Gonadotropins during natural cycles: the galaxy we know

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Disclosures

- **Faculty: Manuela Simoni, MD, PhD**
- **Relationships with commercial interest in the last year: grants and consulting fees from:**

  - Merck
  - Ferring
  - IBSA

Disclaimer: I am only a humble endocrinologist

Educational objectives

- Discuss recent advances in the understanding of the physiology of gonadotropins
- Describe molecular and pathophysiological effects of gonadotropins and their receptors
- Explain FSH and LH dimerization: molecular pathways and possible clinical implications

Agenda

- Gonadotropins in the natural and multiovulatory cycle
- LH and hCG have different scopes and effects
- FSH potentiates LH and hCG action (including apoptosis, via cAMP)
- Estrogen counteracts pro-apoptotic effects of gonadotropin-dependent cAMP increase:
  a new player in the game

Agenda

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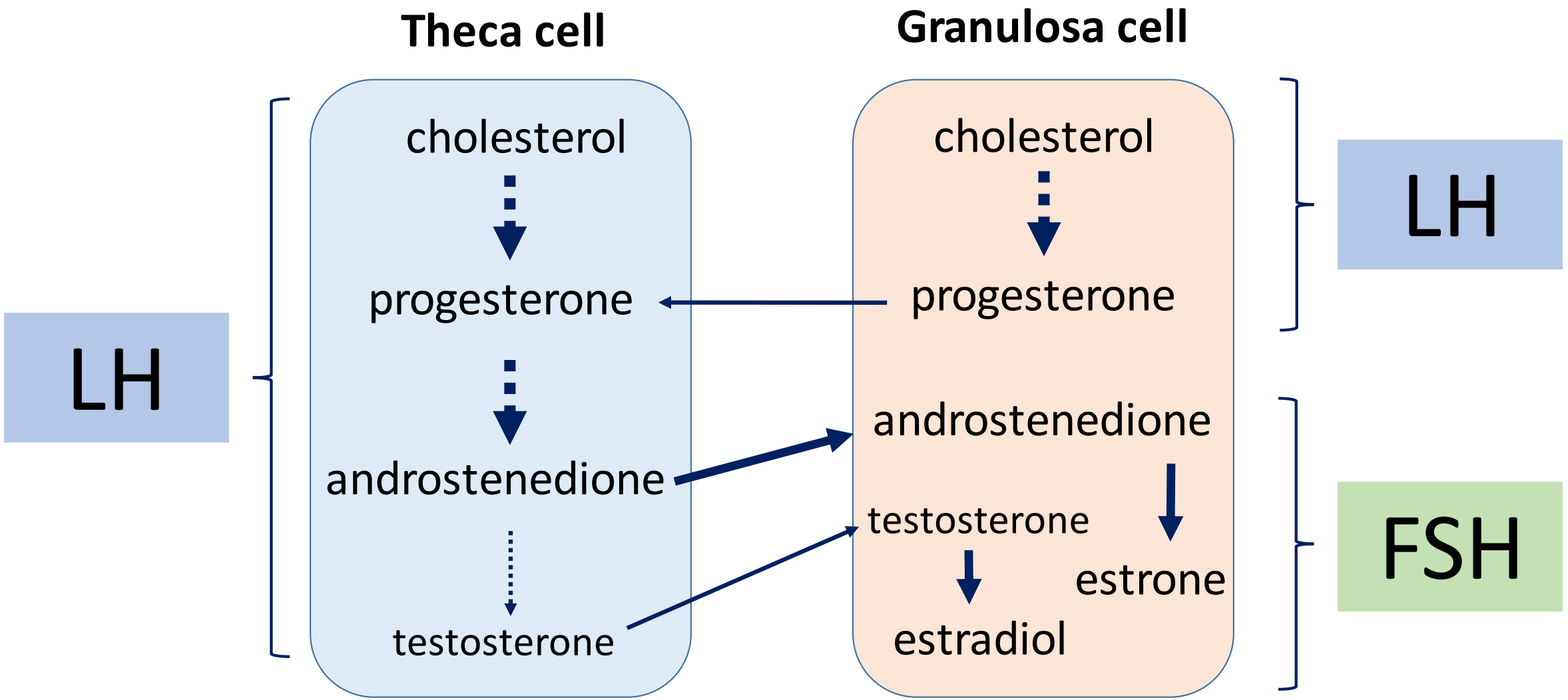
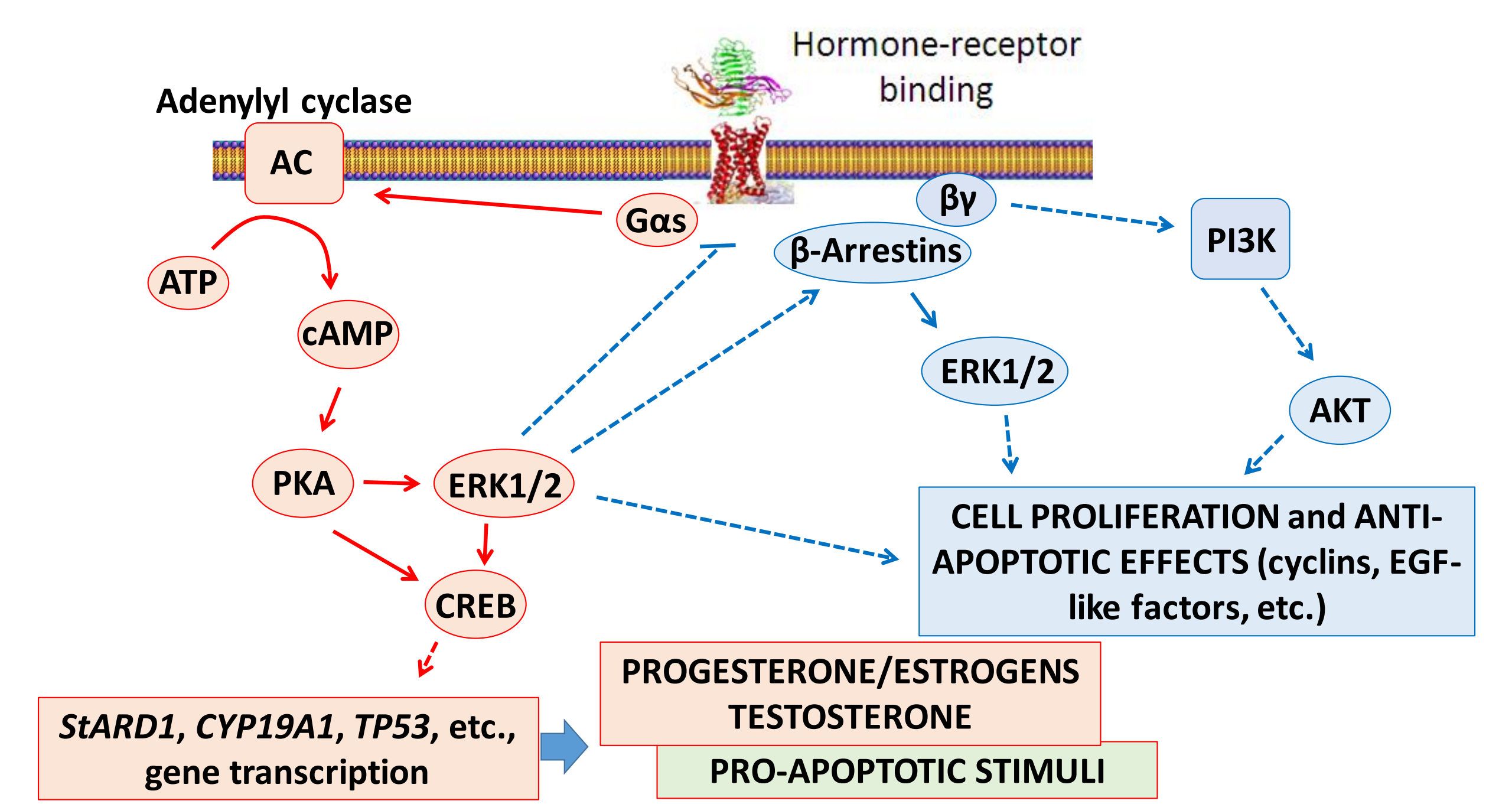
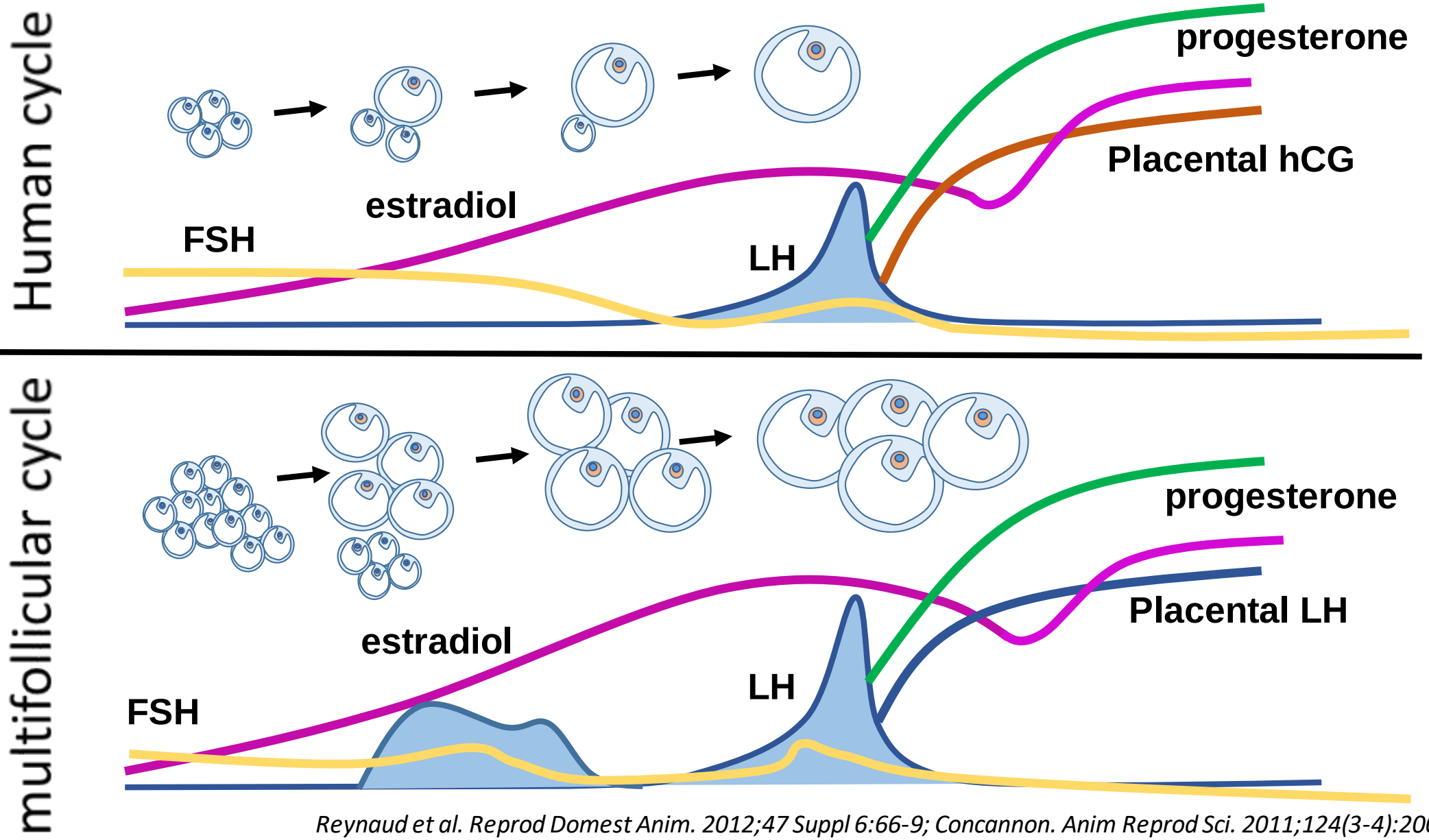
Two cells/two gonadotropins theory





Casarini L, Crépieux P. Front. Endocrinol. 2019. doi.org/10.3389/fendo.2019.00305.

MONO- *versus* MULTI-follicular growth

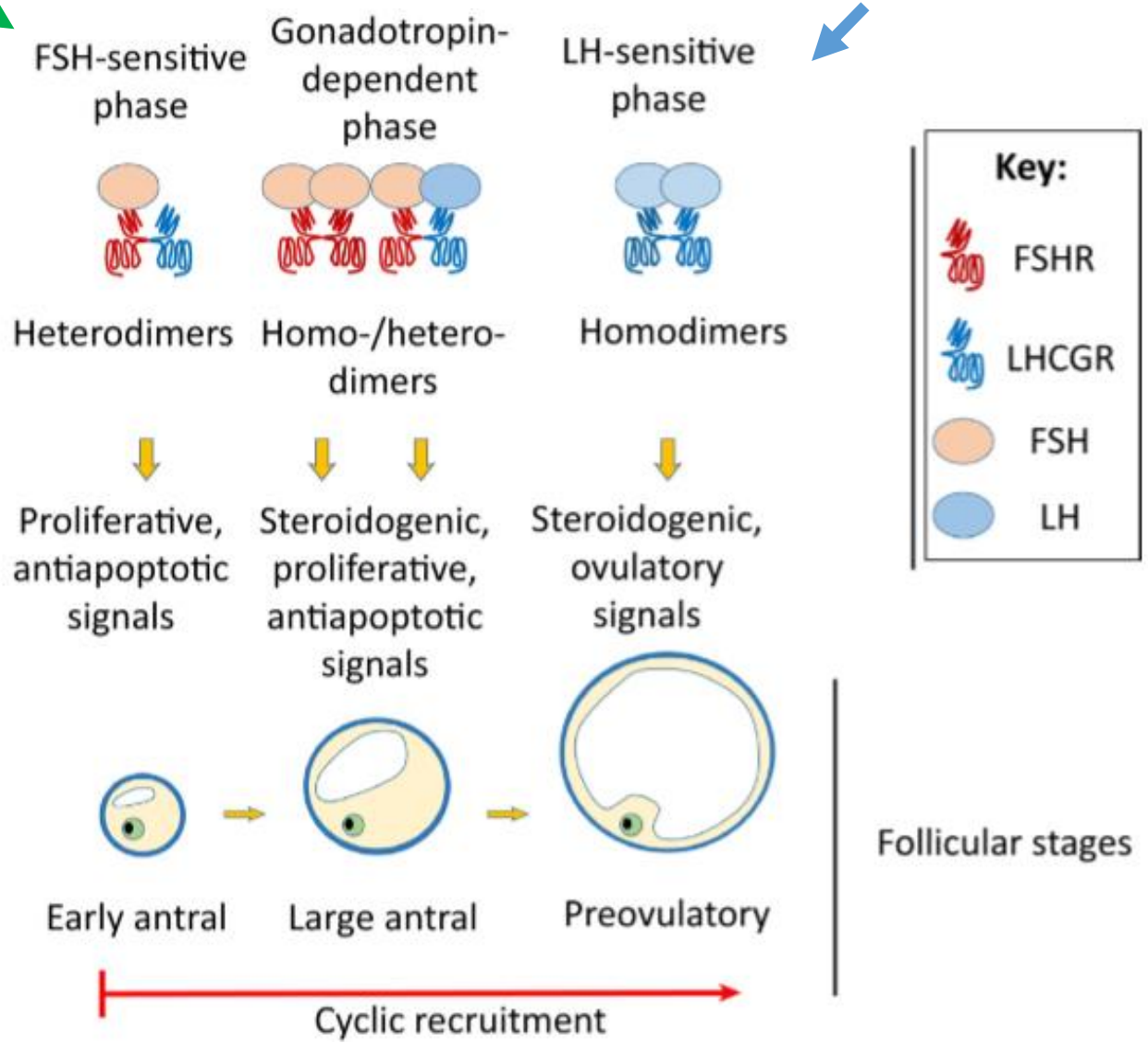


Reynaud et al. Reprod Domest Anim. 2012;47 Suppl 6:66-9; Concannon. Anim Reprod Sci. 2011;124(3-4):200-10.

Low FSHR = Low LHCGR

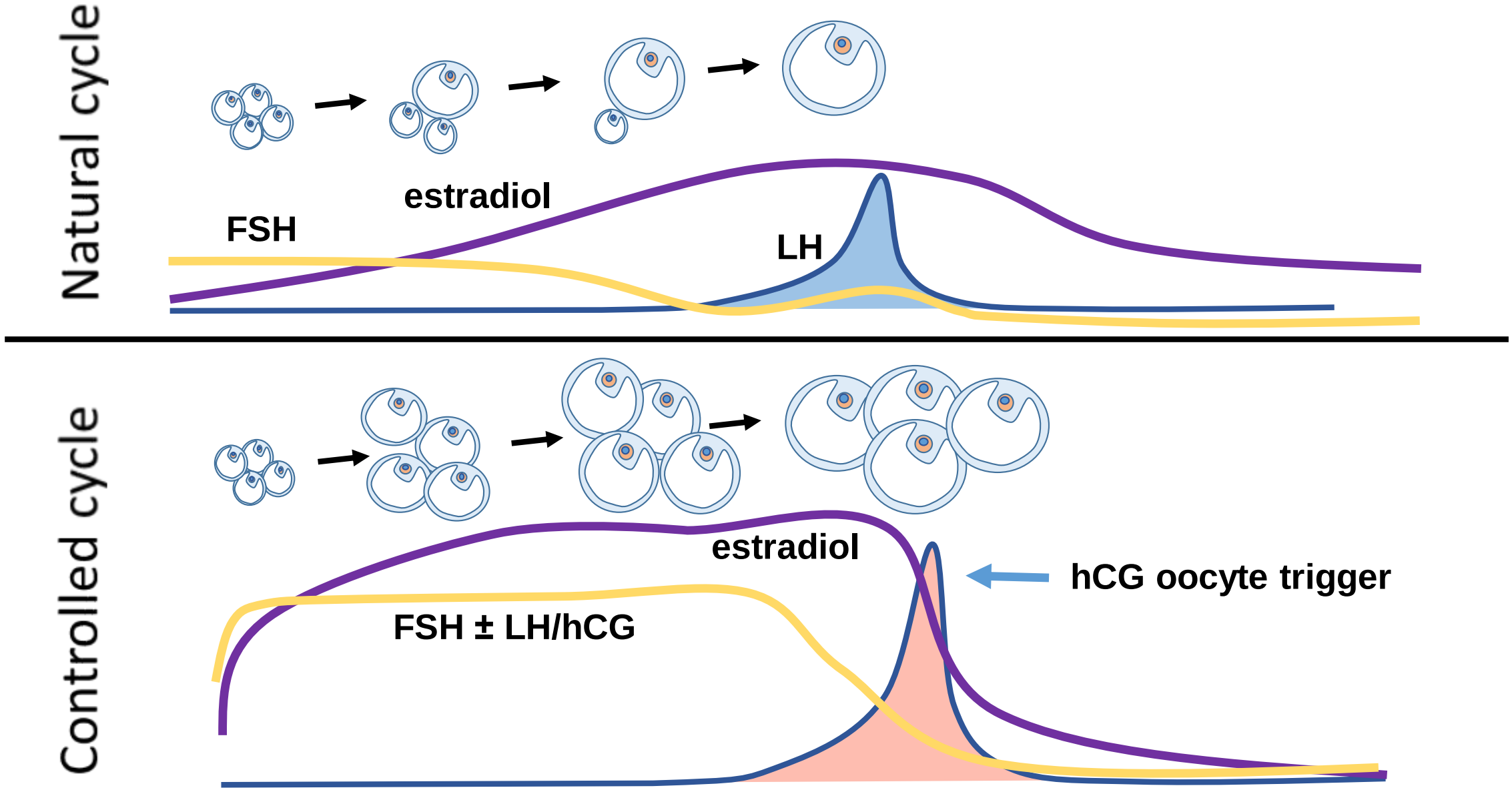
FSHR = LHCGR X 1000

FSHR X 1000 = LHCGR



Casarini et al. Trends Endocrinol Metab. 2018;29(4):208-217.

Natural *versus* controlled follicle growth



Complexity of the hormonal control of follicular growth

- The same receptor (LHCGR) is expressed in two cell types (theca and granulosa)
- The same receptor (LHCGR) responds to two hormones (LH and hCG)
- One cell type (granulosa) expresses two receptors (FSHR and LHCGR)
- Follicular growth depends on:
  - Estrogen (LH action on theca and FSH action on granulosa)
  - FSH action on granulosa (recruitment + apoptosis/atresia)
  - LH action on granulosa (follicular survival, dominance, maturation)

LH activity is fundamental!

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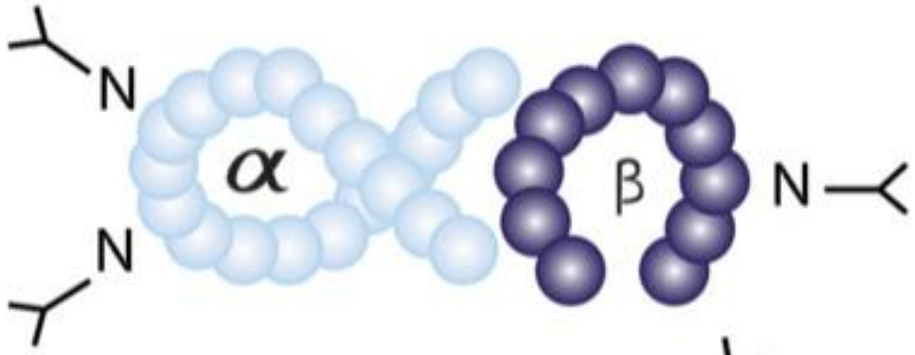
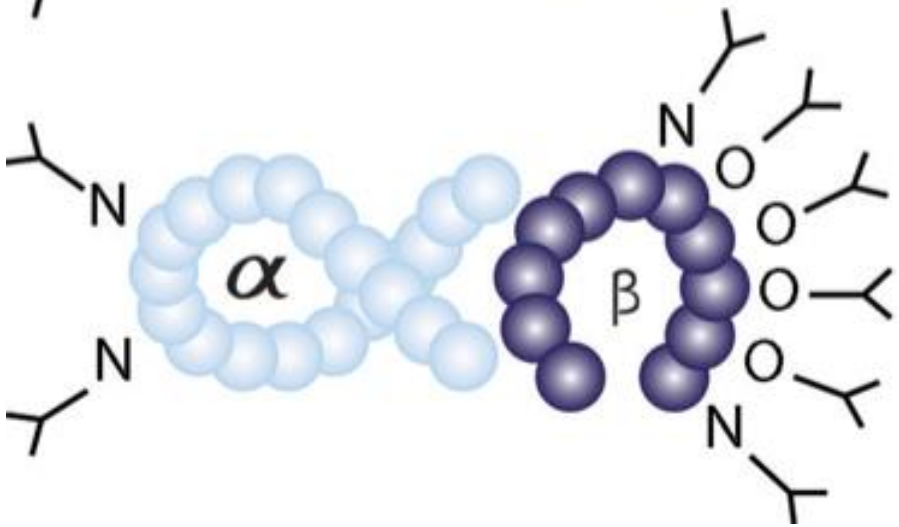
LH/hCG biochemical properties

LH



Molecular weight: 26-32 KDa
Half-life: ~20 min-12 hours

hCG

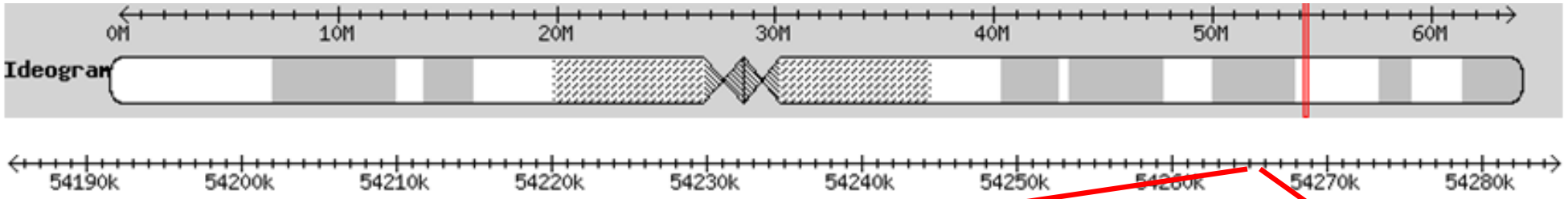


Molecular weight: 37 KDa
Half-life: ~15-460 hours

Esteves and Alviggi. 2015. *Springer*. doi: 10.1007/978-81-322-1686-5_16

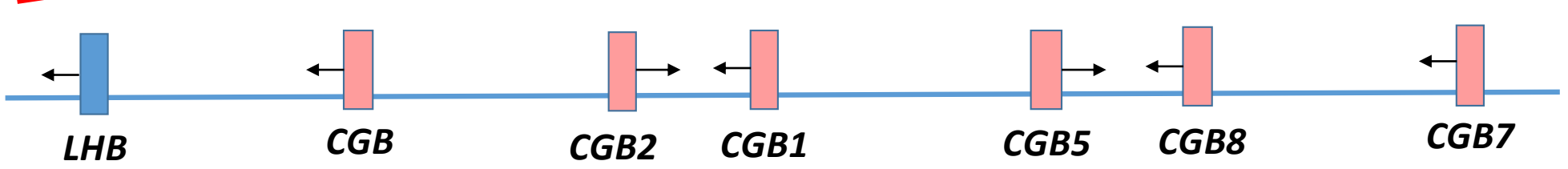
Structure of human *LHB/CGB* gene cluster

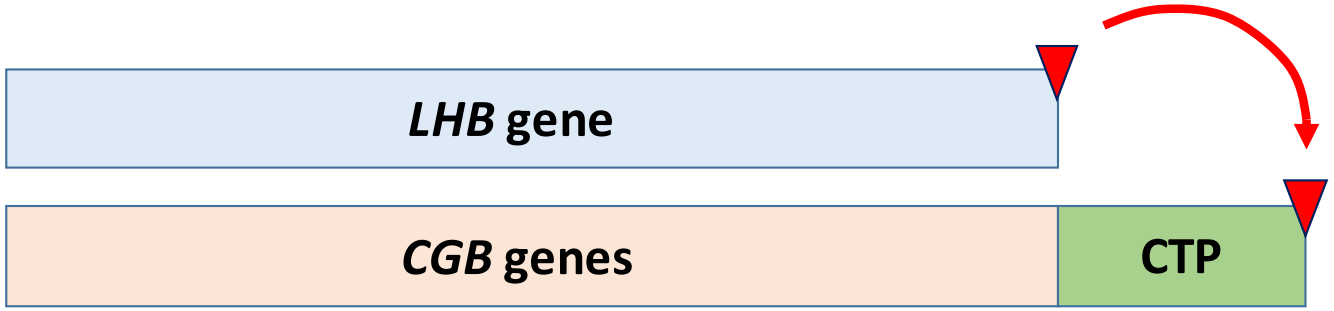
Chromosome 19



LHB gene

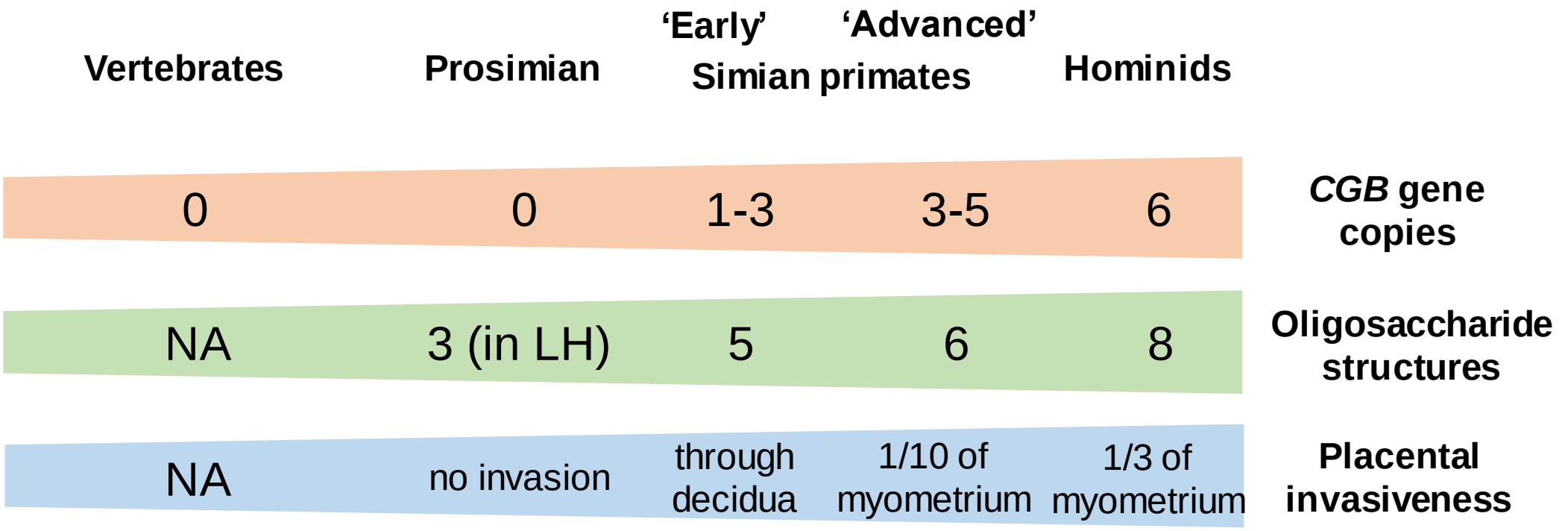
CGB genes





Hallast et al. *Genome Res*. 2005. 15(11):1535-46.

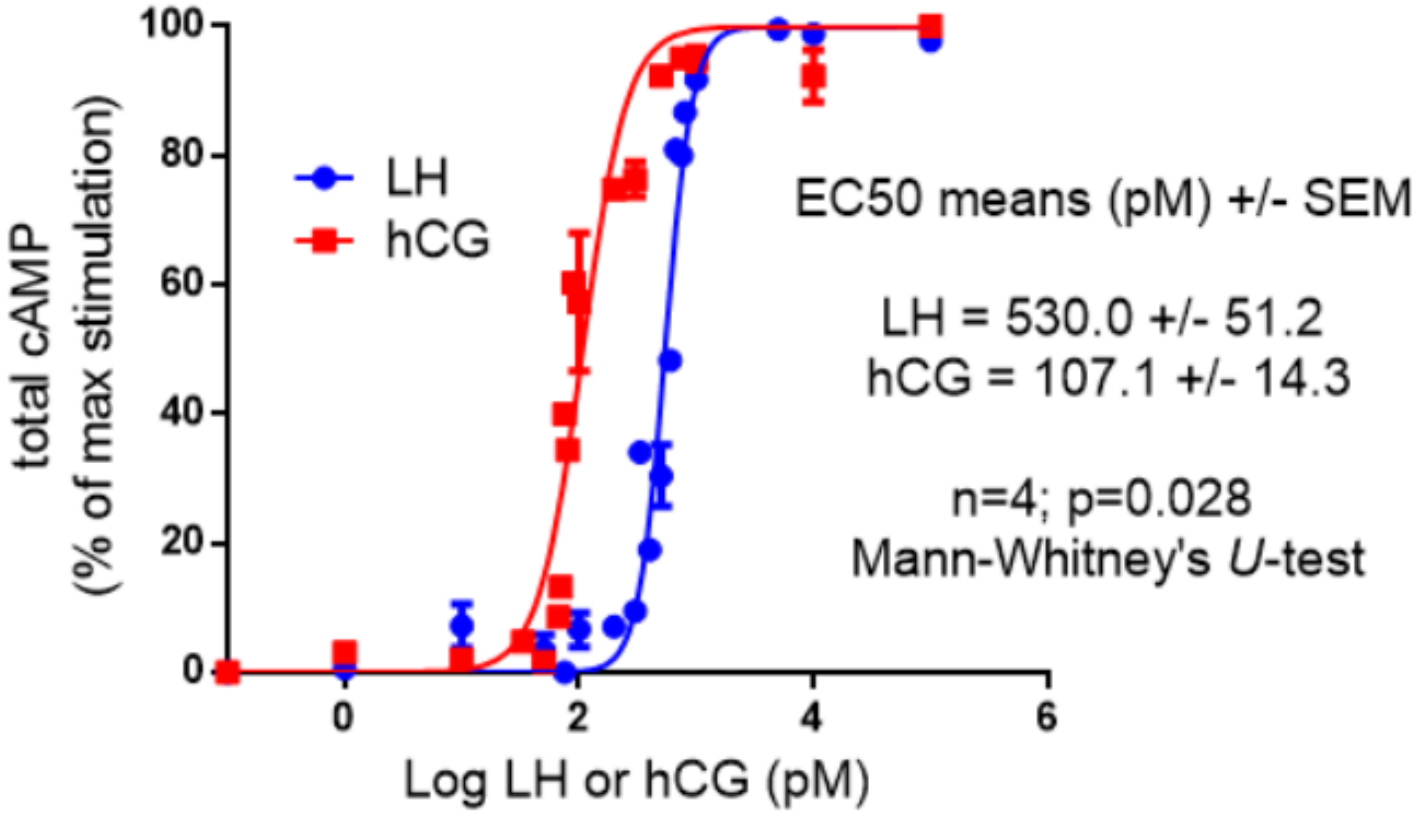
Complexity of the LH/CG system



Henke and Gromoll. *Mol Cell Endocrinol*. 2008. 291(1-2):11-9; Cole. *J Reprod Immunol*. 2009. 82(2):112-8.

LH vs hCG:
cAMP, cell survival, and effects of FSH

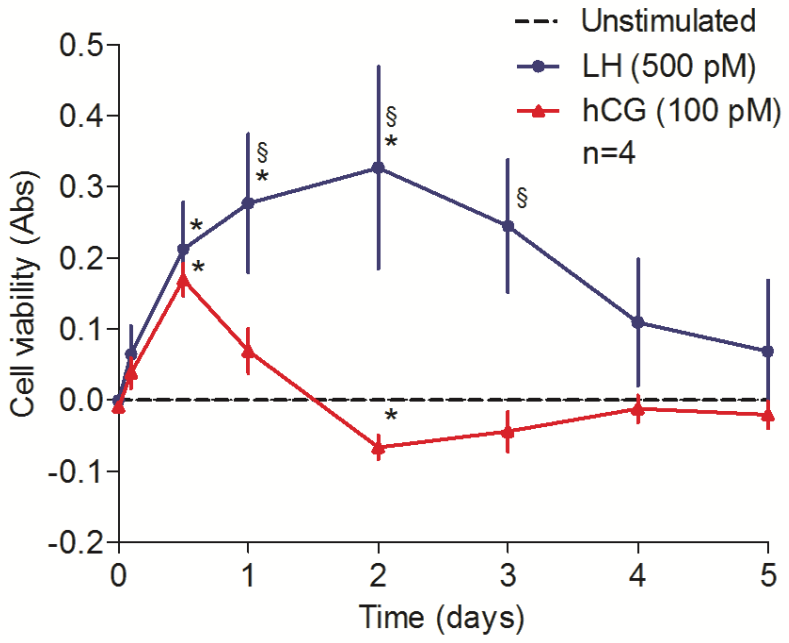
cAMP production

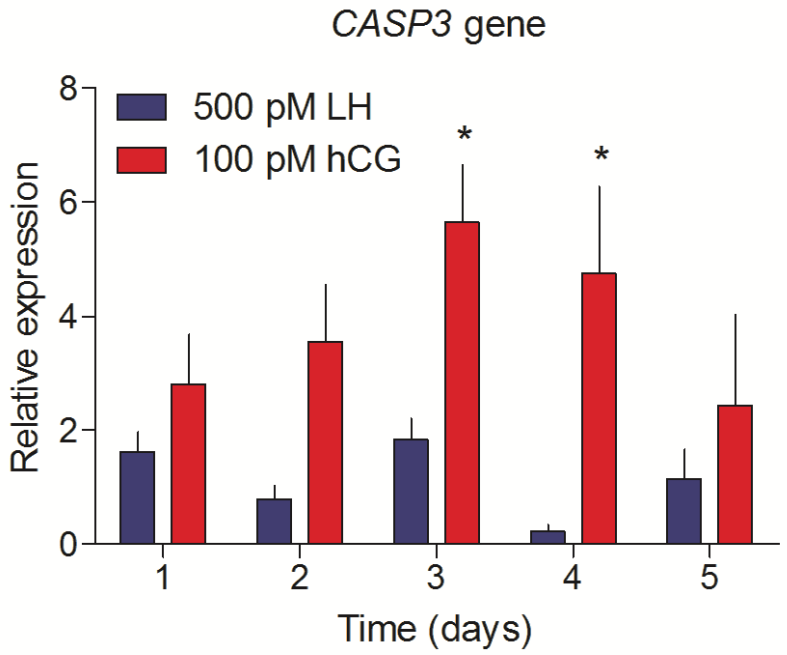


Cave: **Excessive cAMP is pro-apoptotic!**

Casarini et al. *PLoS One*. 2012. 7(10):e46682.

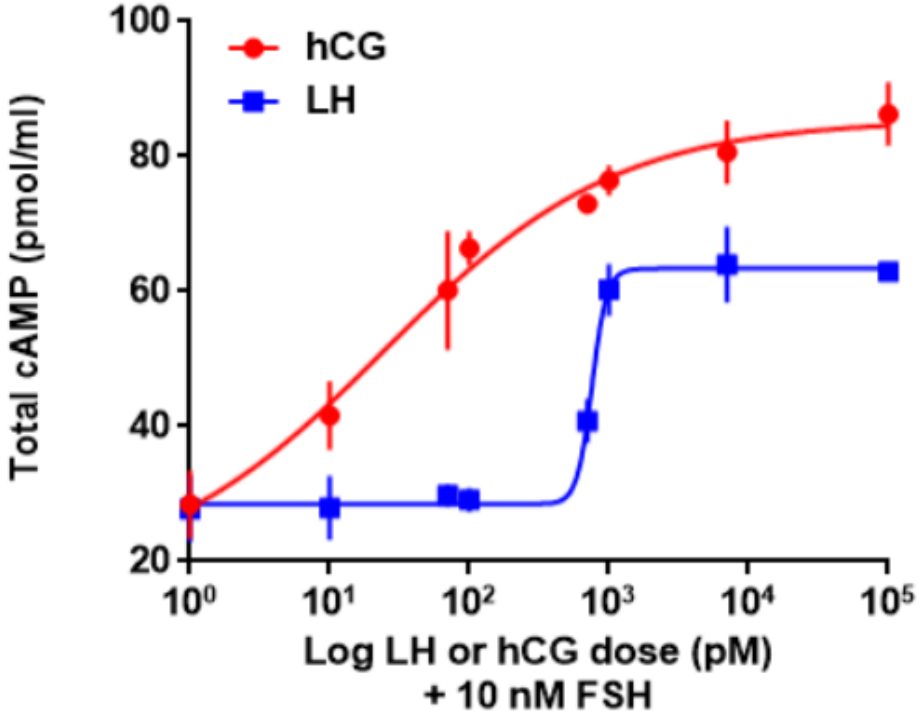
hCG reduces cell viability over time and stimulates Caspase-3 expression in hGL5/LHCGR cells





Casarini et al., Int. J. Mol. Sci, 2017

FSH increases hCG-dependent cAMP activation



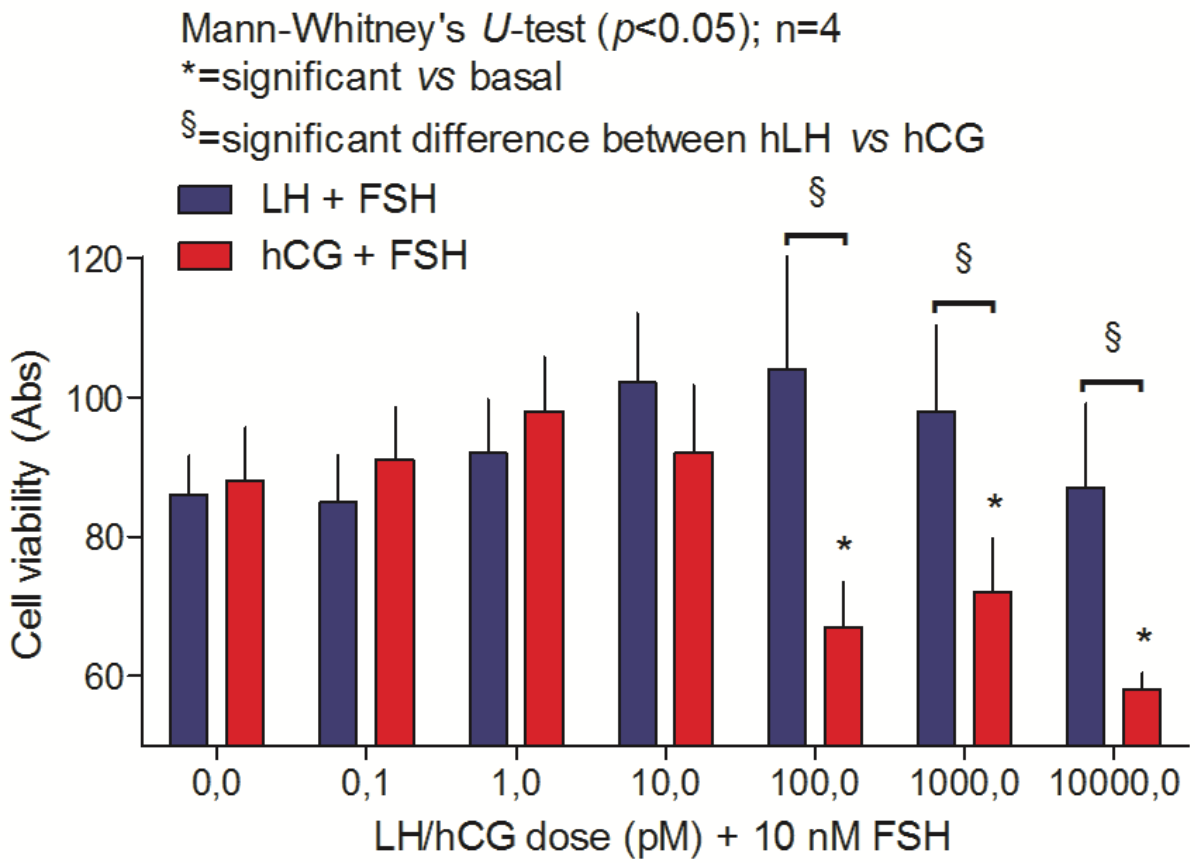
Comparison between LH and hCG EC50 and H-slopes from cAMP dose–response curves, in the presence (present data) or absence (Casarini et al., 2012) of 1×10^1 nM FSH.

EC50 (means±SD)			
LH	hCG	+FSH (nM)	Reference
475.8 ± 137.4	101.8 ± 44.6	0.0	Casarini et al., 2012 (n = 4)
440.9 ± 271.4	20.3 ± 1.2	10^1	Present article (n = 6)
1.1	5.0		Fold difference

Cave: **Excessive cAMP is pro-apoptotic!**

Casarini et al. *Mol Cell Endocrinol*. 2016. 422:103-14.

LH increases and hCG reduces cell viability of hGLC in the presence of FSH

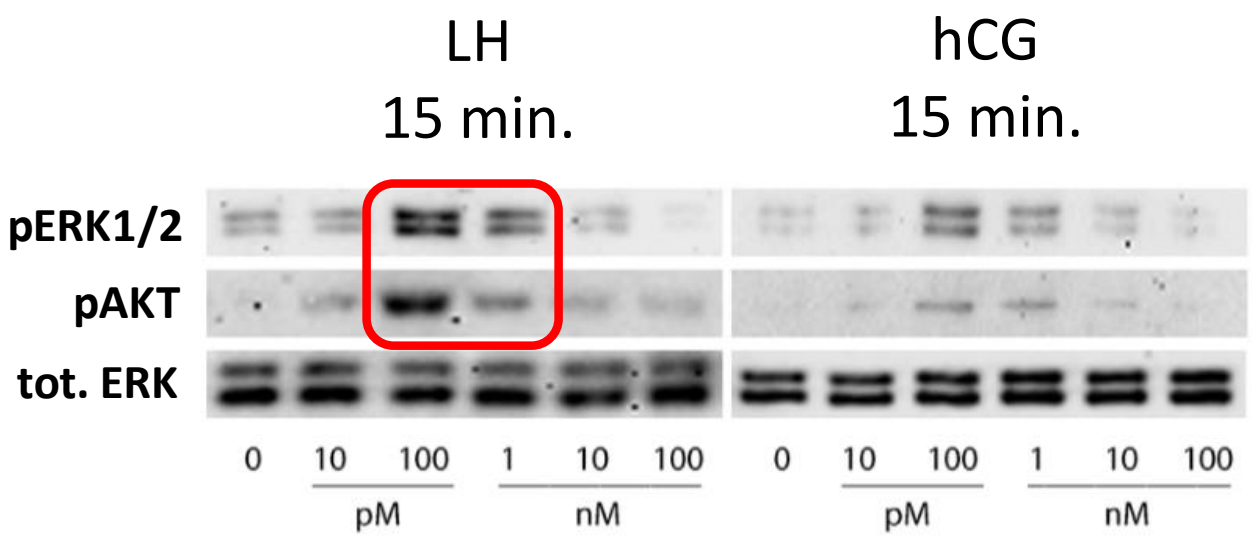


Casarini et al., Mol & Cell Endo, 2016

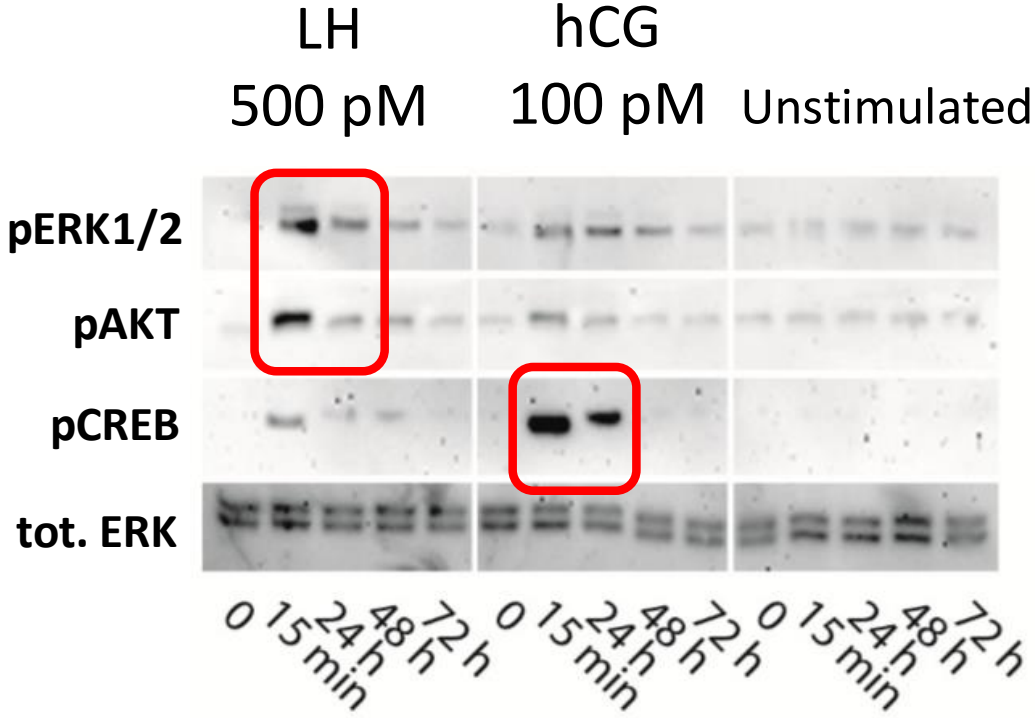
LH vs hCG signal transduction: phosphoprotein activation, steroidogenesis and effects of FSH

LH and hCG signal transduction: phosphoprotein activation

Dose-response

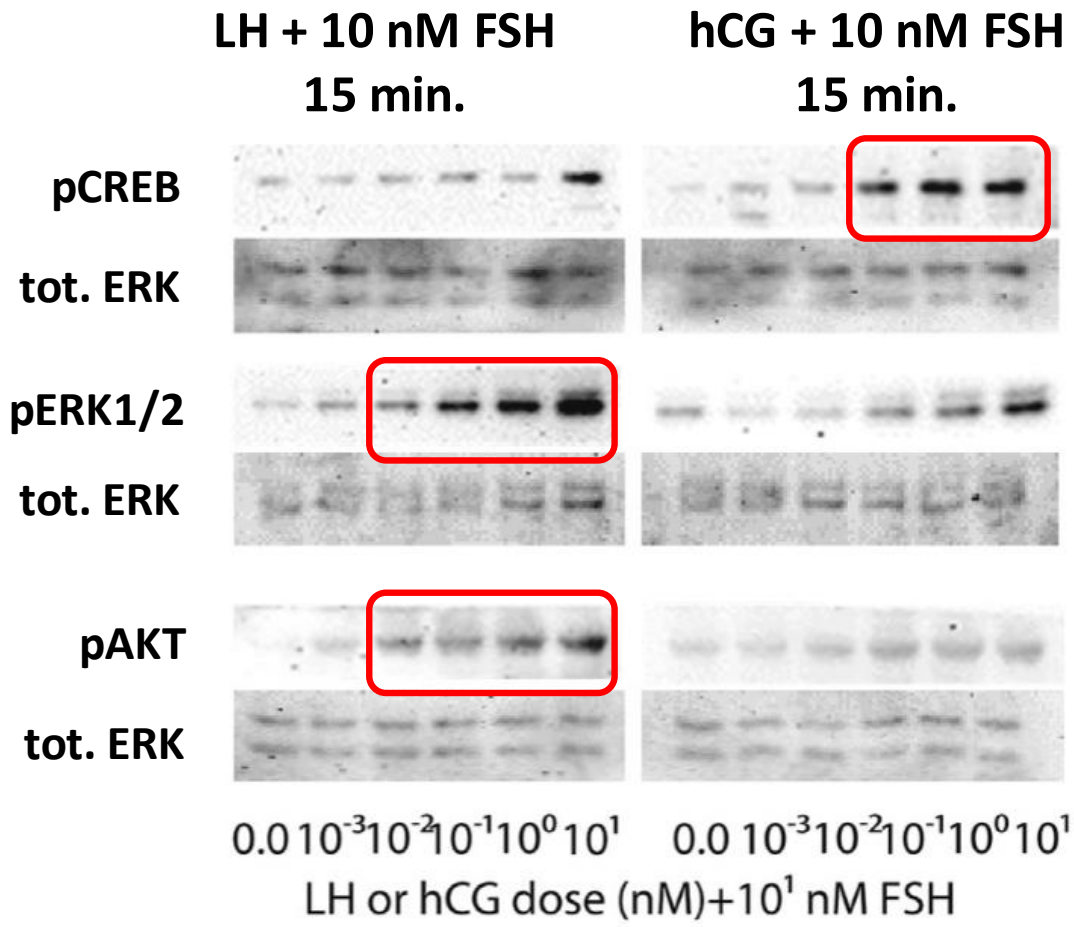


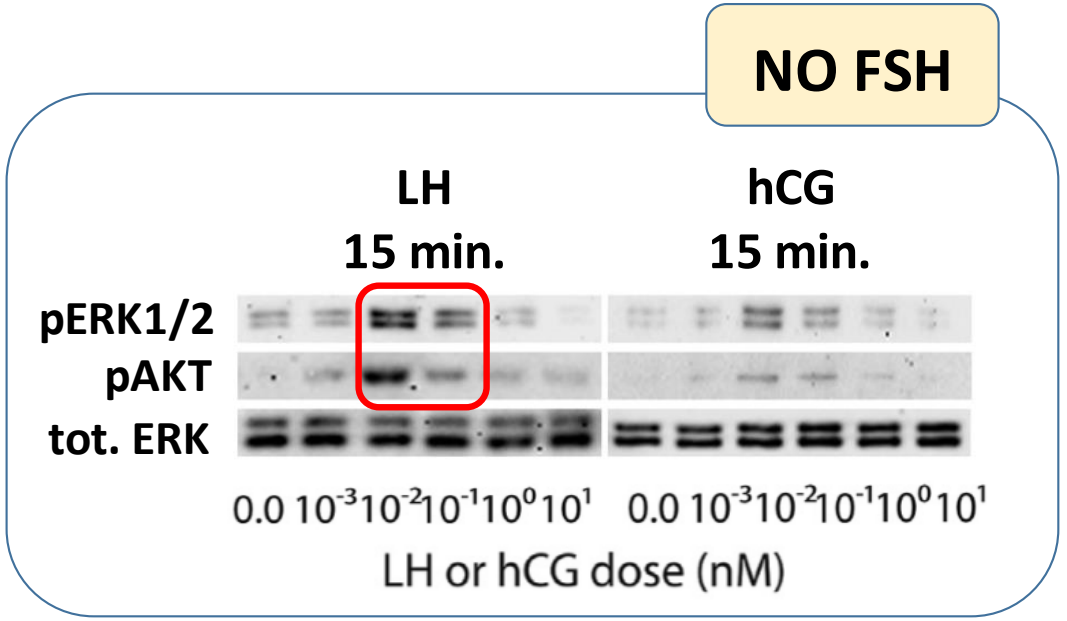
Kinetics



Casarini et al. *PLoS One*. 2012. 7(10):e46682; Casarini et al. *Int J Mol Sci*. 2017. 18(5):E926.

FSH increases LH- and hCG-specific phosphoprotein activation

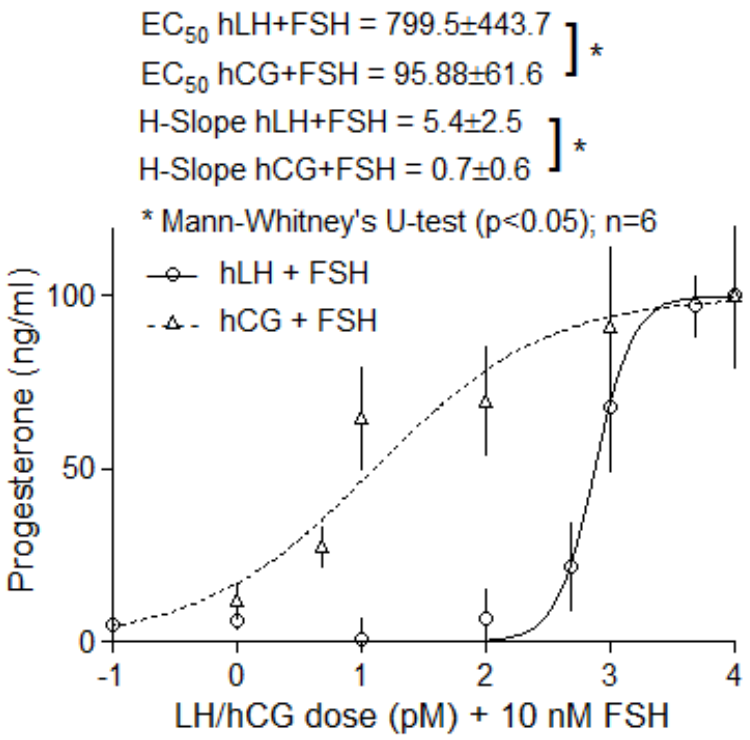




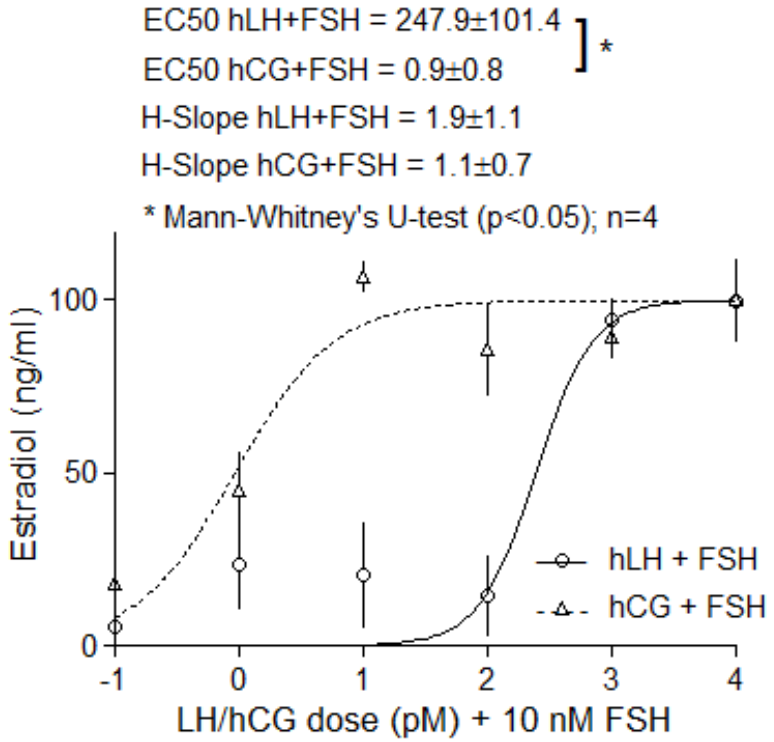
Casarini et al. *Mol Cell Endocrinol*. 2016. 422:103-14.

FSH increases and changes the kinetics of LH- and hCG-dependent steroid synthesis

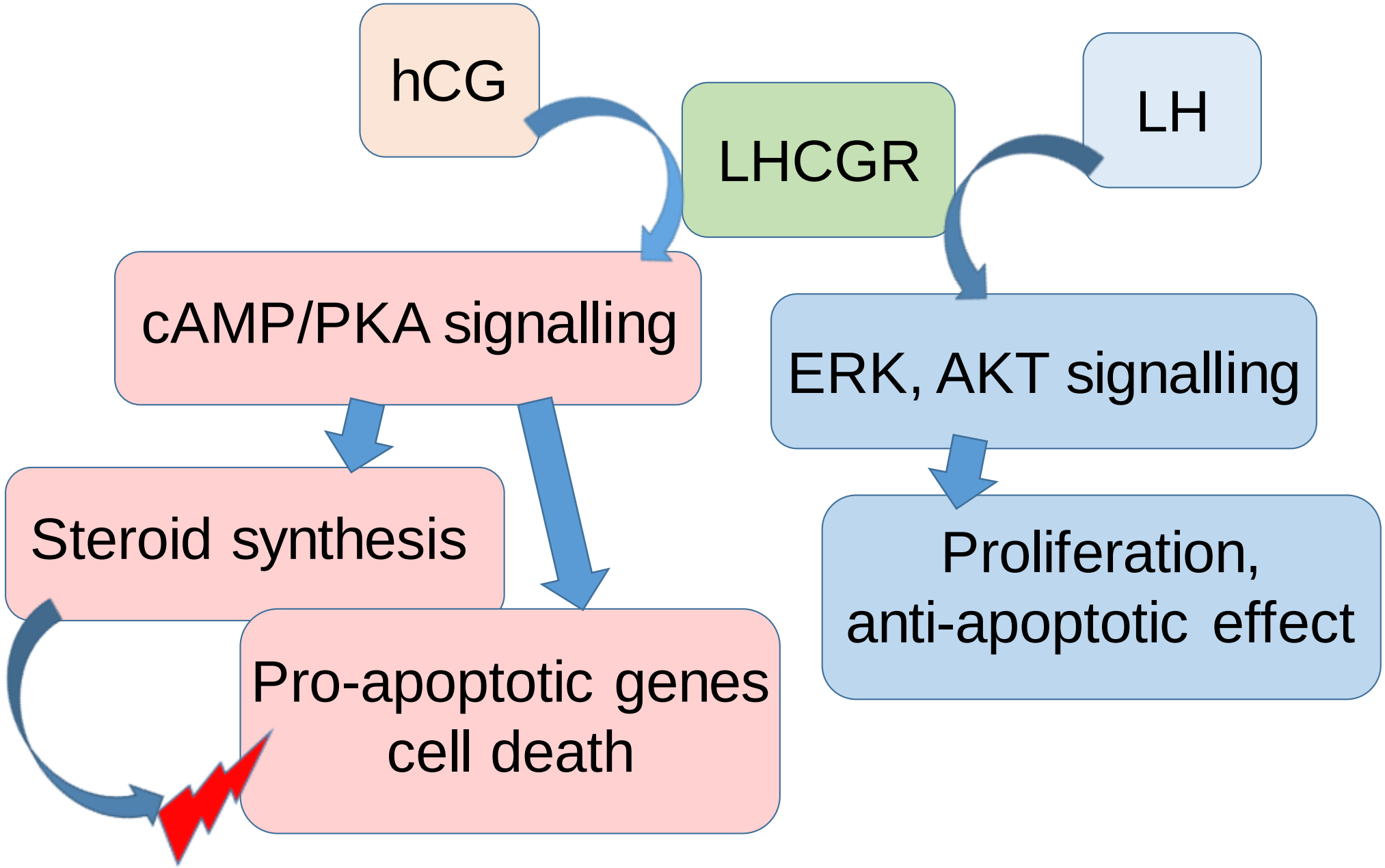
Progesterone



Estradiol



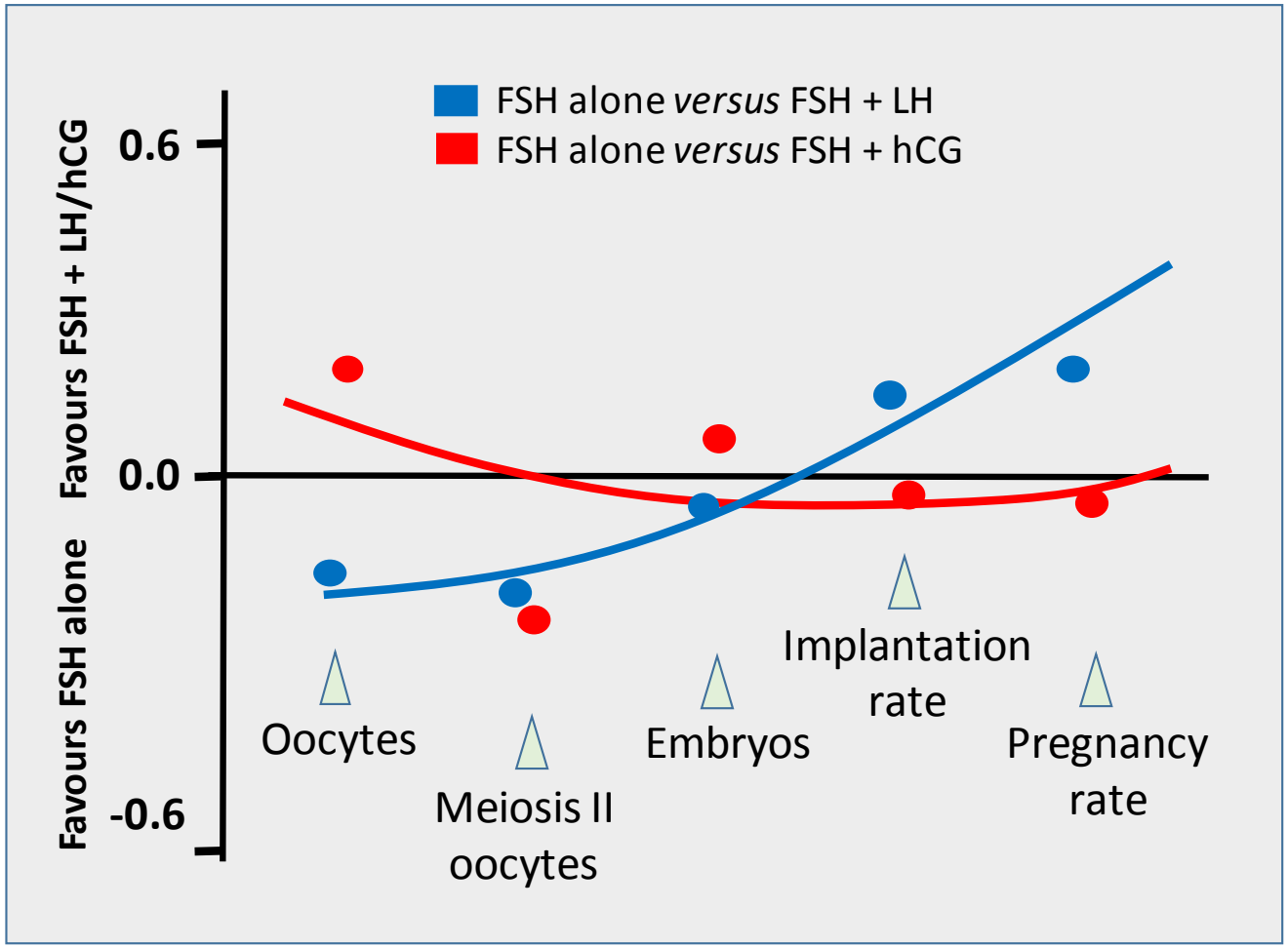
Casarini et al., Mol & Cell Endo, 2016



Casarini et al. Endocr Rev. 2018;39(5):549-592.

Clinical relevance: LH- and hCG-specific ART outcomes

LH		hCG
↑	Proliferative, anti-apoptotic effects	↓
↓	Steroidogenic (and pro-apoptotic) potential	↑
↑	Oocyte quality (and pregnancy rate)	↓



Santi et al. *Front Endocrinol (Lausanne)*. 2017. 8:114.

SUMMARY: LH vs hCG in COS

Molecular/evolutionary determinants of LH and hCG *non*-equivalency

LH

+ Proliferative and anti-apoptotic potential

Granulosa/theca cell system

Follicular growth and oocyte maturation

hCG

+ Steroidogenic potential

Lutein granulosa cells

Pregnancy support

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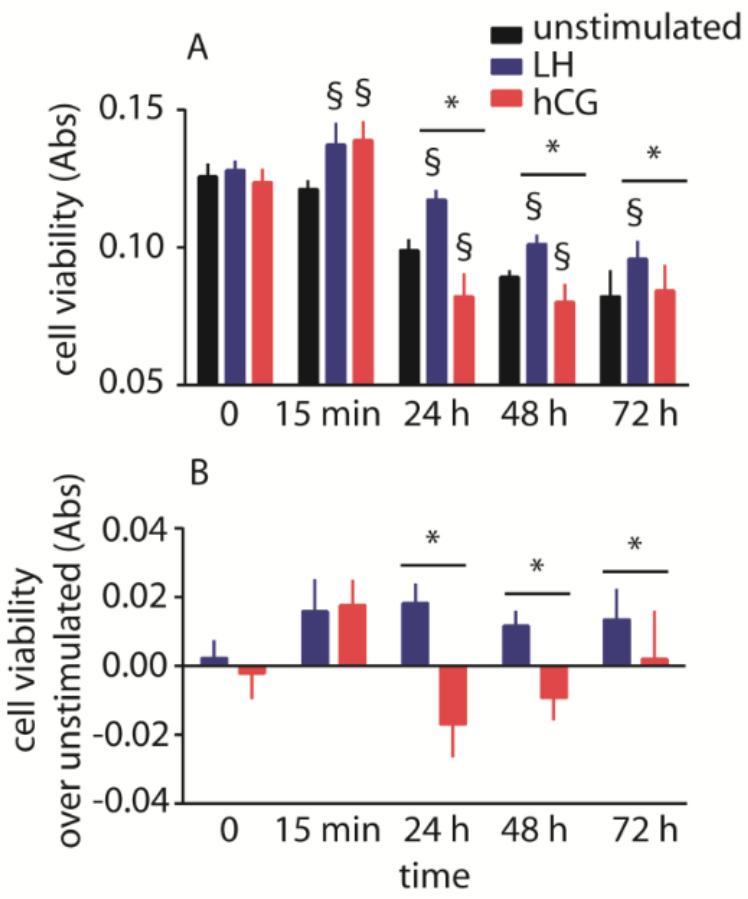
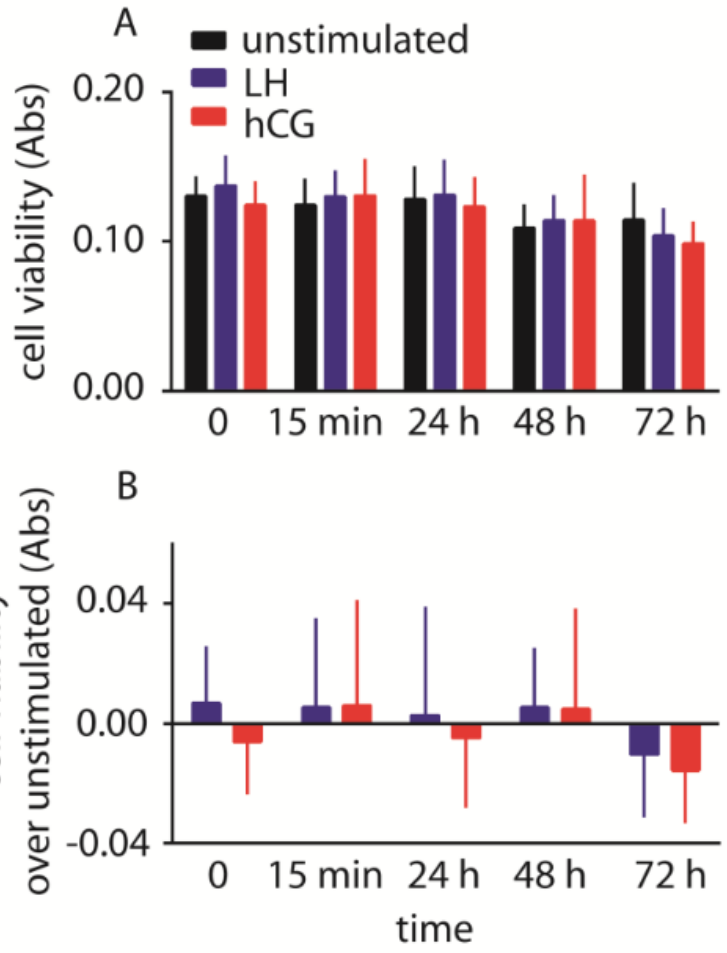
Estrogen counteracts pro-apoptotic effects of gonadotropin-dependent cAMP increase

Cell viability of hGLC

without E2



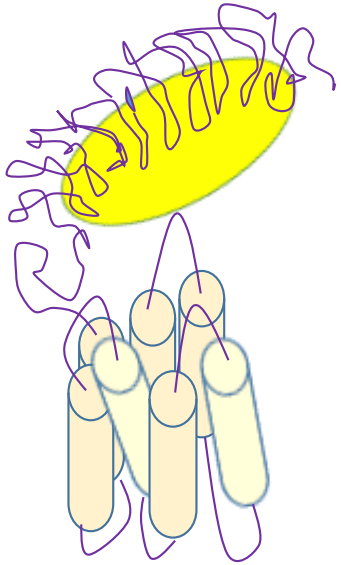
with E2



LH/hCG: 100 pM
E2: 200 pg/ml – n=10

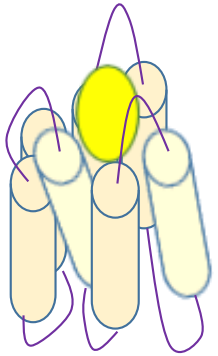
Casarini et al., Int. J. Mol. Sci, 2017

Glycoprotein hormone receptors



Gαs protein/cAMP signalling

G protein-coupled estrogen receptor (GPR30; GPER)



Ca^{2+}, pERK1/2, pAKT signalling

Images reproduced from*: van Durme et al. 2006. Mol Endocrinol. 20(9):2247-55.*

Revankar et al. 2005. Science. 307(5715):1625-30; Chen et al. J Biol Chem. 2011;286(25):22441-55.

Is GPER (GPR30) involved in counteracting the apoptotic effects of FSH (hCG)?

GPER-mediated signals in the ovary

Genistein increases progesterone secretion by elevating related enzymes in chicken **granulosa** cells.

Xiao YQ, Shao D, Tong HB, Shi SR.

Poult Sci. 2019 Apr 1;98(4):1911-1917. doi: 10.3382/ps/pey411.

PMID: 30239854

Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of **granulosa** tumor spheroids via **GPR30** and IGF1R but not via the classic estrogen receptors.

Gogola J, Hoffmann M, Ptak A.

Chemosphere. 2019 Feb;217:100-110. doi: 10.1016/j.chemosphere.2018.11.018. Epub 2018 Nov 4.

PMID: 30414542

The G-protein-coupled estrogen receptor (GPER/**GPR30**) in ovarian **granulosa** cell tumors.

Heublein S, Mayr D, Friese K, Jarrin-Franco MC, Lenhard M, Mayerhofer A, Jeschke U.

Int J Mol Sci. 2014 Aug 27;15(9):15161-72. doi: 10.3390/ijms150915161.

PMID: 25167139 **Free PMC Article**

Stimulation of ovarian cell proliferation by tetrabromobisphenol A but not tetrachlorobisphenol A through G protein-coupled receptor 30.

Hoffmann M, Gogola J, Kotula-Balak M, Ptak A.

Toxicol In Vitro. 2017 Dec;45(Pt 1):54-59. doi: 10.1016/j.tiv.2017.08.009. Epub 2017 Aug 12.

PMID: 28811233

Cell proliferation!

Conclusions

- **Follicular growth and maturation depend on the interplay of LH and FSH**
- **LH stimulates theca cells to produce androgen and acts on granulosa cells to promote proliferation, survival and maturation**
- **hCG is essentially a steroidogenic factor, acting primarily on progesterone, less so on androgen (*in vitro*)**
- **hCG and FSH are (potentially) pro-apoptotic factors (via cAMP)**
- **Follicular growth and maturation depend on estrogen!**
- **Estrogen is anti-apoptotic via GPER (blocks FSHR-mediated cAMP)**

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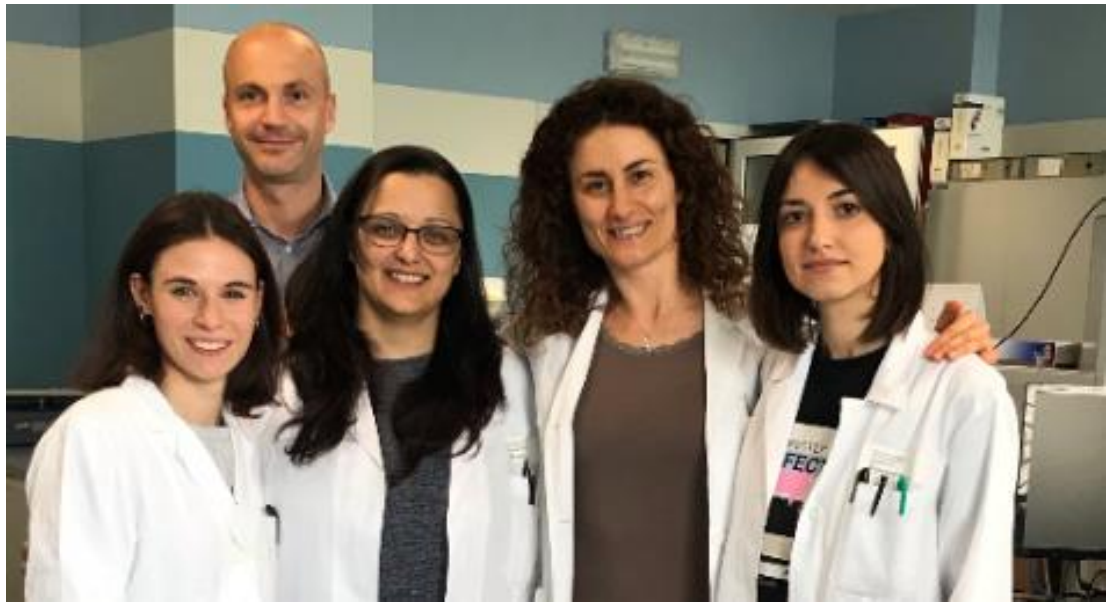

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