Females

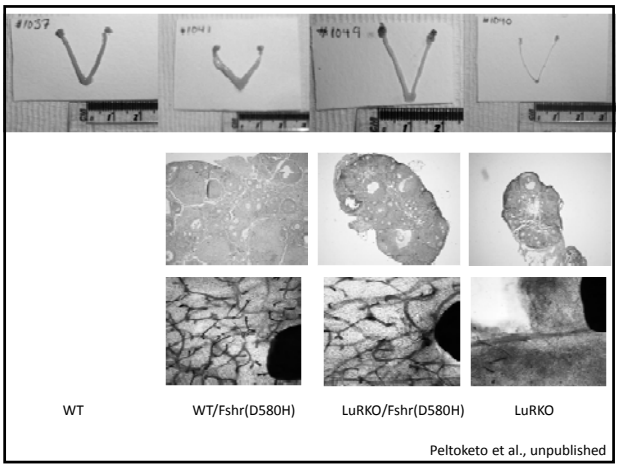
Zhang et al. Mol Endo 2001

How to exploit the LHR-KO mouse: Studies beyond the knockout phenotype

- Can strong FSH stimulation compensate missing LH action?

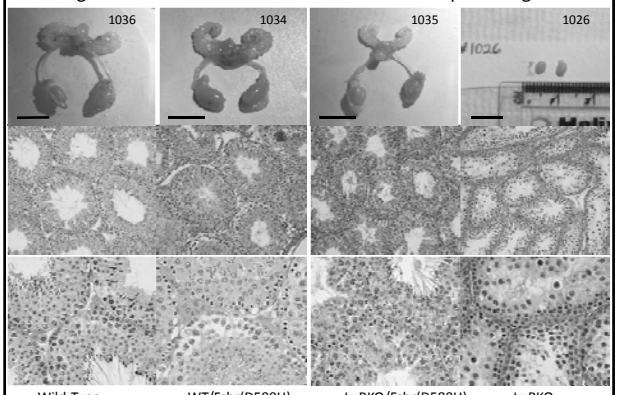
Crossing of FSHR-CAM and LuRKO Mice

- Can high FSH action compensate for missing LH action?
  - Follicular maturation, estrogen production and ovulation in females
  - Testosterone production and spermatogensis in males

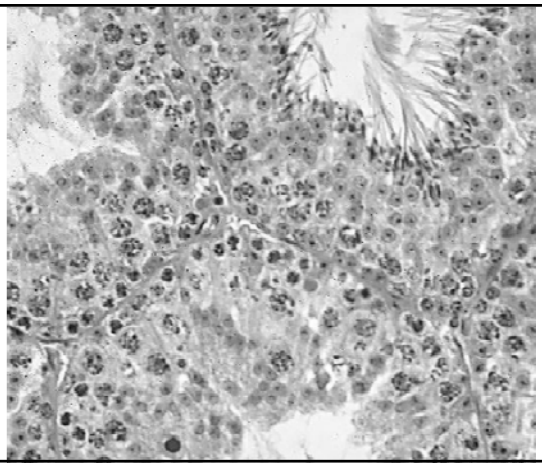


Peltoketo et al., unpublished

Strong FSHR stimulation alone is able to maintain spermatogenesis



Peltoketo et al., unpublished



Strong FSHR Simulation in the Absence of LH Action Can:

IN MALE MICE:

- Stimulate Leydig cell androgen production (via paracrine Sertoli->Leydig cell link ?)
- Induce full spermatogenesis
- Induce fertility

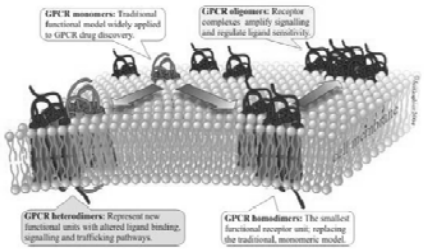
IN FEMALE MICE:

- Increase ovarian size
- Advance follicular maturation to large antral stage
- Increase estrogen production (-> enlarged uterus and mammary gland development)

How to exploit the LHR-KO mouse: Studies beyond the knockout phenotype

- **Do gonadotrophin receptors form functional dimers?**

Diversification of GPCR function and cellular response by dimerization/oligomerization



Why is it important to know if G-protein coupled receptors (GPCRs) dimerise?

- GPCRs are the largest gene family in the human genome (~900, 3% or all genes)
- They regulate the senses of smell, touch, taste, vision, and mediate actions of neurotransmitters and hormones
- About 40% of currently used drugs function through GPCRs
- Dimerisation could contribute to multiple functions of a specific GPCR, and make it possible to develop selective blockers or activators of specific actions

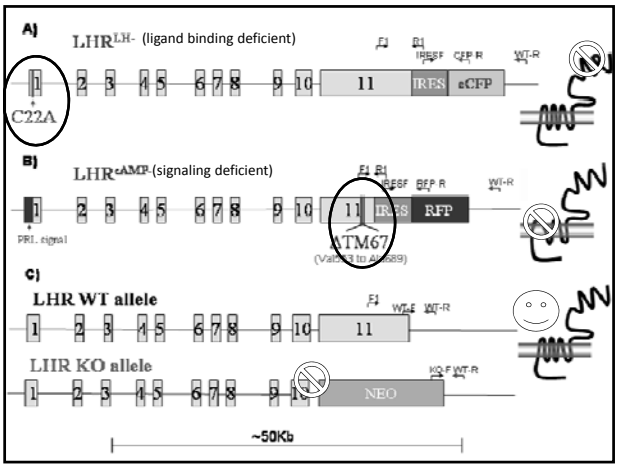
  (biased agonism -> strengthening of wanted effects and/or elimination of side effects)

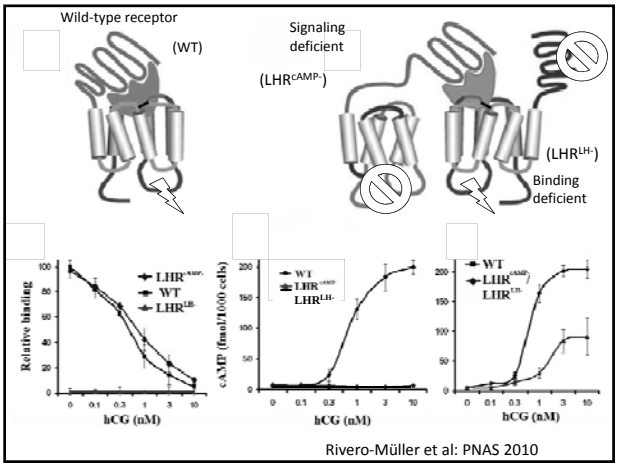
GPCR dimerisation: some topical questions

- How does ligand binding to one protomer affect an associated protomer?
- What is the functional unit that activates downstream signaling molecules?
- What parts of the receptor form the interfaces between protomers?
- Where along the pathway from synthesis to degradation do dimers form?
- Do they ever dissociate?
- **Does dimerisation occur in vivo?**

Is LHR dimerisation a physiologically meaningful mode of GPCR function?

Can we rescue the hypogonadal phenotype of LHR-KO mice through functional complementation (i.e. compulsory dimerisation) of binding- and signaling-deficient LHR mutants?



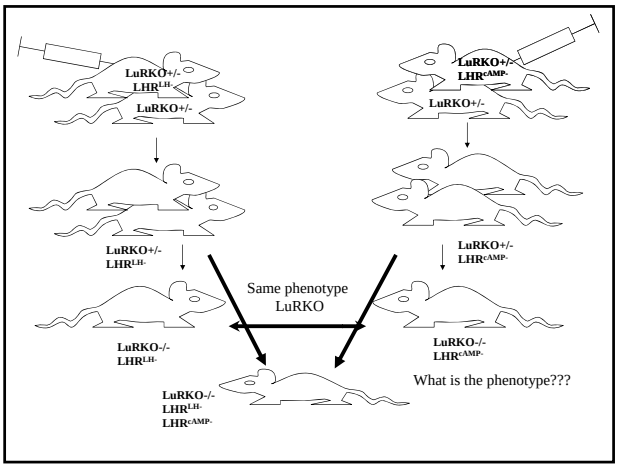


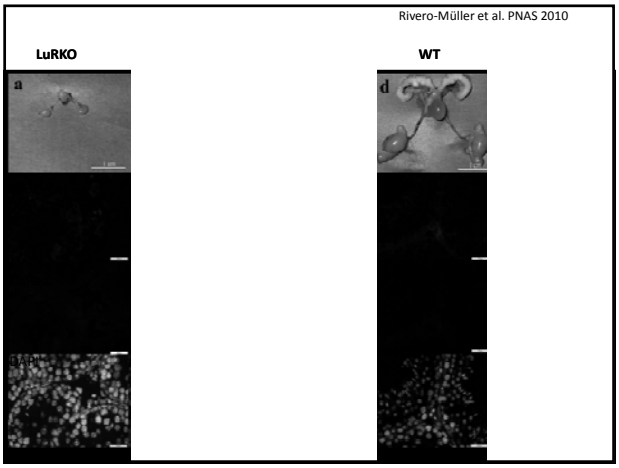
Rivero-Müller et al: PNAS 2010

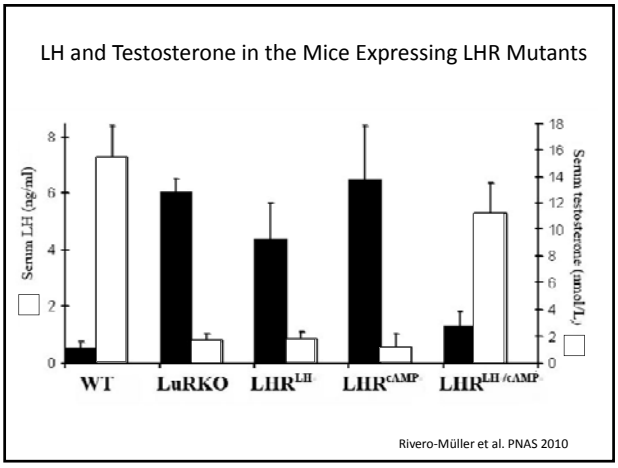
LHR (LuRKO) KO mice
- **no LHR expression**
- **hypogonadal**

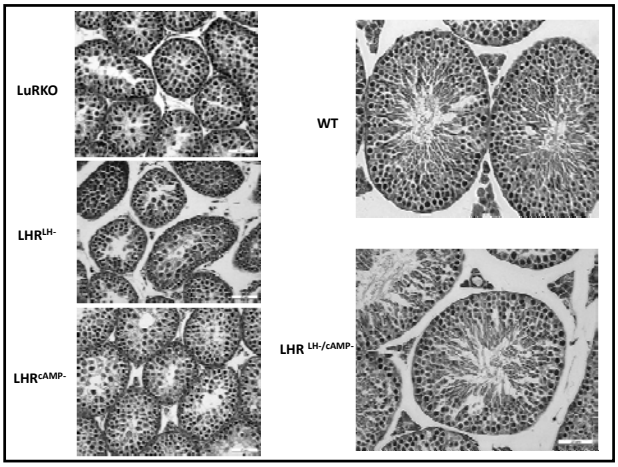
LHR^{LH-} BAC-TG mice
- **express ligand binding-deficient LHR**

LHR^{cAMP-} BAC-TG mice
- **express signaling-deficient LHR**


LuRKO+/-
LHR^{LH-}
LuRKO+/-
LuRKO+/-
LHR^{cAMP-}
LuRKO+/-
LuRKO+/-
LHR^{LH-}
LuRKO+/-
LHR^{cAMP-}
Same phenotype
LuRKO
LuRKO-/-
LHR^{LH-}
LuRKO-/-
LHR^{cAMP-}
What is the phenotype???
LuRKO-/-
LHR^{LH-}
LHR^{cAMP-}


Rivero-Müller et al. PNAS 2010
LuRKO
WT
a
d


LH and Testosterone in the Mice Expressing LHR Mutants
Serum LH (ng/ml)
Serum testosterone (nmol/L)
WT
LuRKO
LHR^{LH-}
LHR^{cAMP-}
LHR$^{LH-/cAMP-}$
Rivero-Müller et al. PNAS 2010



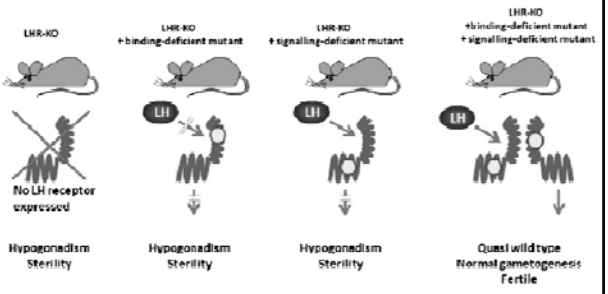
Testis and Seminal Vesicle Weights, and Fertility of the LuRKO mice expressing LHR^{LH-}, LHR^{-cAMP} or Both

	Testis (mg ± SD)	Seminal vesicles (mg ± SD)	**Pups sired**
LuRKO	53.6 ± 5.7[a,*]	< 2 mg[a]	**N/A**
LHR^{LH-}/LuRKO	52.1 ± 8.3[a]	< 2 mg[a]	**N/A**
LHR^{cAMP-}/LuRKO	54.9 ± 6.7[a]	< 2 mg[a]	**N/A**
LHR$^{LH-/cAMP-}$/LuRKO	145.3 ± 44.0[b]	539.8 ± 37.7[b]	**7.5 ± 1.3 (n=5)**
WT	147.9 ± 40.2[b]	535.2 ± 26.6[b]	**7.1 ± 1.9 (n=4)**

*a vs. b: $p < 0.01$

Rivero-Müller et al. PNAS 2010

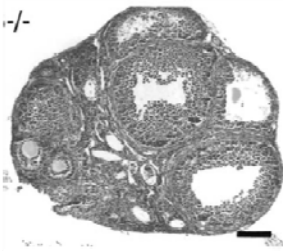
The blind and the lame: An in vivo demonstration of functional G protein–coupled receptor dimers



Rivero-Müller at al.: PNAS 2010:107:2319.
(commentary by Vassart: PNAS 2010,107:1820)

LHR $^{LH-/cAMP-}$/LHR-KO ovary

LHR-KO ovary



Rivero-Müller et al: Unpublished

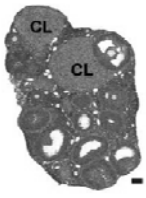
How about females? (preliminary data)

- *LHR^{LH-}/LHR^{cAMP-}/LHR-KO mice are infertile*

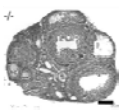
- incomplete signaling upon functional complementation does not activate the whole complement of LH actions in the ovary

- *LHR^{LH-}* ***OR*** *LHR^{cAMP-} mutation in the WT background show ovarian hyperstimulation*

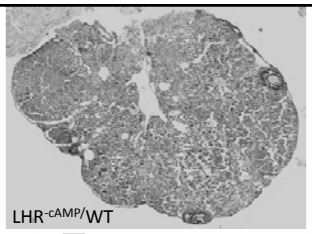
- enhanced WT-LHR activity due to positive allosteric influence of the inactive receptor protomer



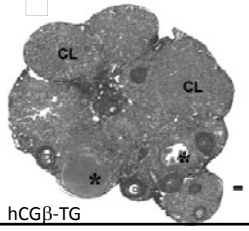
WT



LuRKO



LHR$^{-cAMP/}$WT



hCGβ-TG

How about females? (preliminary data)

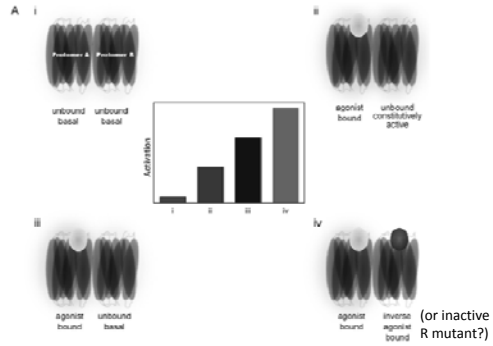
- *LHR^{LH-}/LHR^{cAMP-}/LHR-KO mice are infertile*

- biased signaling upon functional reconstitution does not activate the whole complement of LH actions in the ovary

- *LHR^{LH-}* ***OR*** *LHR^{cAMP-} mutation in the WT background show ovarian hyperstimulation*

- WT-LHR activity may be enhanced through positive allosteric influence of the inactive receptor protomer

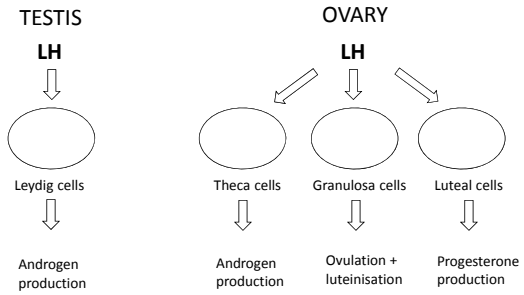
Allosteric modulation of intrinsic efficacy of GPCR dimers by ligand binding



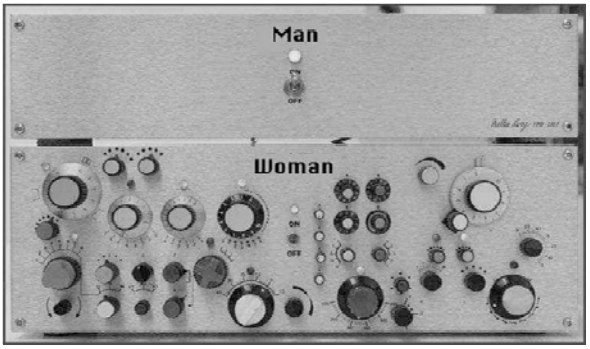
(or inactive R mutant?)

Smith & Milligan: Pharm Rev 2010

LH Actions in Testis and Ovary



Sex Differences in LH Action ;-)



NOVEL MECHANISMS AND ACTIONS OF GONADOTROPHINS

TAKE-HOME MESSAGES:

1. Mouse model for activating FSHR mutation predicts a strong female but very mild male phenotype in humans.
2. Strong FSHR stimulation can take over some LH functions in the absence of functional LHR.
3. Functional complementation of binding- and signaling-deficient LHR mutants is able to rescue normal male, but not female, reproductive phenotype in LHR-KJO background.

Dept. of Physiology, University of Turku

- Adolfo Rivero-Müller
- Fu-Ping Zhang
- Ashutosh Trehan
- Matti Poutanen

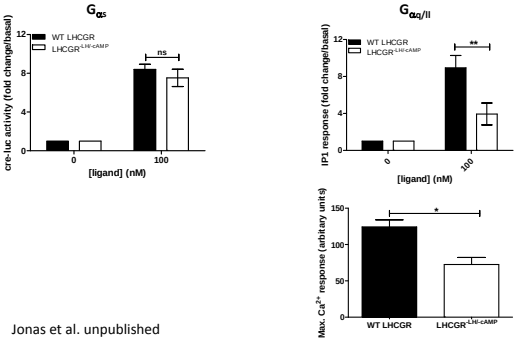
Dept. of Reprod. Biol., Imperial College London

- Hellevi Peltoketo
- Yen-Yin Chou
- Aylin Hanyaloglu
- Kim Jonas
- Layi Oduwole

Grants

- The Academy of Finland
- Sigrid Jusélius Foundation
- MRC
- The Wellcome Trust
- BBSRC
- European Union

LHR trans-activation is compatible only with normal $G_{\alpha s}$, but not with $G_{\alpha q/11}$ response



Jonas et al. unpublished