Gonadotropin role & administration strategies in 2009: bases & new trends

Salvador, Brazil – May 8-9, 2009

Physiological aspects of ovarian LH/ FSH receptors

Manuela Simoni

Fertility Merck Serono Meeting

Individualized Treatment

MERCK SERONO FERTILIDADE

Physiological aspects of ovarian LH/FSH receptors

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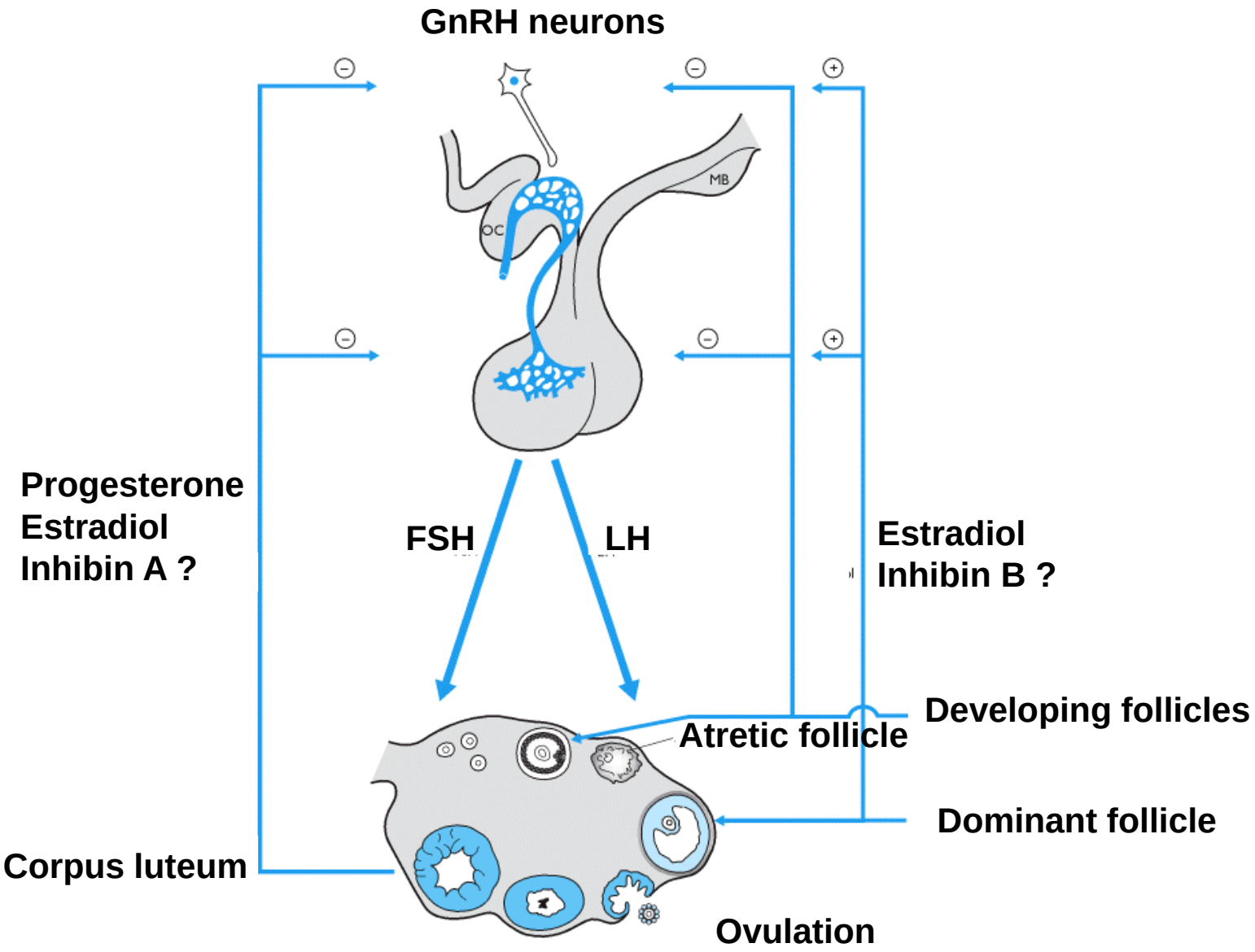
Hormonal control of ovarian function



Outline

- **Physiology of follicular maturation**
- **FSH and the *FSHR***
- **Mutations and polymorphisms**
- **Physiological effects of *FSHR* SNP**
- **LH, hCG and the *LHCGR***
- **Mutations and polymorphisms**
- **Can the *LHCGR* distinguish between LH and hCG?**

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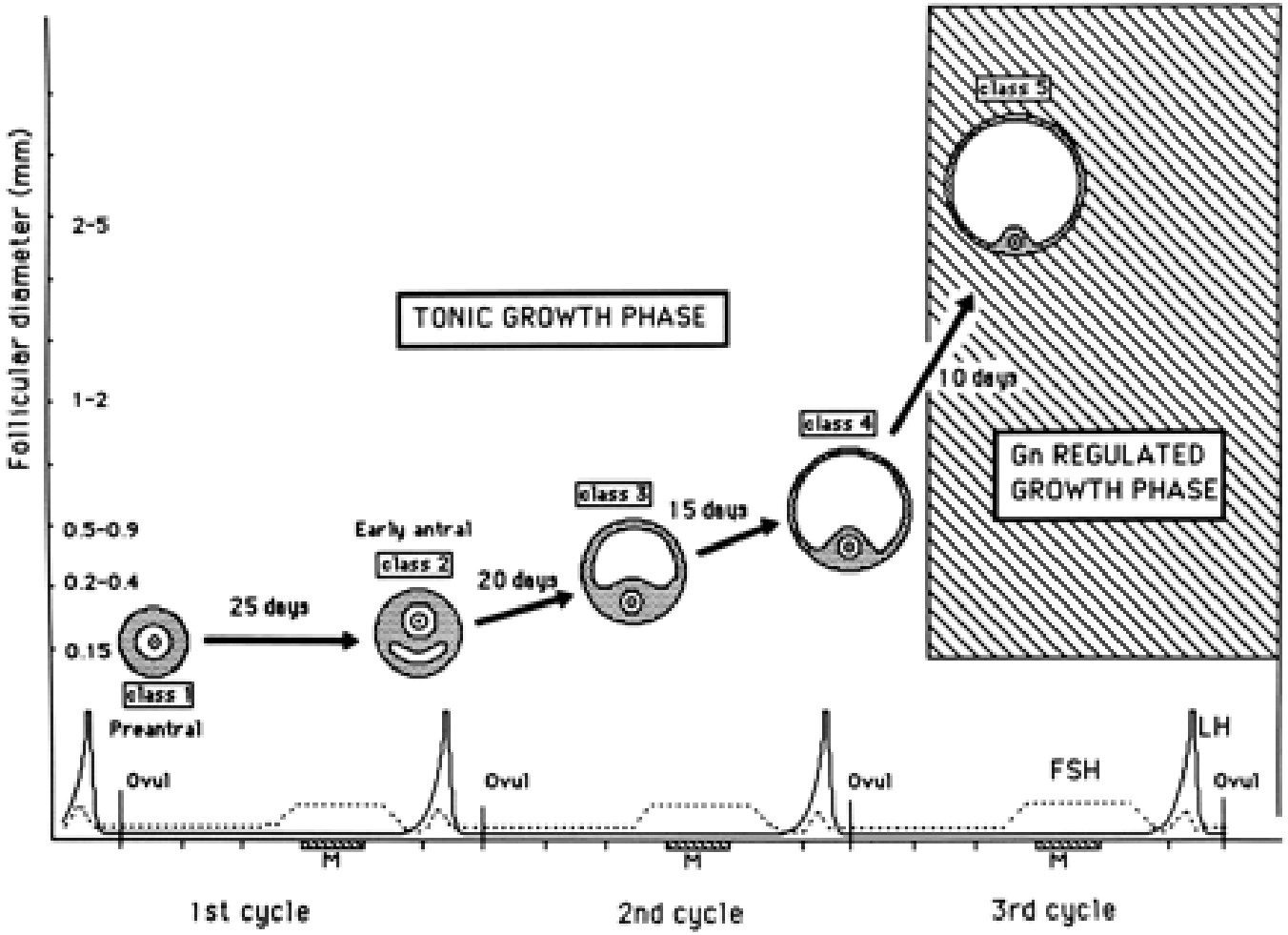
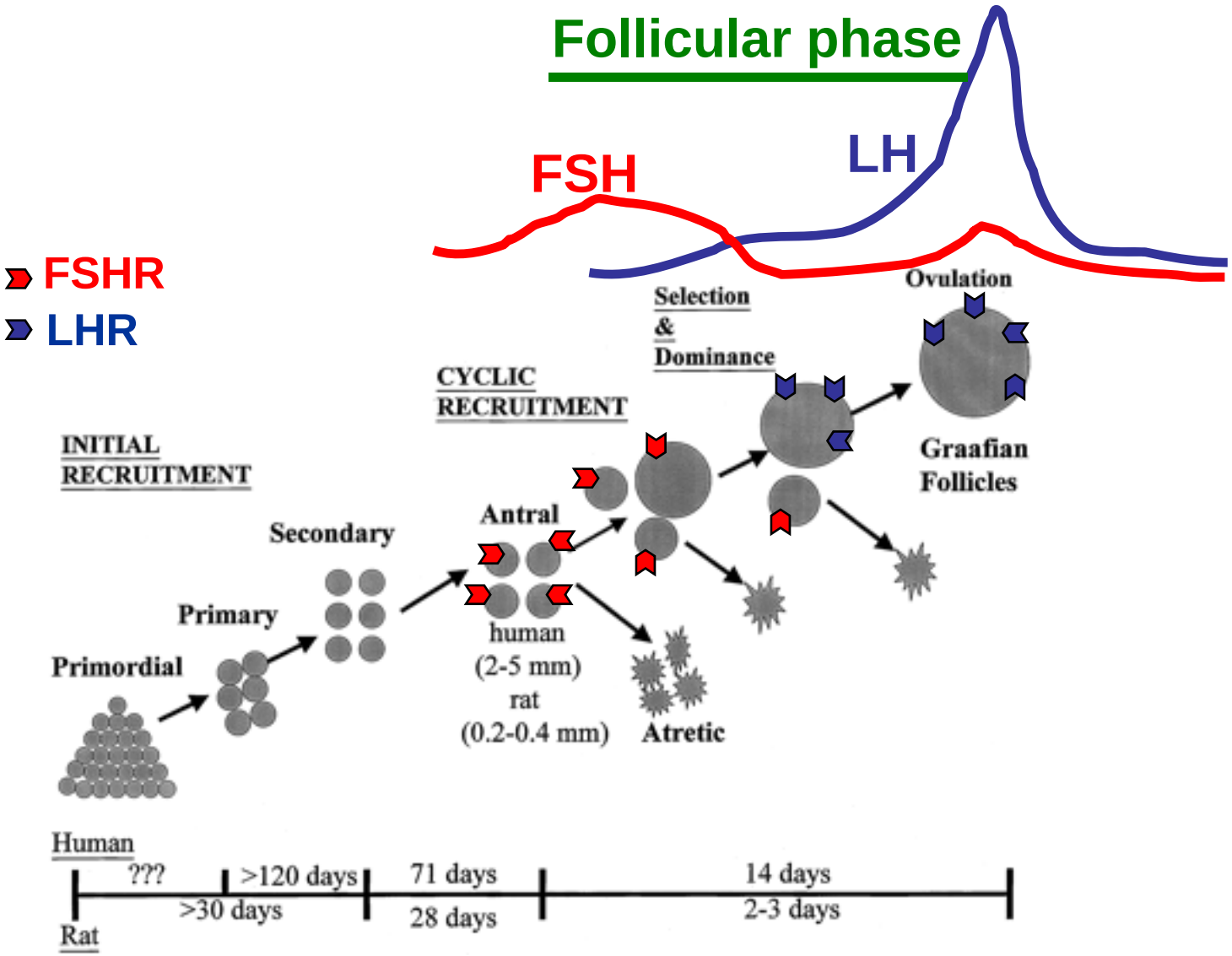
Follicular selection is gonadotropin-dependent



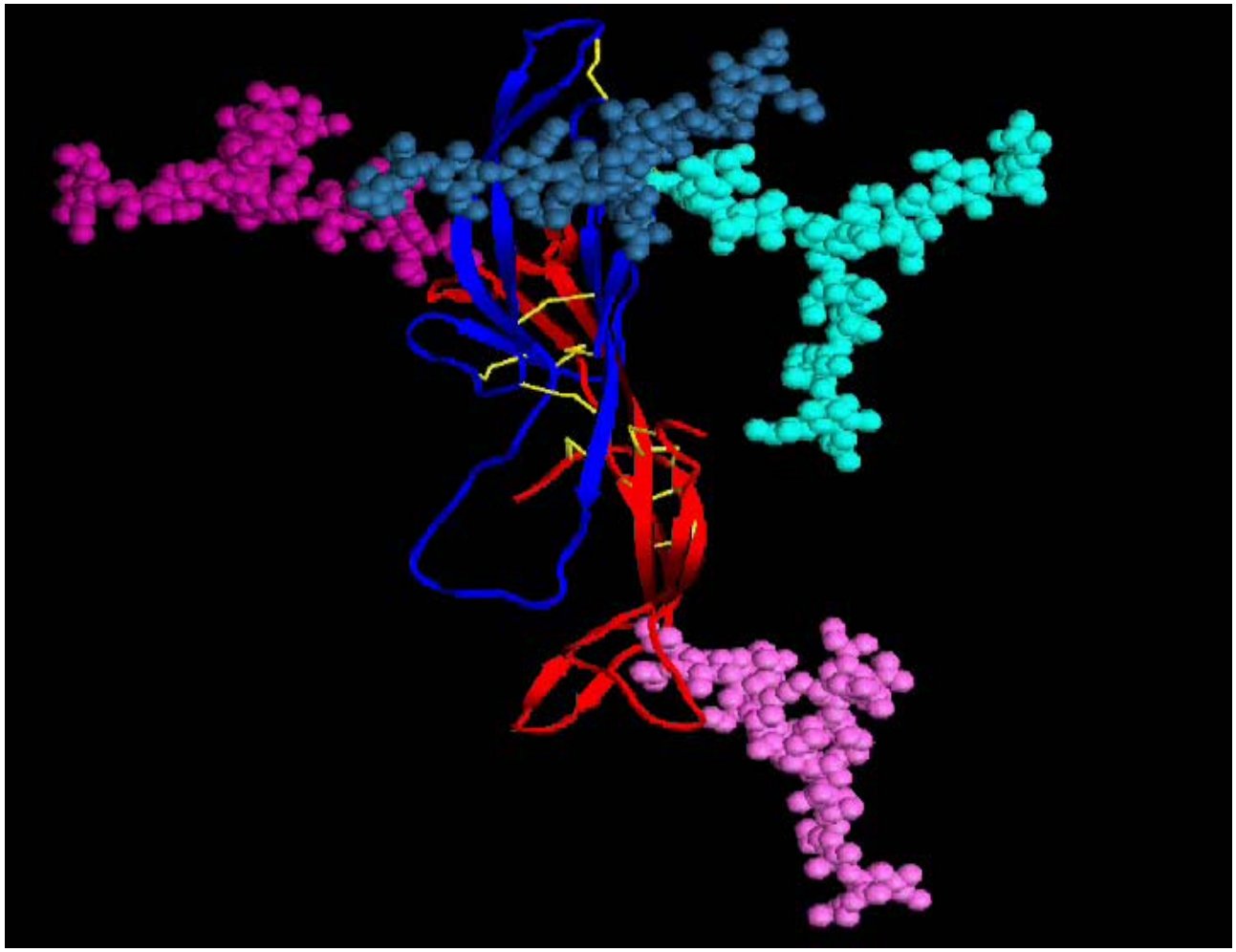
Fauser & Van Heusden, Endocr. Reviews 18:71, 1997

Follicle recruitment and selection

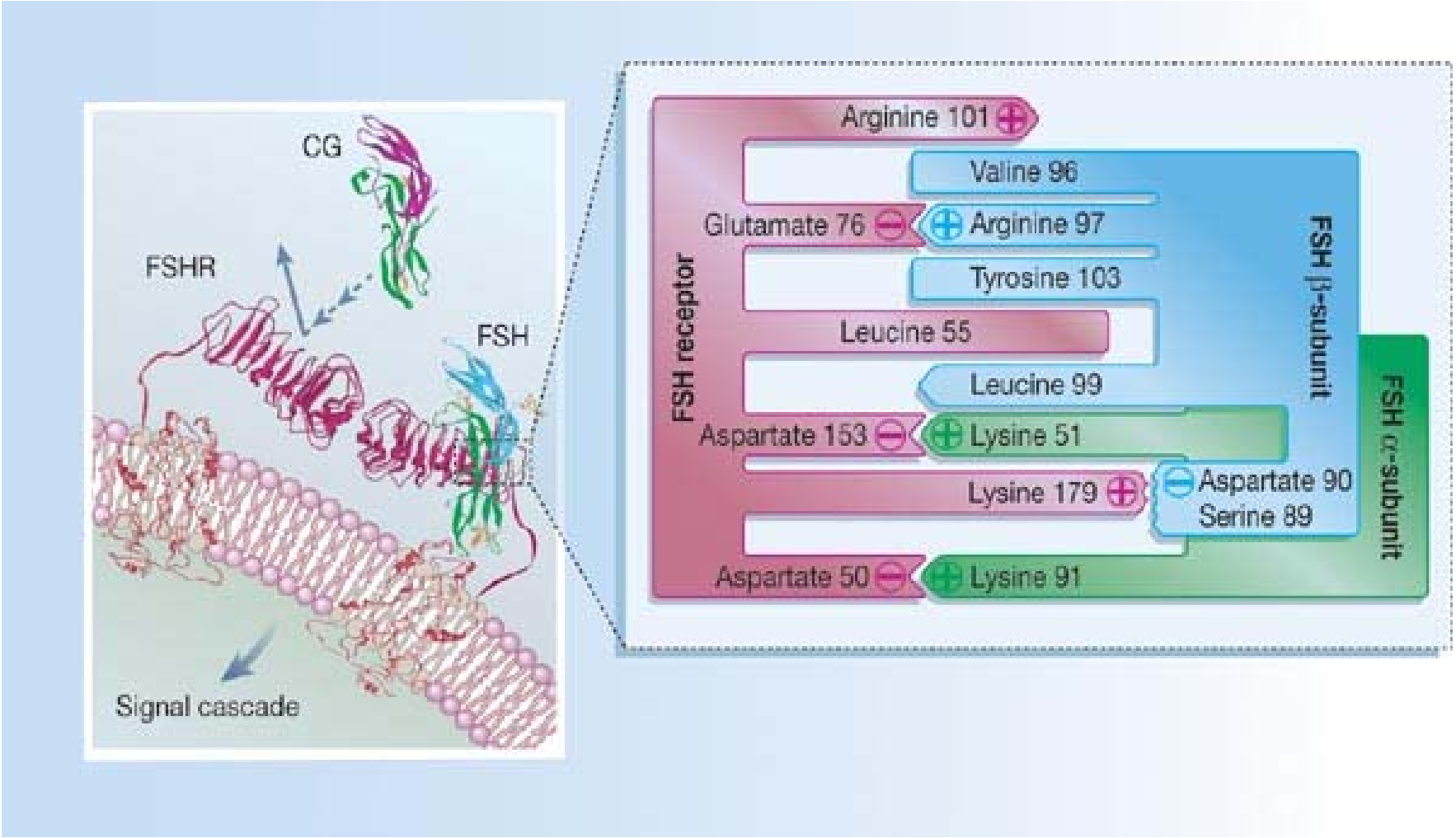


McGee & Hsueh, Endocr. Reviews 21:200, 2000

FSH: 3D structure



FSH-FSHR interaction

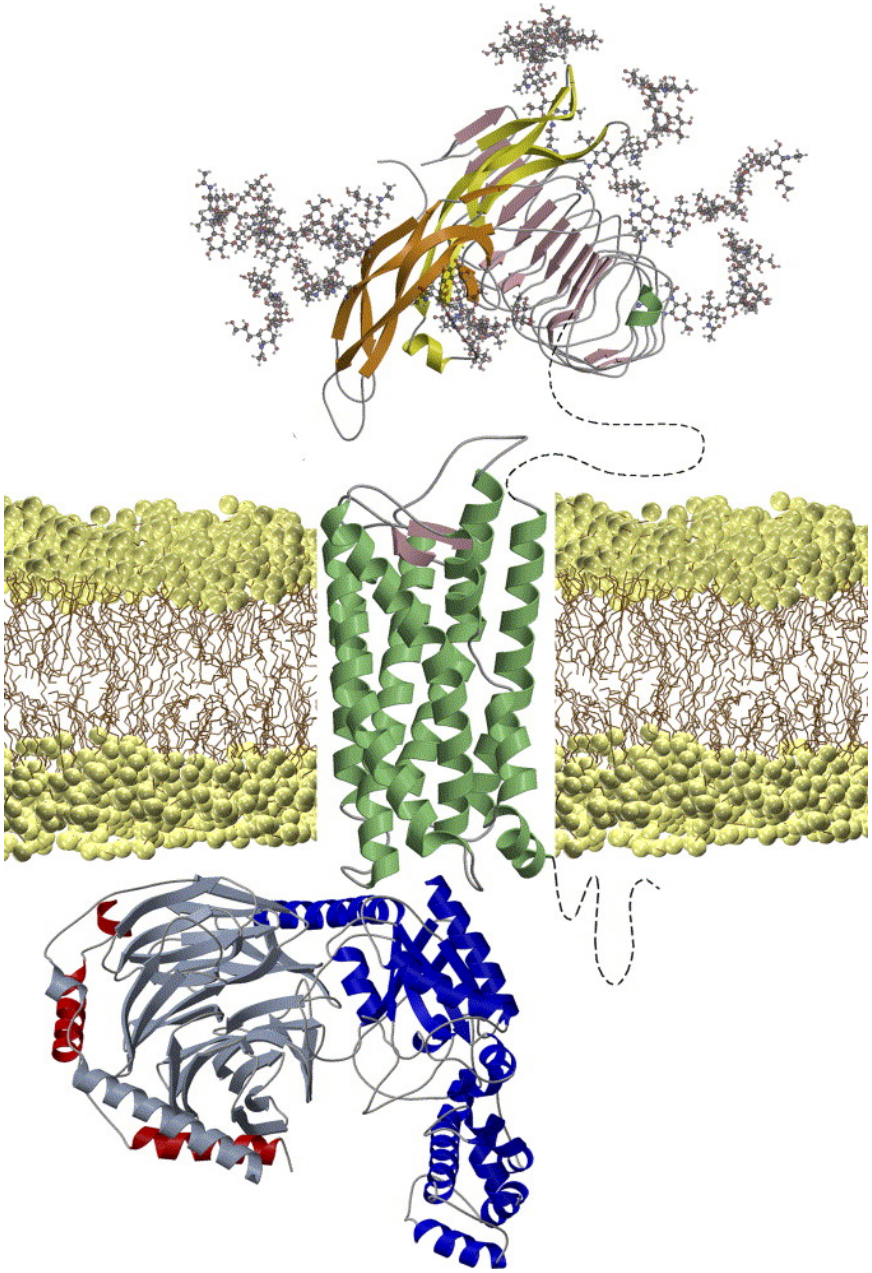


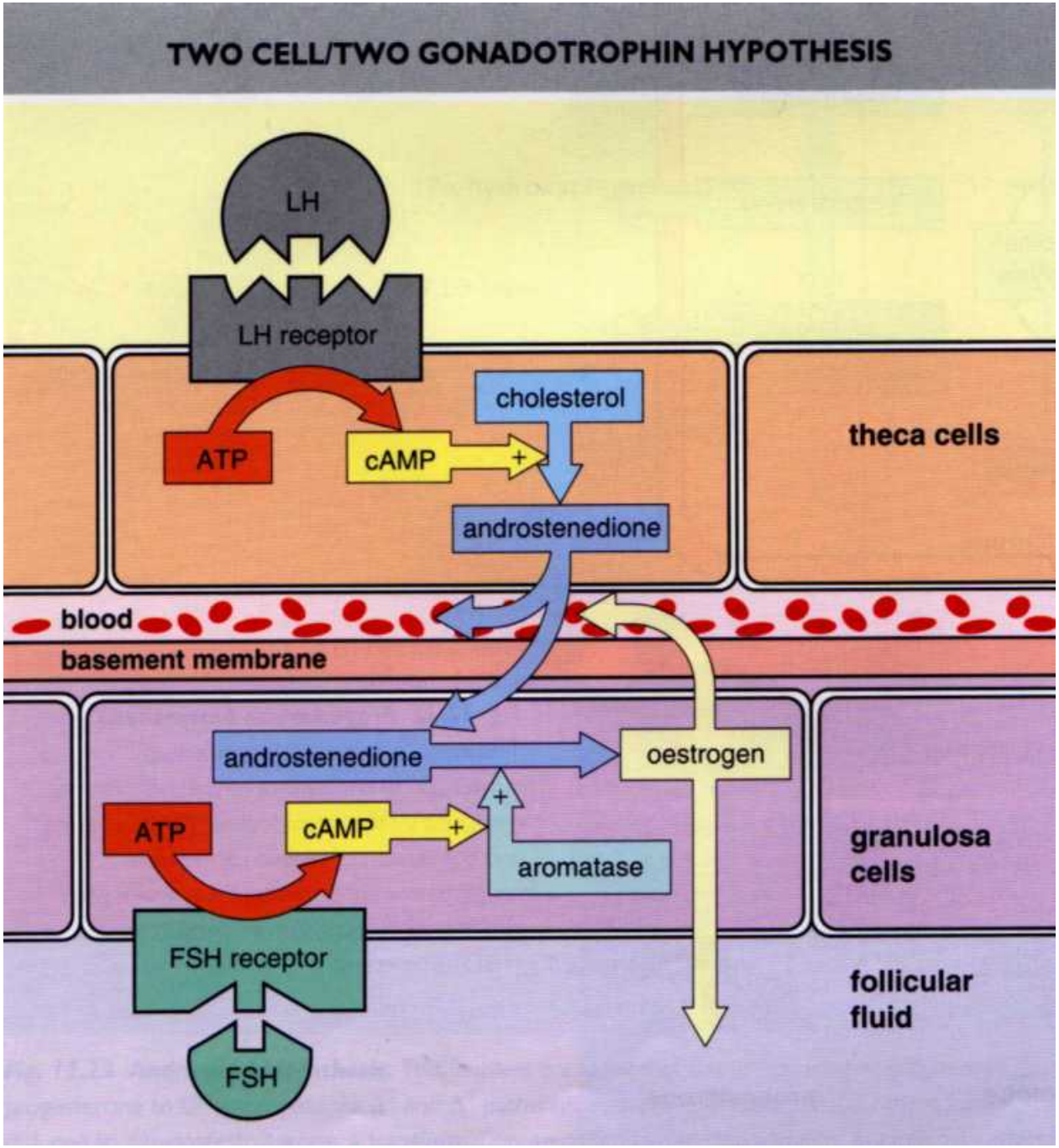
FSH-FSHR interaction

extracellular domain

transmembrane domain

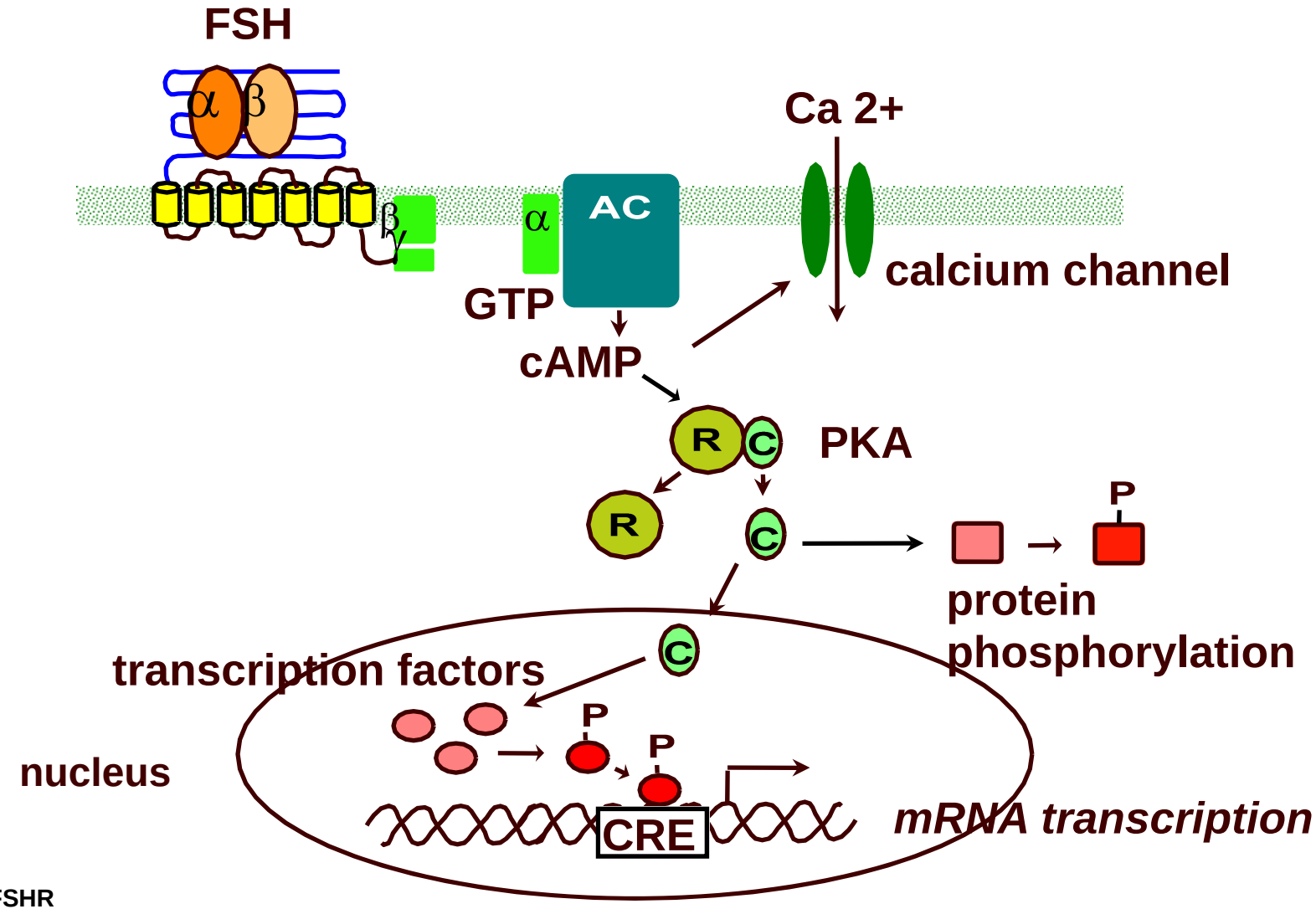
intracellular domain – G protein




TWO CELL/TWO GONADOTROPHIN HYPOTHESIS
LH
LH receptor
ATP
cAMP
+
cholesterol
androstenedione
theca cells
blood
basement membrane
androstenedione
oestrogen
ATP
cAMP
+
+
aromatase
granulosa cells
FSH receptor
FSH
follicular fluid

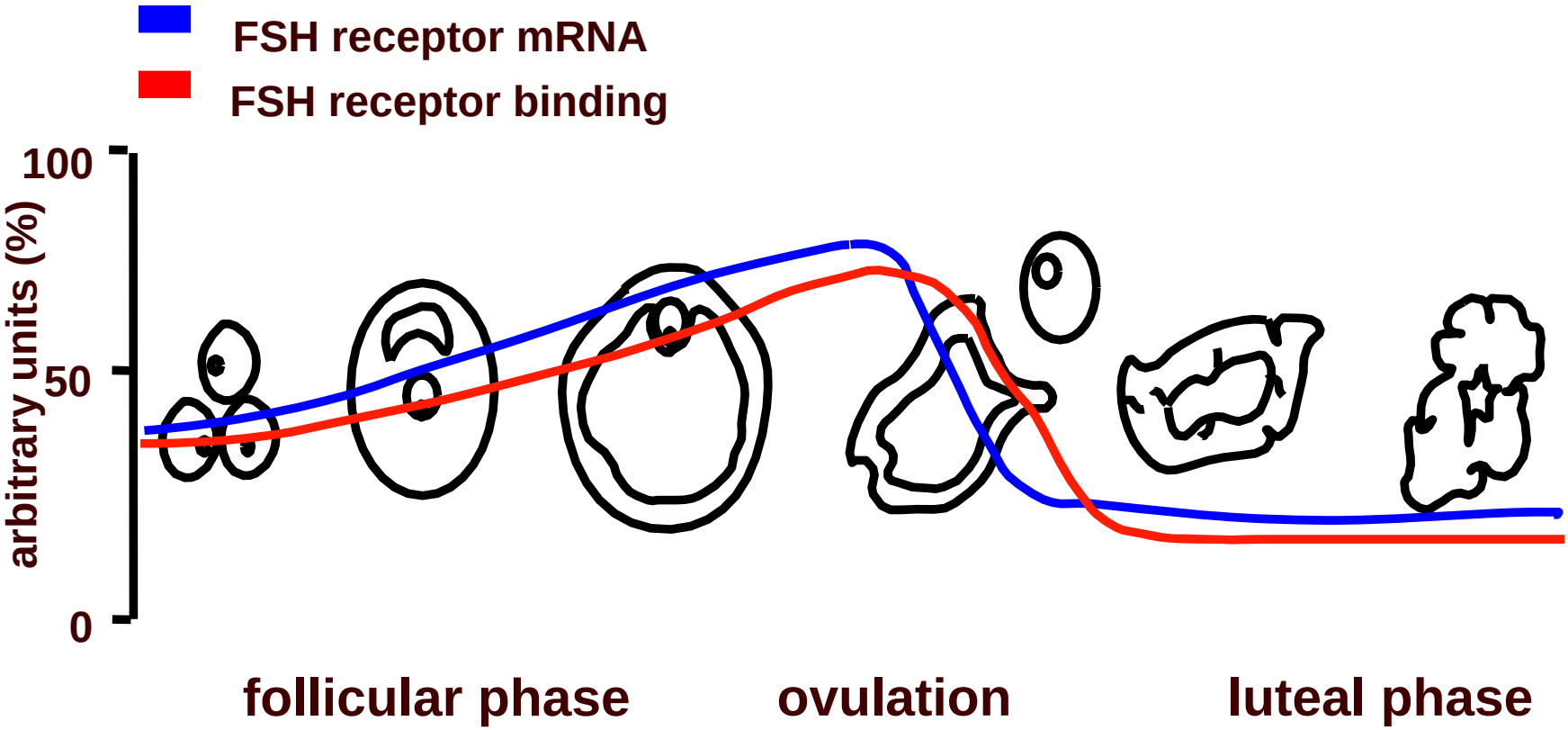
genrepro

Signal transduction pathway of the FSH receptor



FSHR

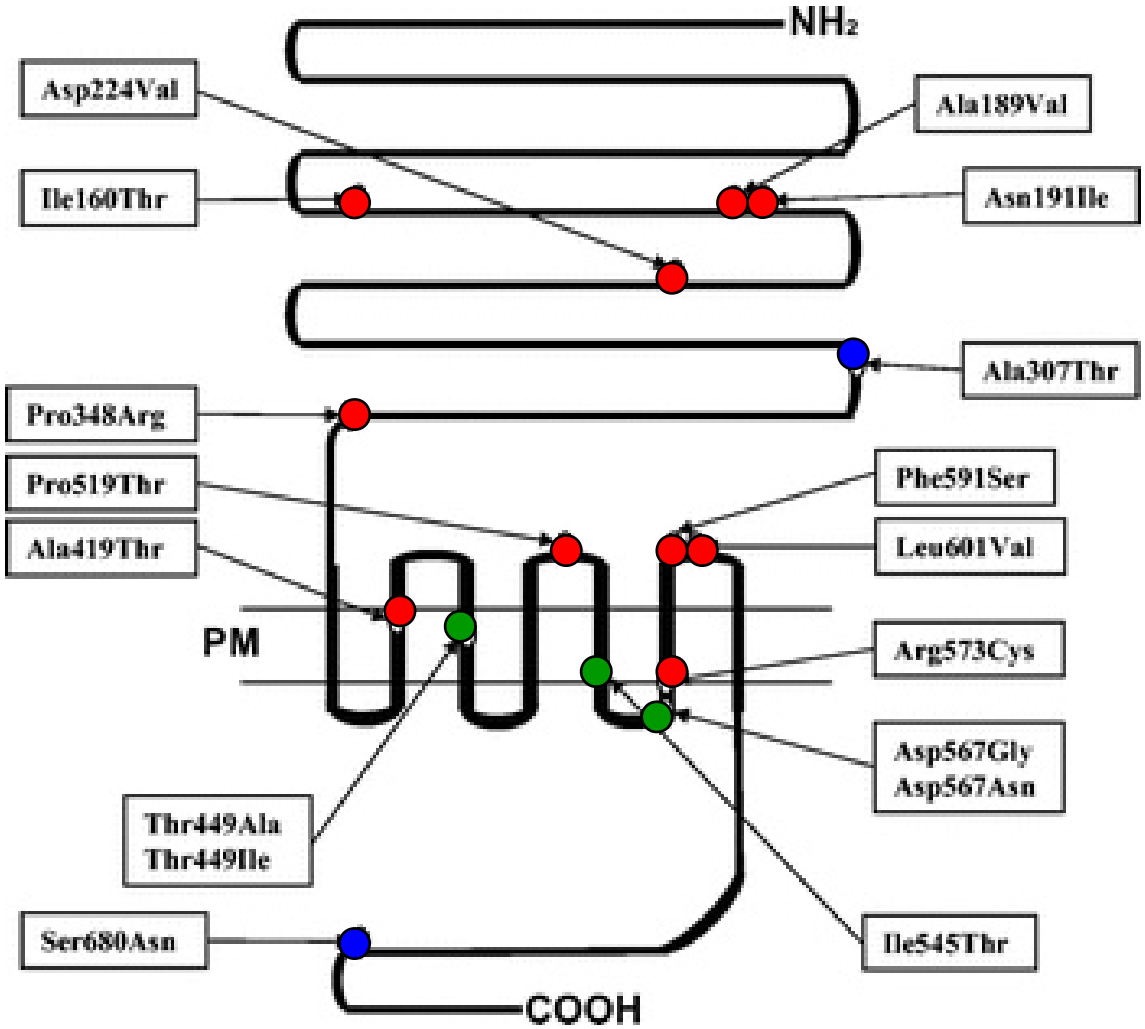
Expression of the FSH receptor during the estrous cycle



FSHR

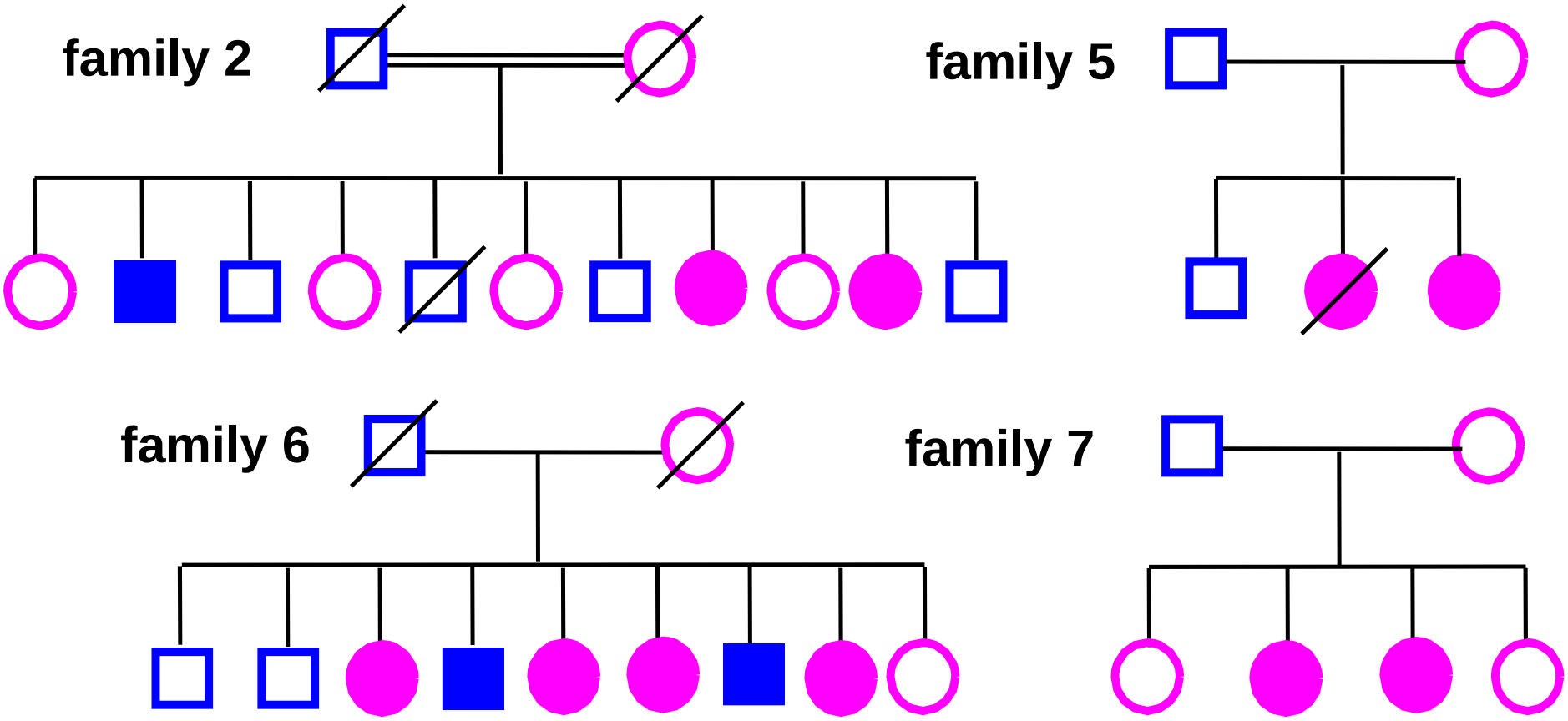
Mutations and polymorphisms of the FSHR

- inactivating
- activating
- polymorphisms



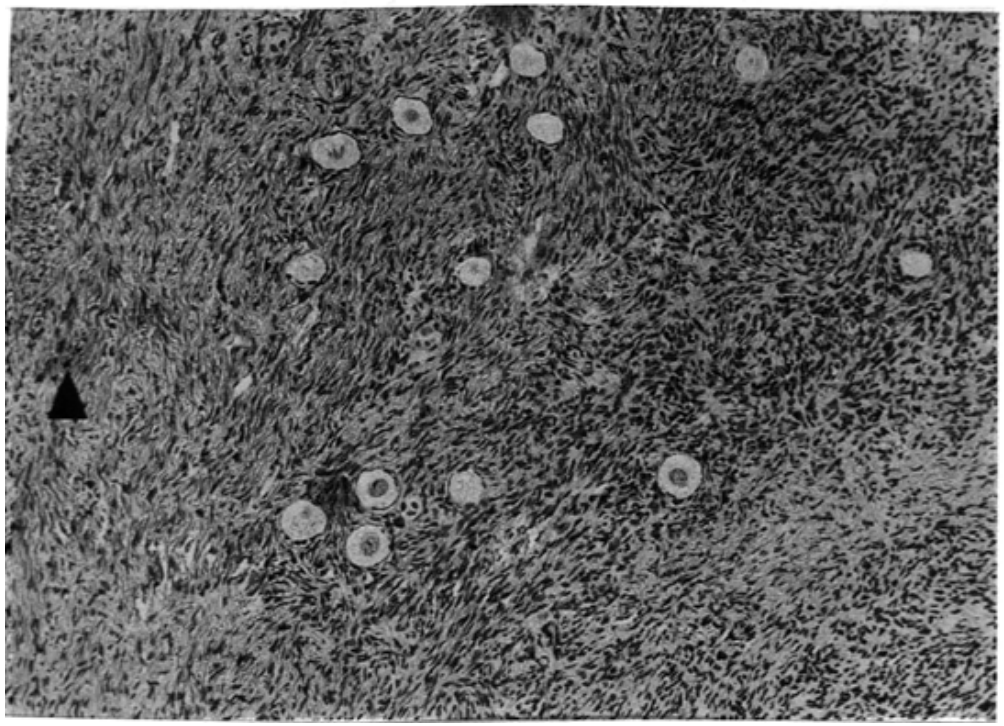
Lussiana et al., 2008

Pedigree of four Finnish families with hereditary hypergonadotropic ovarian failure



FSHR

Primary follicles in the ovaries of women with inactivating mutations of the FSHR



Aittomäki et al., JCE&M 1996

Consequences of inactivating mutations of the *FSHR*

Ala189→Val	Altered signal transduction (cAMP, IP_3) after FSH-FSHR binding
	Hypergonadotropic amenorrhea (homozygous)
	Secondary amenorrhea (heterozygous)
Asn191→Ile	Altered signal transduction (cAMP) after FSH-FSHR binding
	Hypergonadotropic amenorrhea (homozygous)
	No clinical signs (heterozygous)
Ile160→Thr	Altered FSHR expression on the cell surface
	Secondary amenorrhea (heterozygous)
Arg573→Cys	Altered signal transduction (cAMP) after FSH-FSHR binding
	Secondary amenorrhea (heterozygous)
Asp224→Val	Altered signal transduction (cAMP) after FSH-FSHR binding
Leu601→Val	Primary amenorrhea (when both mutations are associated)
Pro348→Arg	Totally impaired FSHR-FSH binding
	Primary amenorrhea and POF (homozygous)
	Minor abnormalities of the internal genitalia (heterozygous)
Ala419→Thr	Altered signal transduction (cAMP) after FSH-FSHR binding
	Primary amenorrhea (heterozygous)
Pro519→Thr	Altered FSHR expression on the cell surface
	Primary amenorrhea and POF (homozygous)
Phe591→Ser	Altered signal transduction (cAMP) after FSH-FSHR binding
	Primary amenorrhea and POF (homozygous)
	Predisposition to sex cord ovarian tumors (heterozygous)

Lussiana et al., 2008

Consequences of activating mutations of the *FSHR*

Asp567→Asn	FSHR increased sensibility to FSH or hCG
	Spontaneous or iatrogenic ovarian hyperstimulation syndrome (OHSS)
Thr449→Ile	FSHR increased sensibility to FSH, hCG or TSH
Thr449→Ala	Spontaneous or iatrogenic ovarian hyperstimulation syndrome (OHSS) even due to hypothyroidism
Ile545→Thr	FSHR increased sensitivity to FSH, hCG or TSH
	Spontaneous or iatrogenic ovarian hyperstimulation syndrome (OHSS) even due to hypothyroidism
Asp567→Gly	Ligand-independent constitutive activation of FSHR
	Normal spermatogenesis in the absence of FSH

Lussiana et al., 2008

Mutations of the FSH receptor

Inactivating:

primary or secondary amenorrhea

Activating:

OHSS, sensitivity to TSH

Inactivating:

infertility

Activating:

normal spermatogenesis without gonadotropins

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- **Physiological effects of *FSHR* SNP**
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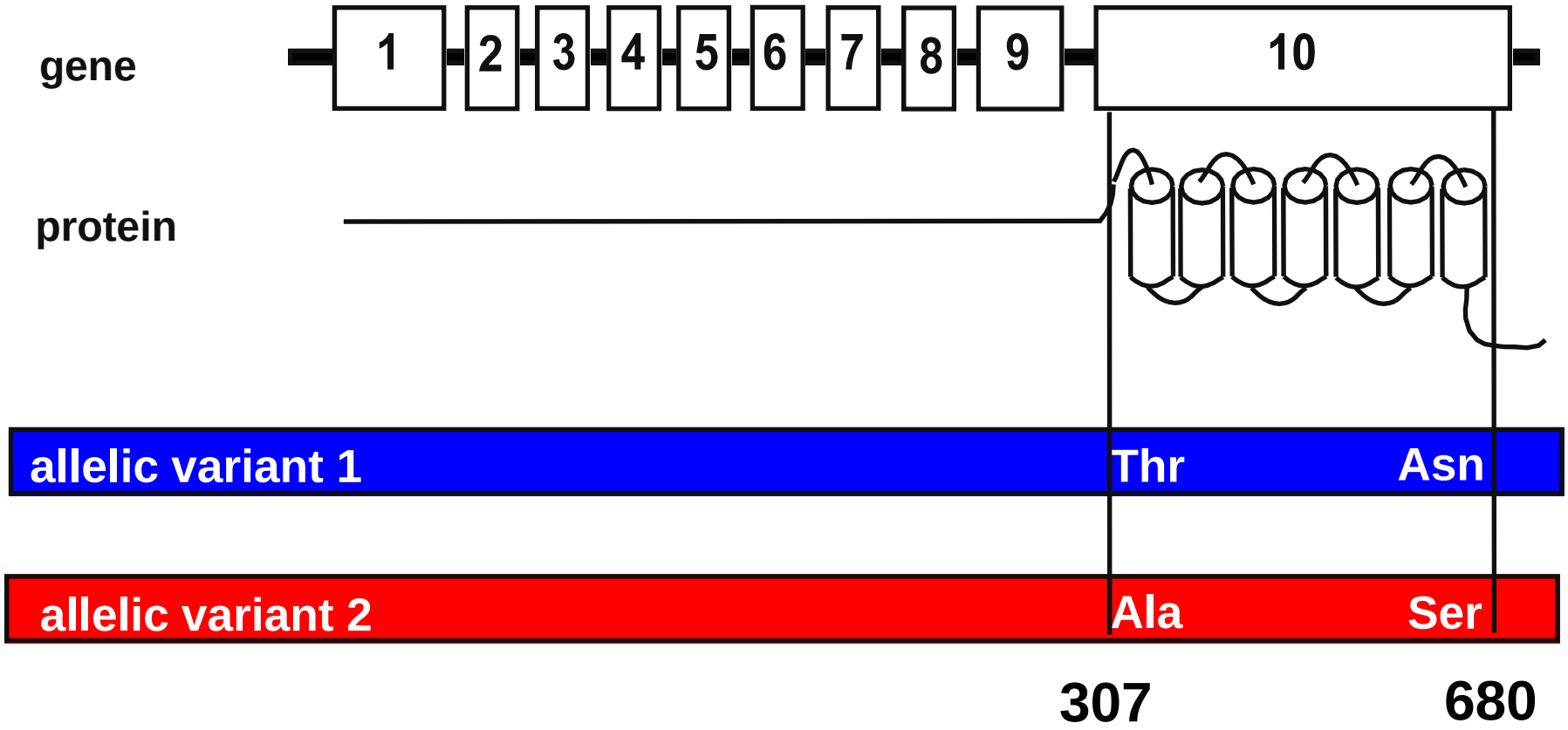
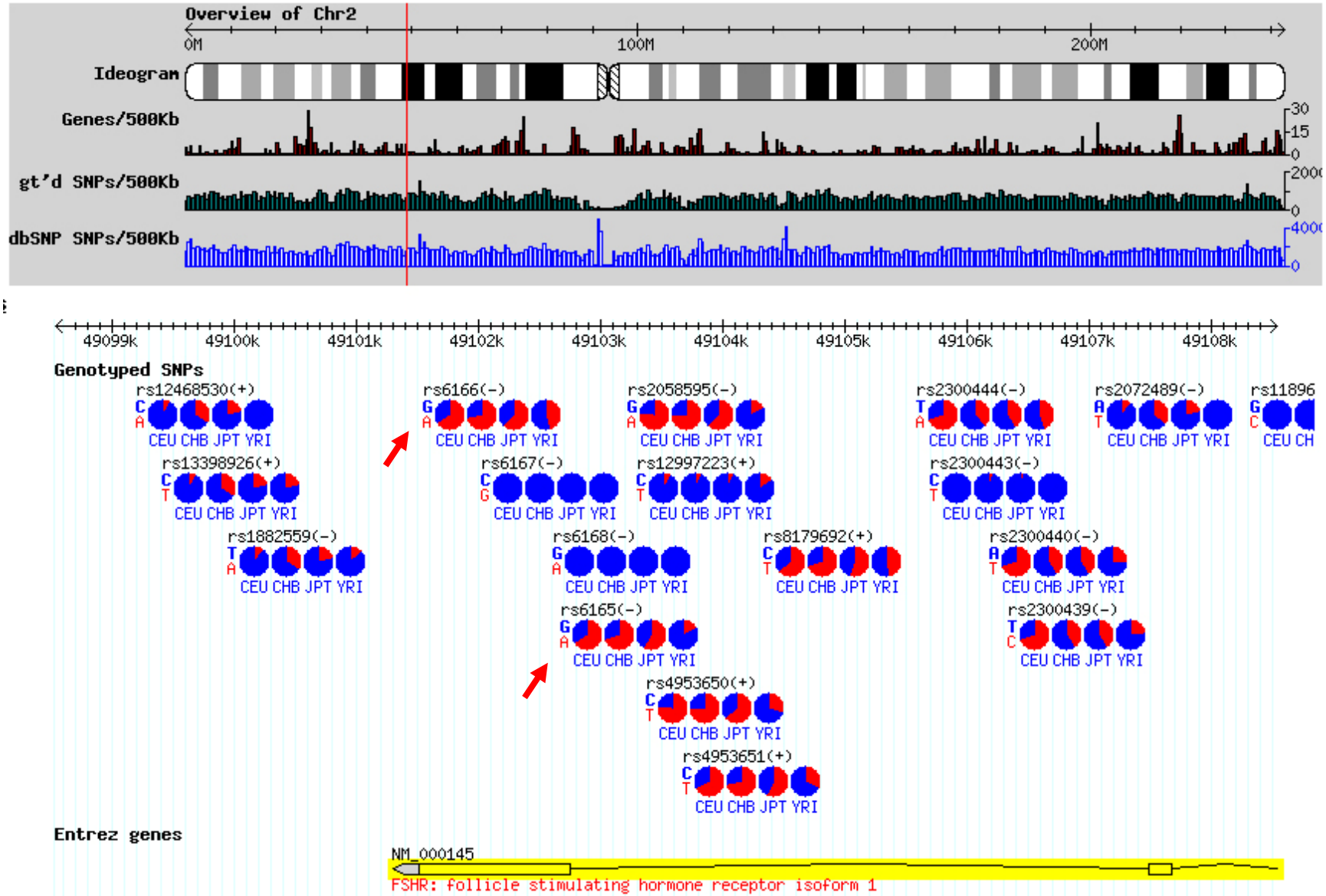
- Pathophsyological effects of *LHCGR* SNP

Allelic variants of the FSH receptor

(Simoni *et al.*, JCEM 84: 751, 1999)



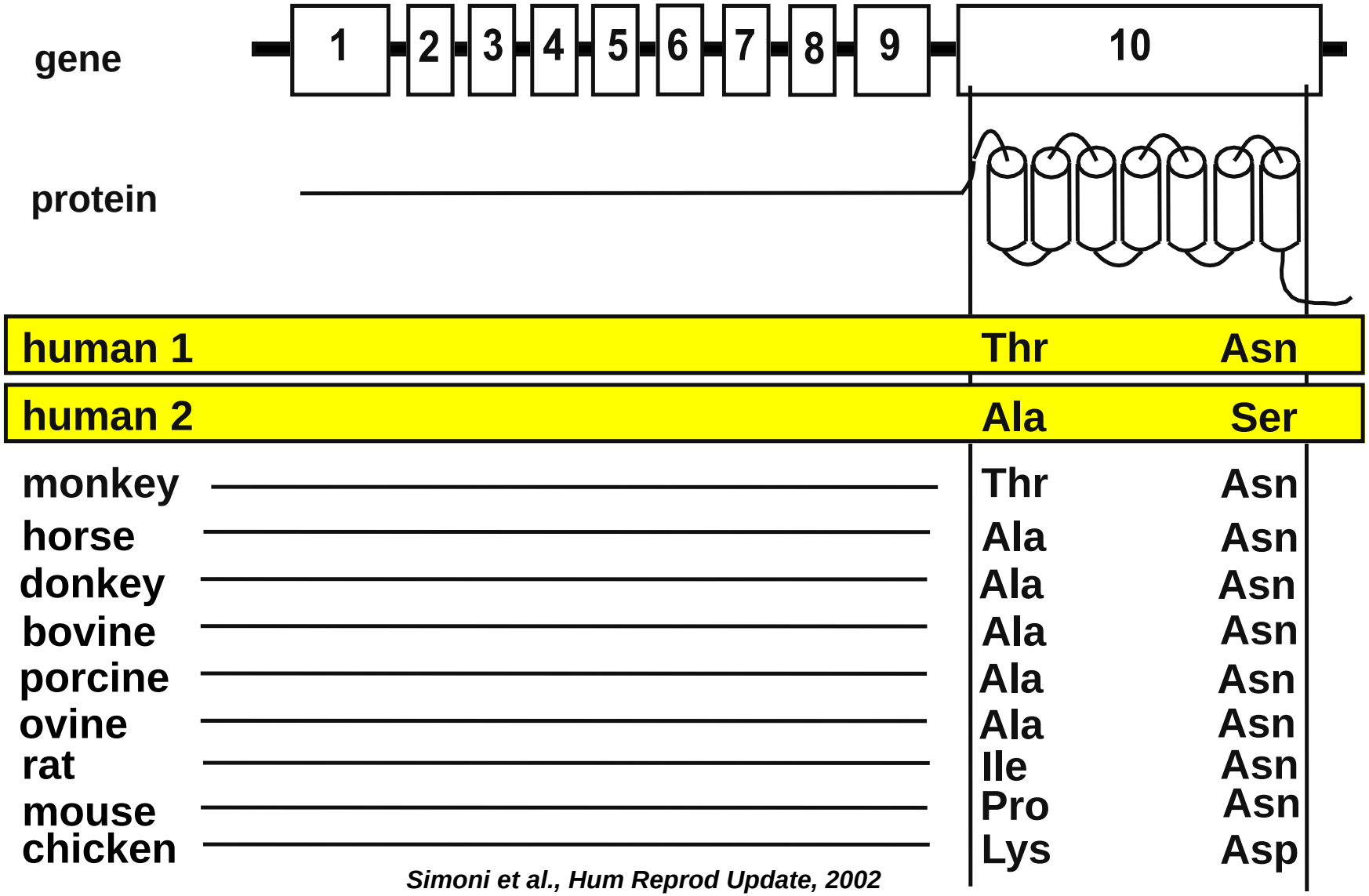
Over 1600 SNPs in the human FSH receptor



Ethnic distribution of SNP at codon 680 of the FSH receptor

	Sample Assertainment					Genotypes				Alleles			
ss#	Population	Individual Group	Sample (2N)	Founder (N)	Source	A/A	A/G	G/G	HWP	A	G	N	Het. +/-std err
ss11457643	CEPH		184		AF					0.670	0.330		
	CHMJ		74		IG				0.001	0.689	0.284	0.027	
ss24250675	AFD_EUR_PANEL	European	48	24	IG	0.417	0.458	0.125	1.000	0.646	0.354		
	AFD_AFR_PANEL	African American	46	23	IG	0.435	0.478	0.087	0.752	0.674	0.326		
	AFD_CHN_PANEL	Asian	48	24	IG	0.375	0.583	0.042	0.150	0.667	0.333		
ss44231258	HapMap-CEU	European	120	60	IG	0.467	0.400	0.133	0.439	0.667	0.333		
	HapMap-HCB	Asian	90	45	IG	0.556	0.311	0.133	0.150	0.711	0.289		
	HapMap-JPT	Asian	88	44	IG	0.364	0.523	0.114	0.479	0.625	0.375		
	HapMap-YRI	Sub-Saharan African	120	60	IG	0.200	0.517	0.283	1.000	0.458	0.542		
	AoD_African_American		90		AF					0.620	0.380		
	AoD_Caucasian		92		AF					0.520	0.480		
ss5586543	P1		200	100	GF	0.390	0.470	0.140	1.000	0.625	0.375		
	CAUC1		60	30	GF	0.500	0.333	0.167	0.200	0.666	0.333		
	AFR1		48	24	GF	0.250	0.625	0.125	0.200	0.562	0.438		
	HISP1		46	22.9999995231629	GF	0.348	0.565	0.087	0.294	0.631	0.370		
	PAC1		46	23	GF	0.435	0.391	0.174	0.479	0.631	0.369		
ss7787	WIAF-CSNP-POP1		84		AF					0.675	0.325		
Total Samples			**1034**	**554**		**0.092**	**0.339**	**0.401**	**0.038**	**0.126**	**0.004**		**0.628**
													0.469+/-0.126

Polymorphism of the human FSH receptor: comparison with other animal species



Simoni et al., Hum Reprod Update, 2002

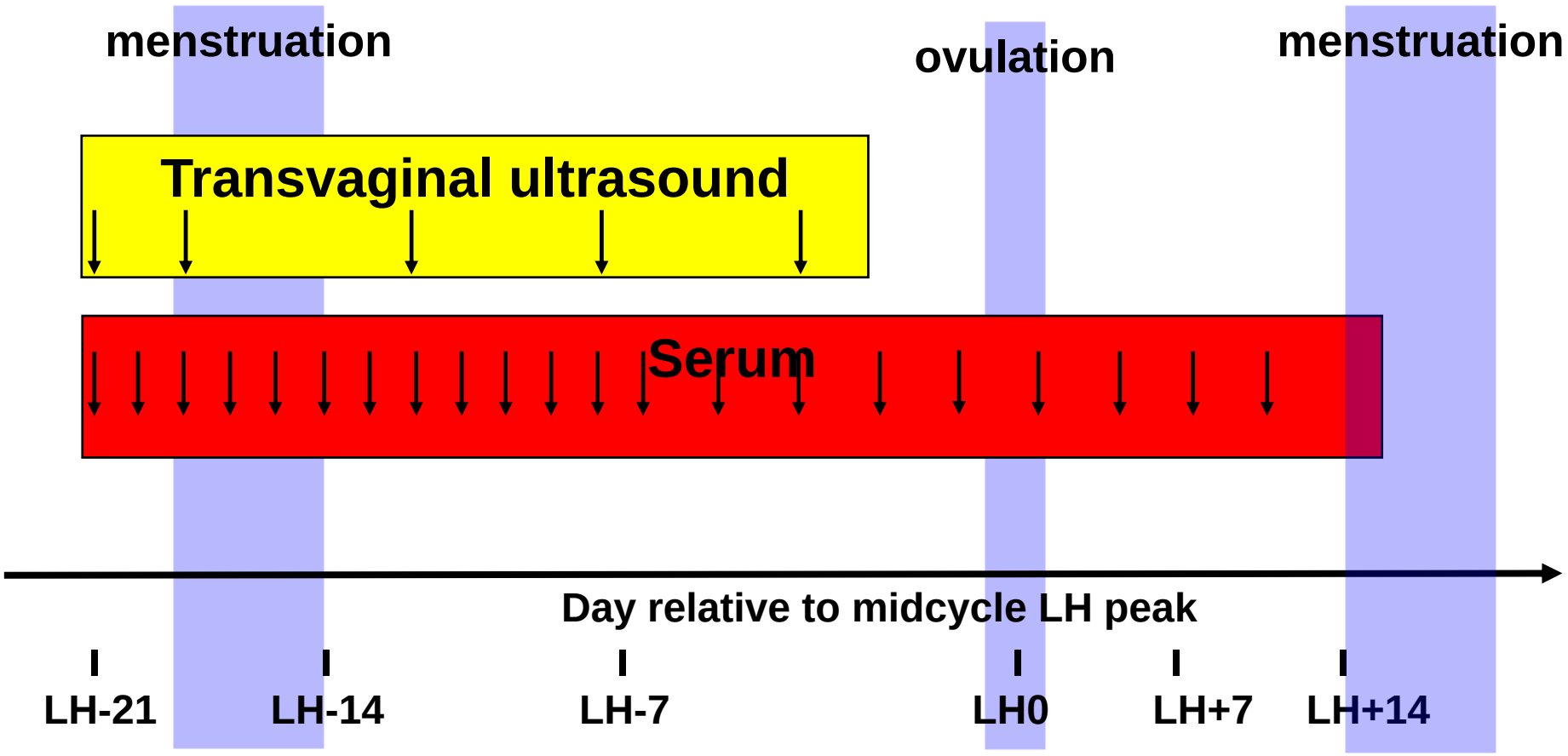
Effects of FSHR SNP 680 on hormonal dynamics of menstrual cycle

Greb et al., JCEM 90:4866, 2005

- **Study design: mc monitoring in volunteers recruited by newspaper adv.**
- **Inclusion criteria: age 18-34, regular mc (25-32 days), BMI 19-28, Caucasian**
- **Screened: 125**
  - **Heterozygous: 64 (excluded)**
  - **Homoz. Asn: 42**
  - **Homoz. Ser 22**
- **Completed mc monitoring: 13 Asn + 10 Ser**
- **Evaluated: 12 Asn + 9 Ser**

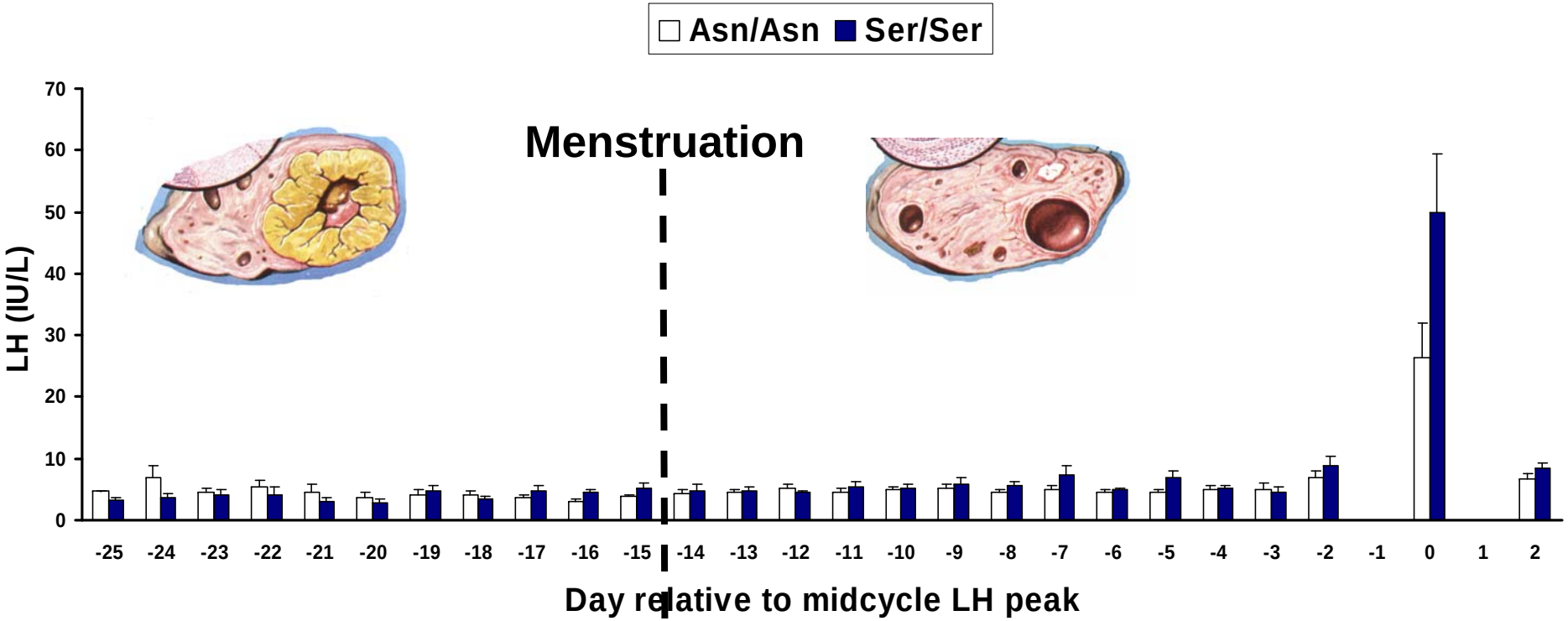
Study Design

(Asn/Asn: n=12, Ser/Ser: n=9)



Greb et al., JCEM 90:4866, 2005

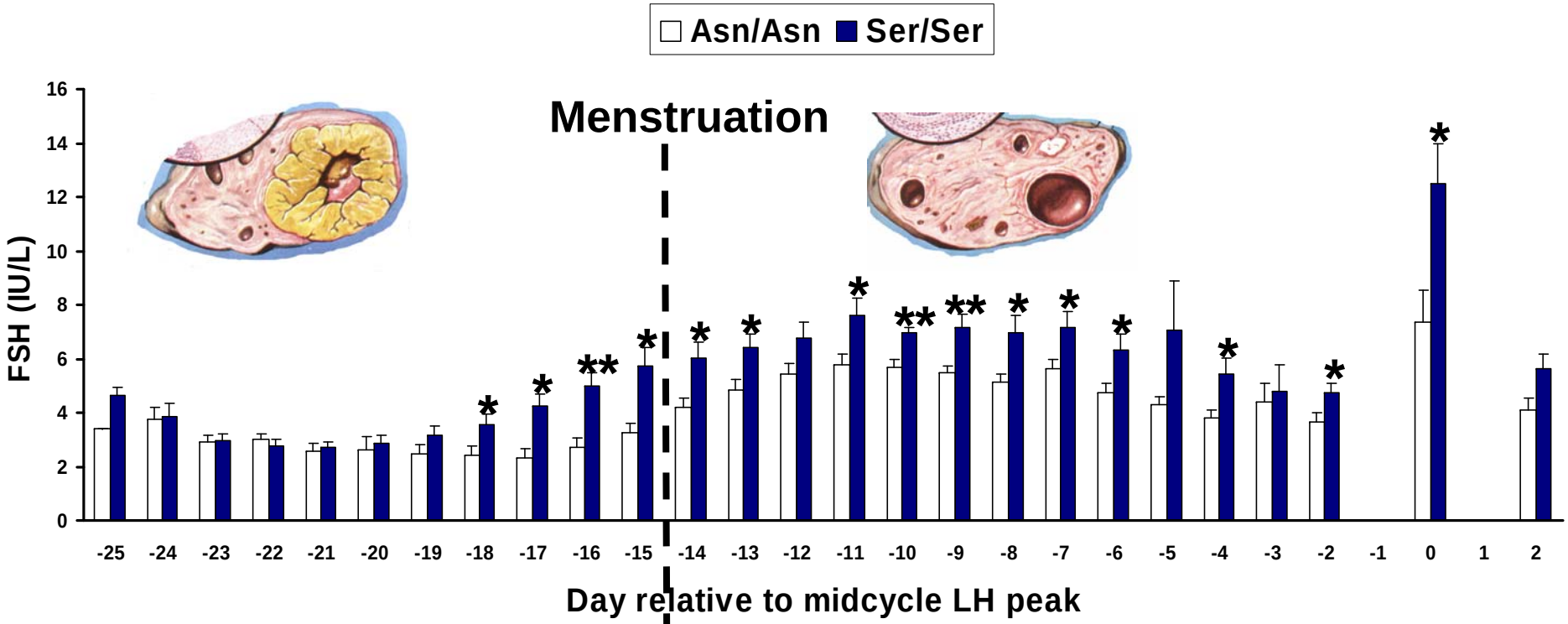
Serum LH levels



N.S.

Greb et al., JCEM 90:4866, 2005

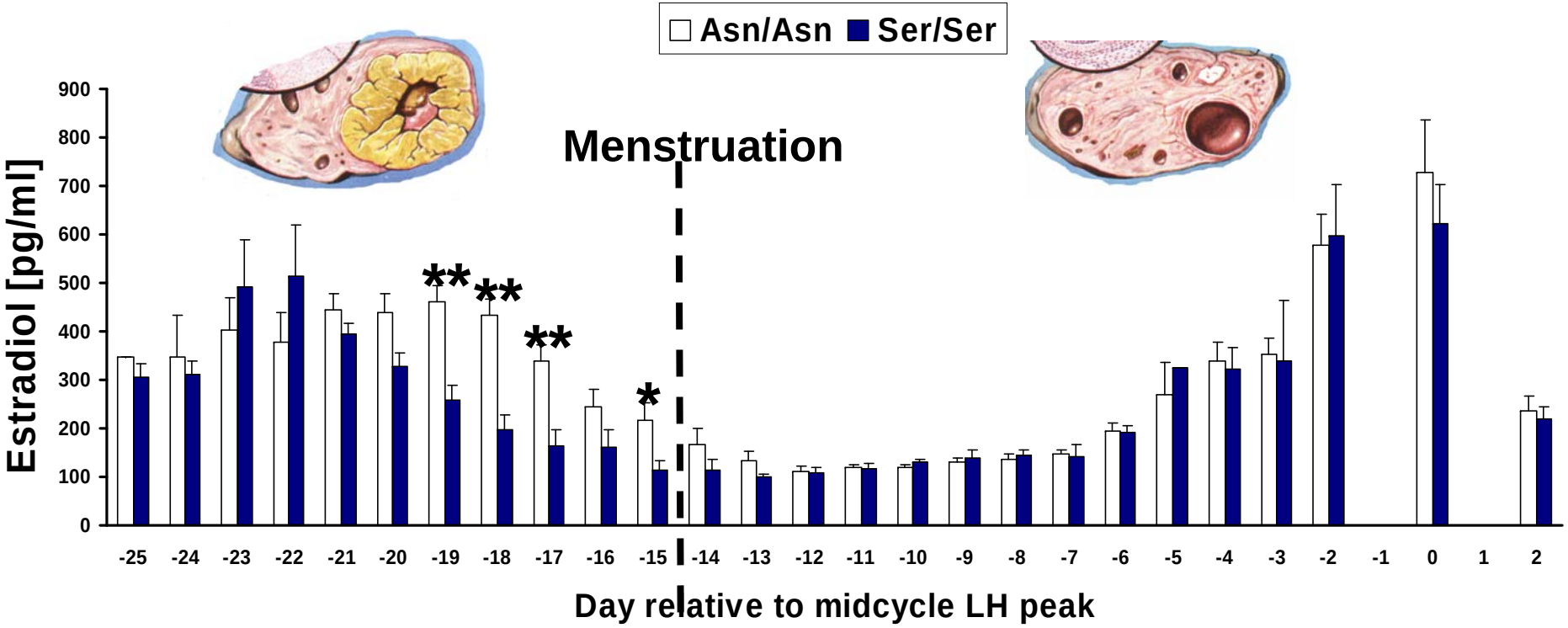
Serum FSH levels



* $P < 0.05$
** $P < 0.005$

Greb et al., JCEM 90:4866, 2005

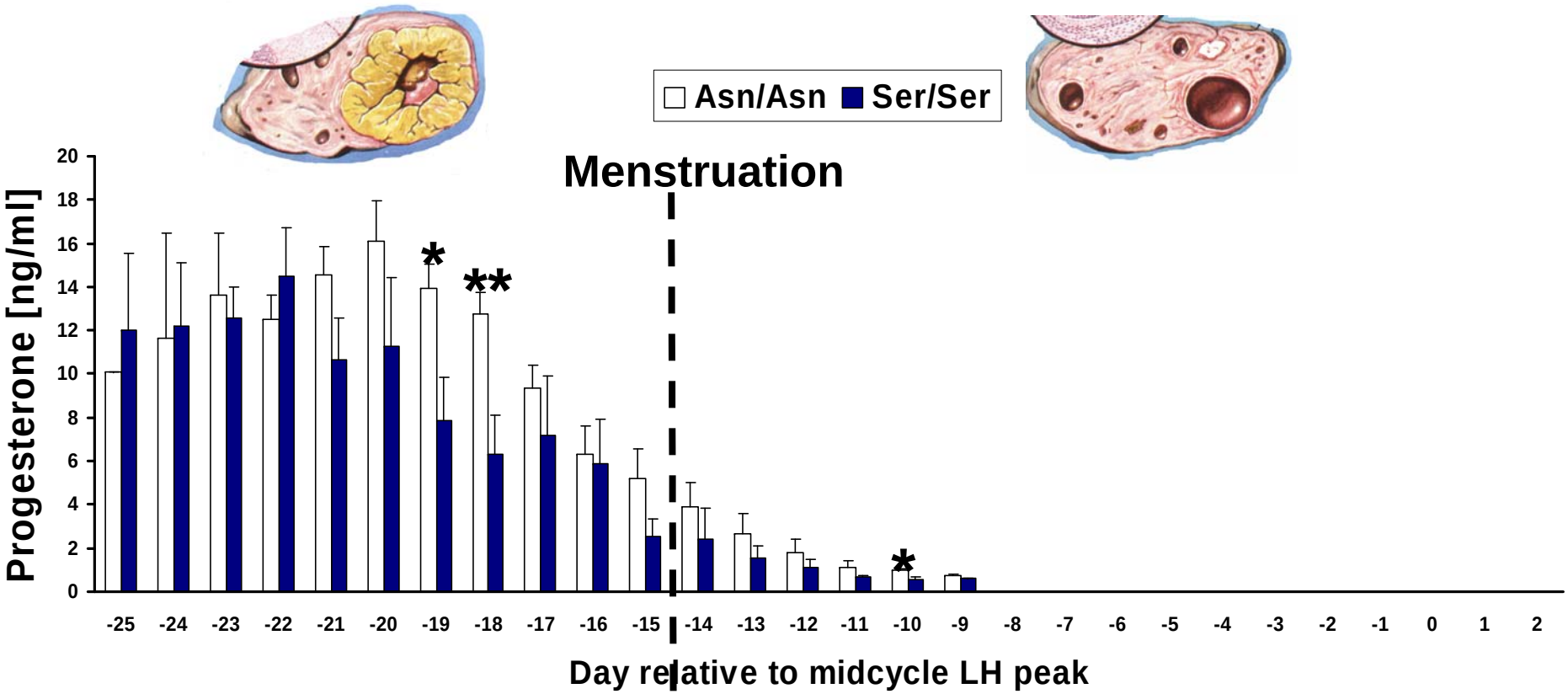
Estradiol levels



* $P < 0.05$
** $P < 0.005$

Greb et al., JCEM 90:4866, 2005

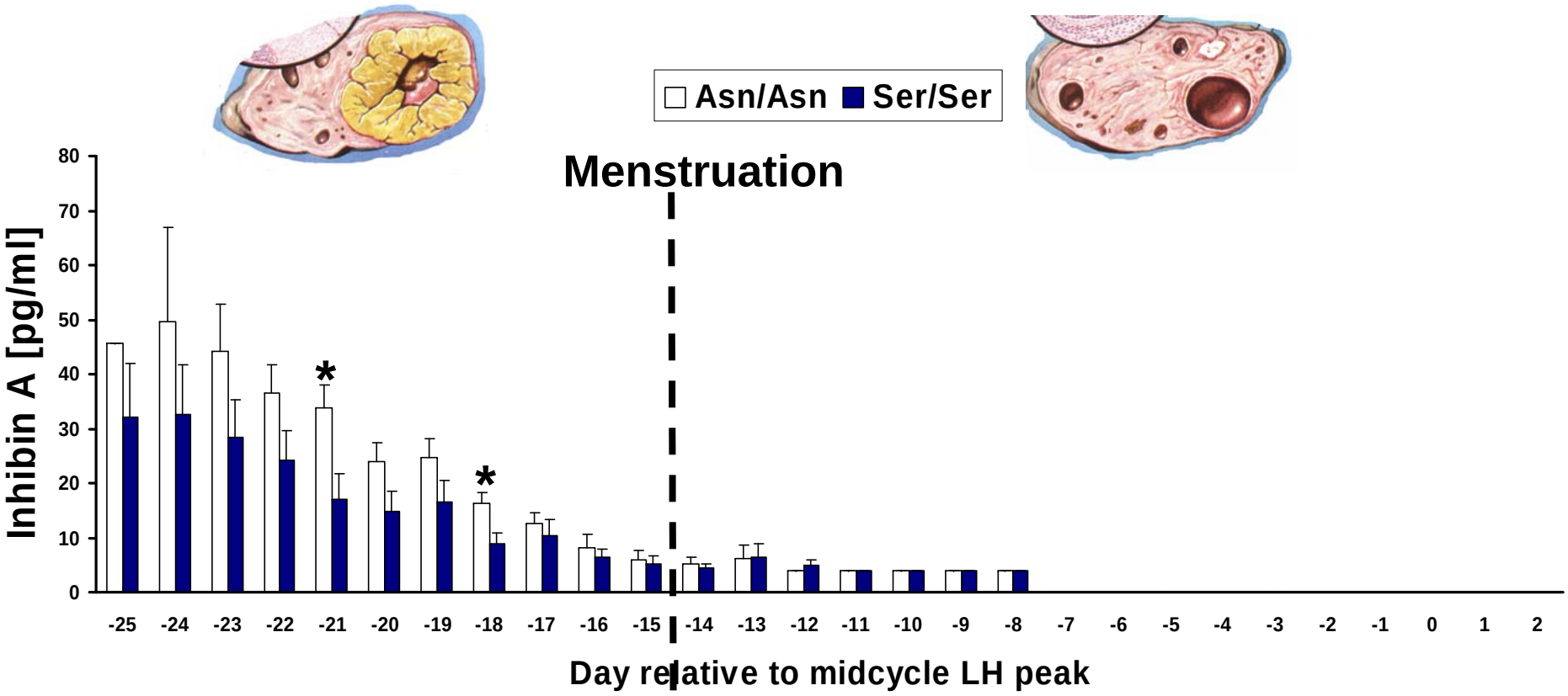
Progesterone levels



* $P < 0.05$
** $P < 0.005$

Greb et al., JCEM 90:4866, 2005

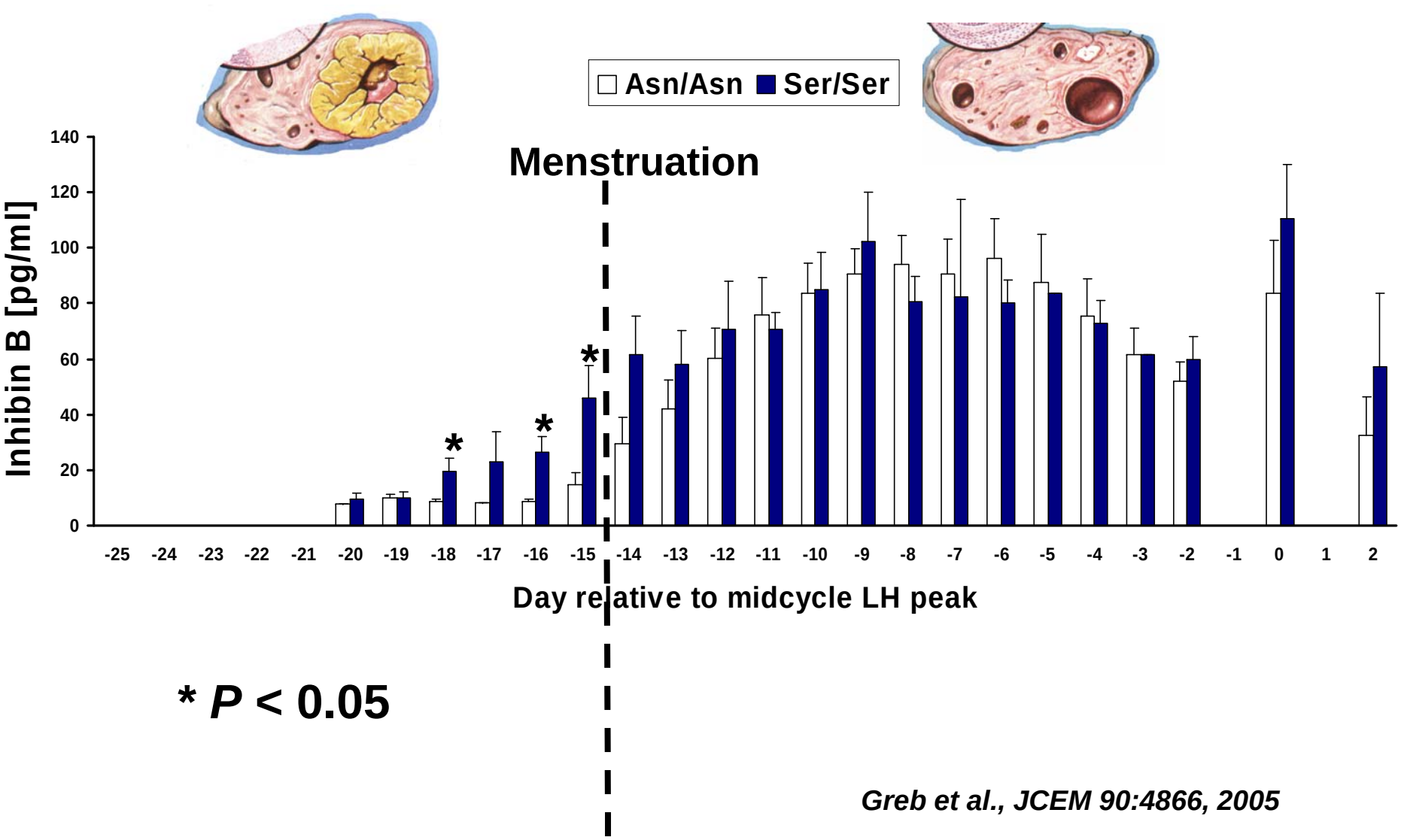
Inhibin A levels



* $P < 0.05$

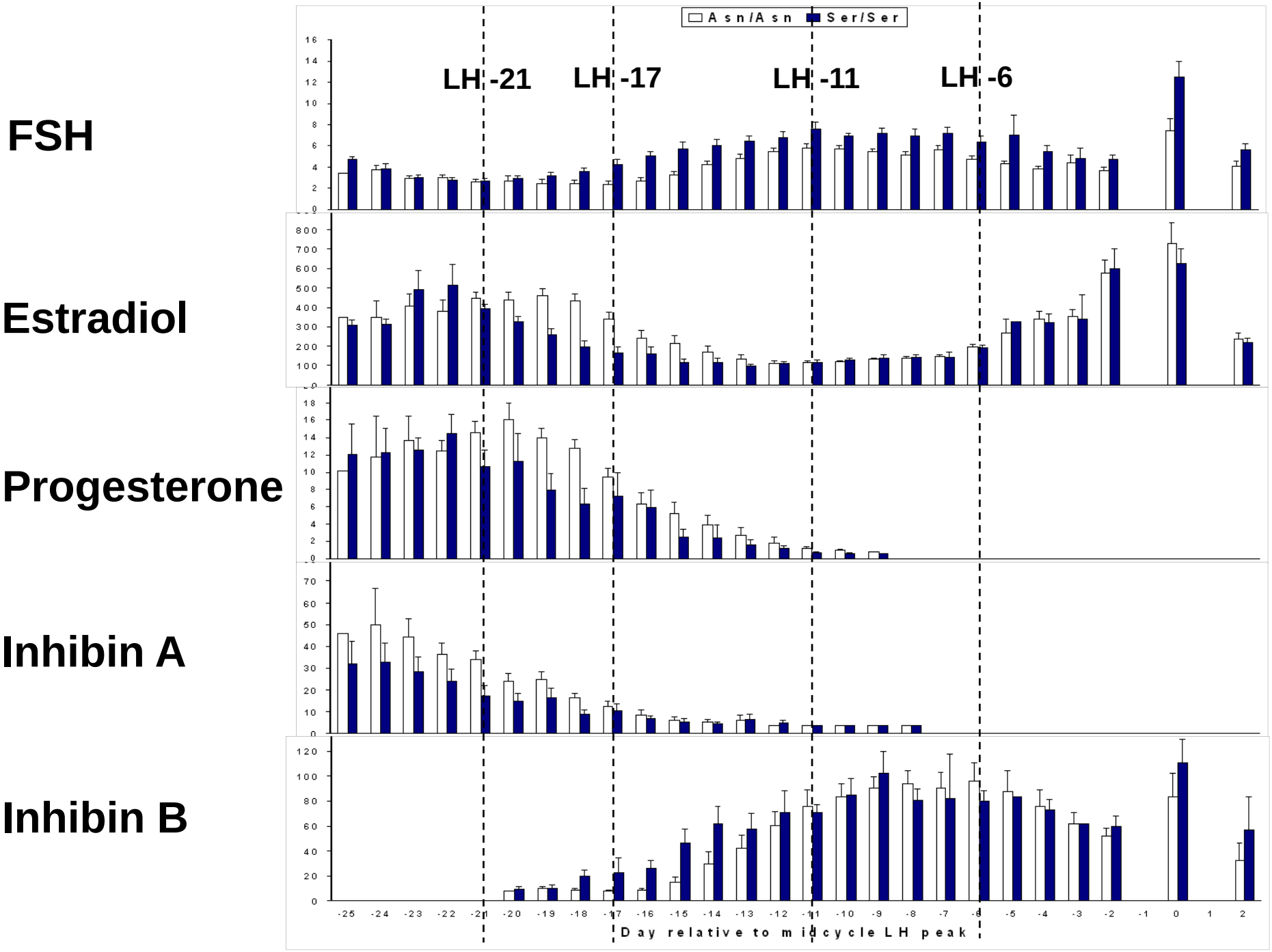
Greb et al., JCEM 90:4866, 2005

Inhibin B levels

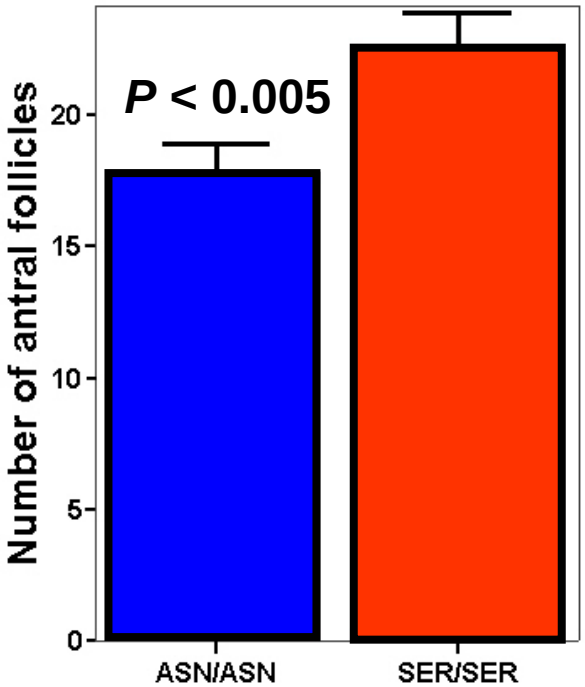


* $P < 0.05$

Greb et al., JCEM 90:4866, 2005


Asn/Asn
Ser/Ser
LH -21
LH -17
LH -11
LH -6
FSH
Estradiol
Progesterone
Inhibin A
Inhibin B
Day relative to midcycle LH peak

Ovarian Response



	Asn/Asn	Ser/Ser	
Menstrual Cycle Length (d)	**25.7 ± 0.6**	**28.7 ± 1.1**	***P* < 0.05**
Luteolysis-Midcycle LH peak (d)	**11.3.0 ± 0.6**	**13.6 ± 1.0**	***P < 0.05.***

Greb et al., JCEM 90:4866, 2005

Conclusions

Ser/Ser is associated with:

- **decreased negative feedback**
- **higher FSH levels**
- **recruitment of a larger number of antral follicles**
- **longer duration of the menstrual cycle**

compared to Asn/Asn

higher FSH threshold!

Greb et al., JCEM 90:4866, 2005

The FSHR genotype is associated with a different length of the menstrual cycle

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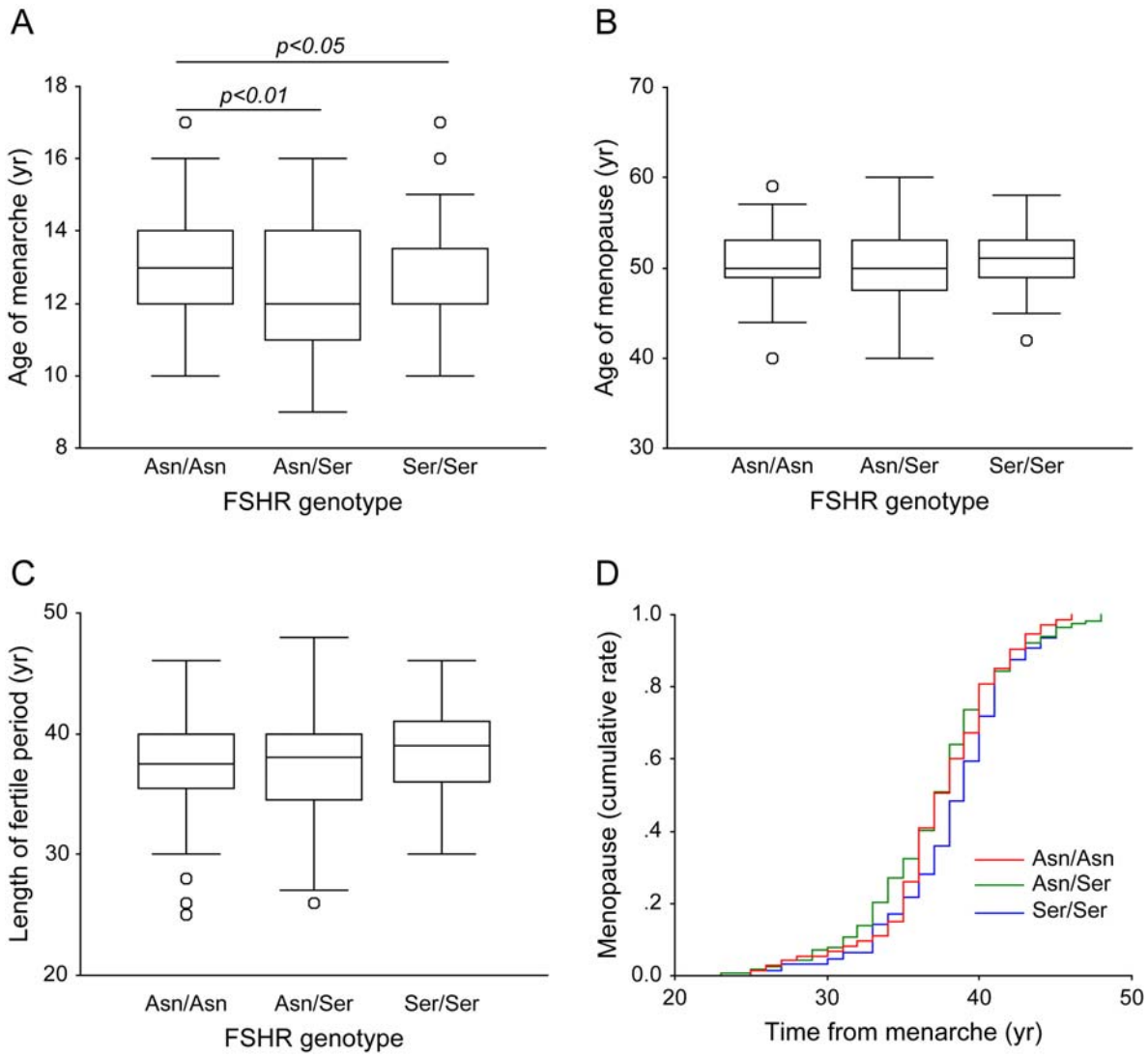
Question: does the FSHR genotype influence the *duration* of fertile age?

FSH receptor genotype and duration of fertile age



Zerbetto et al., Fertil & Steril 2008

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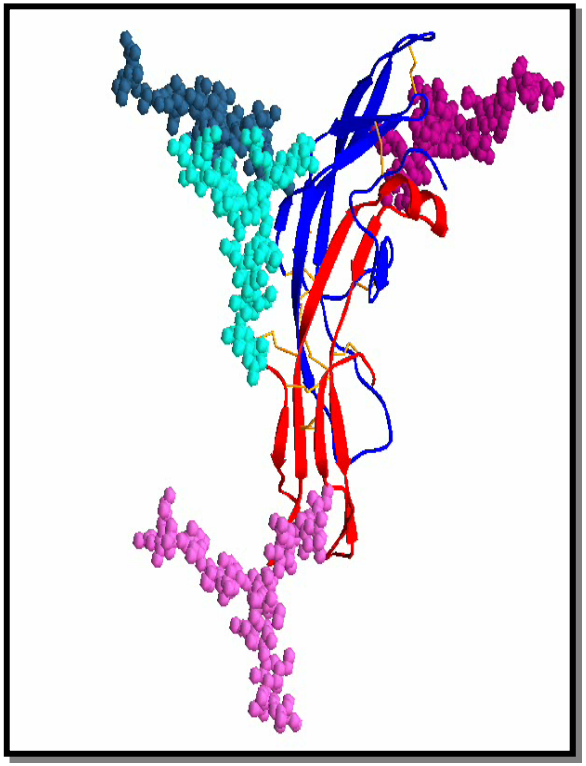
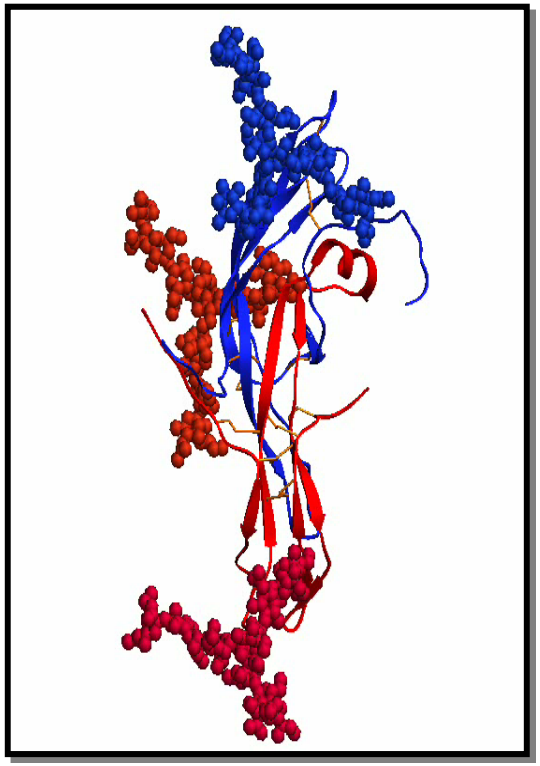
- Can the *LHCGR* distinguish between LH and hCG?

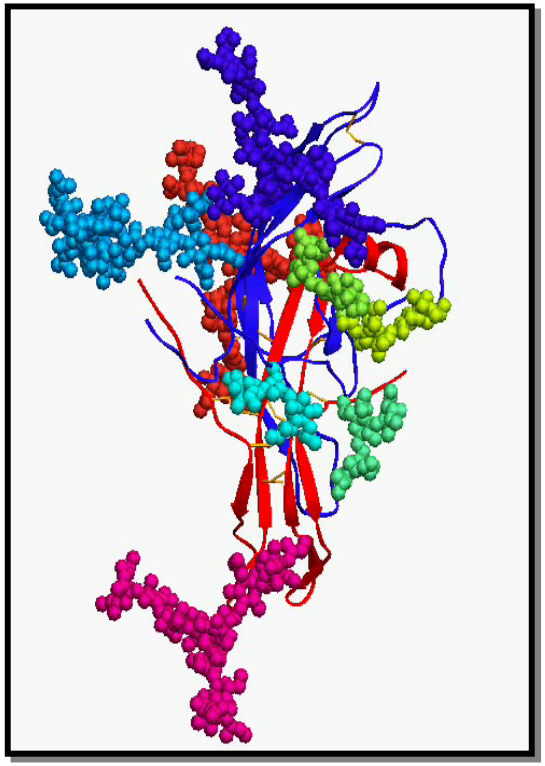
LH and hCG are similar to FSH



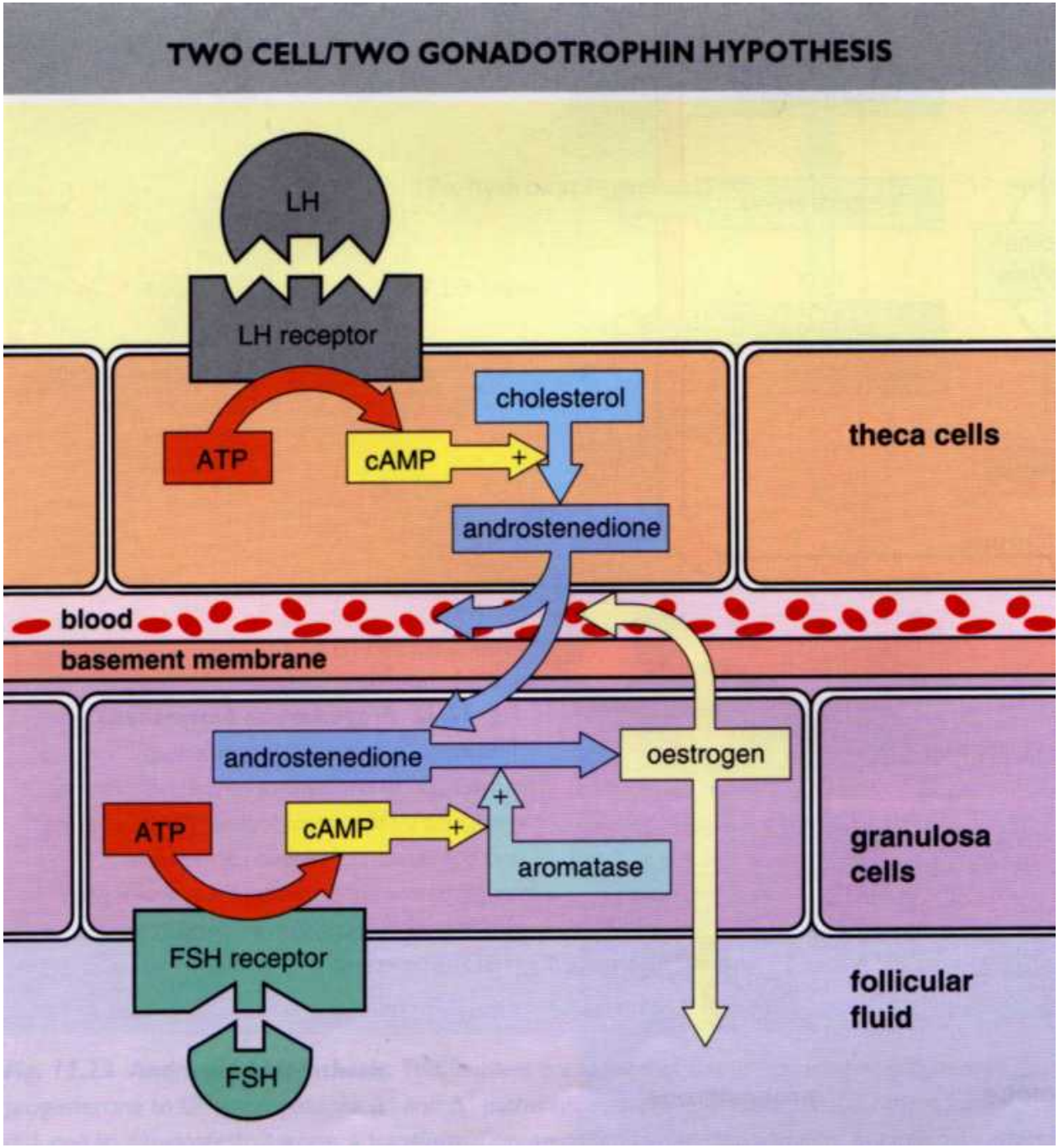
r-hFSH



r-hLH

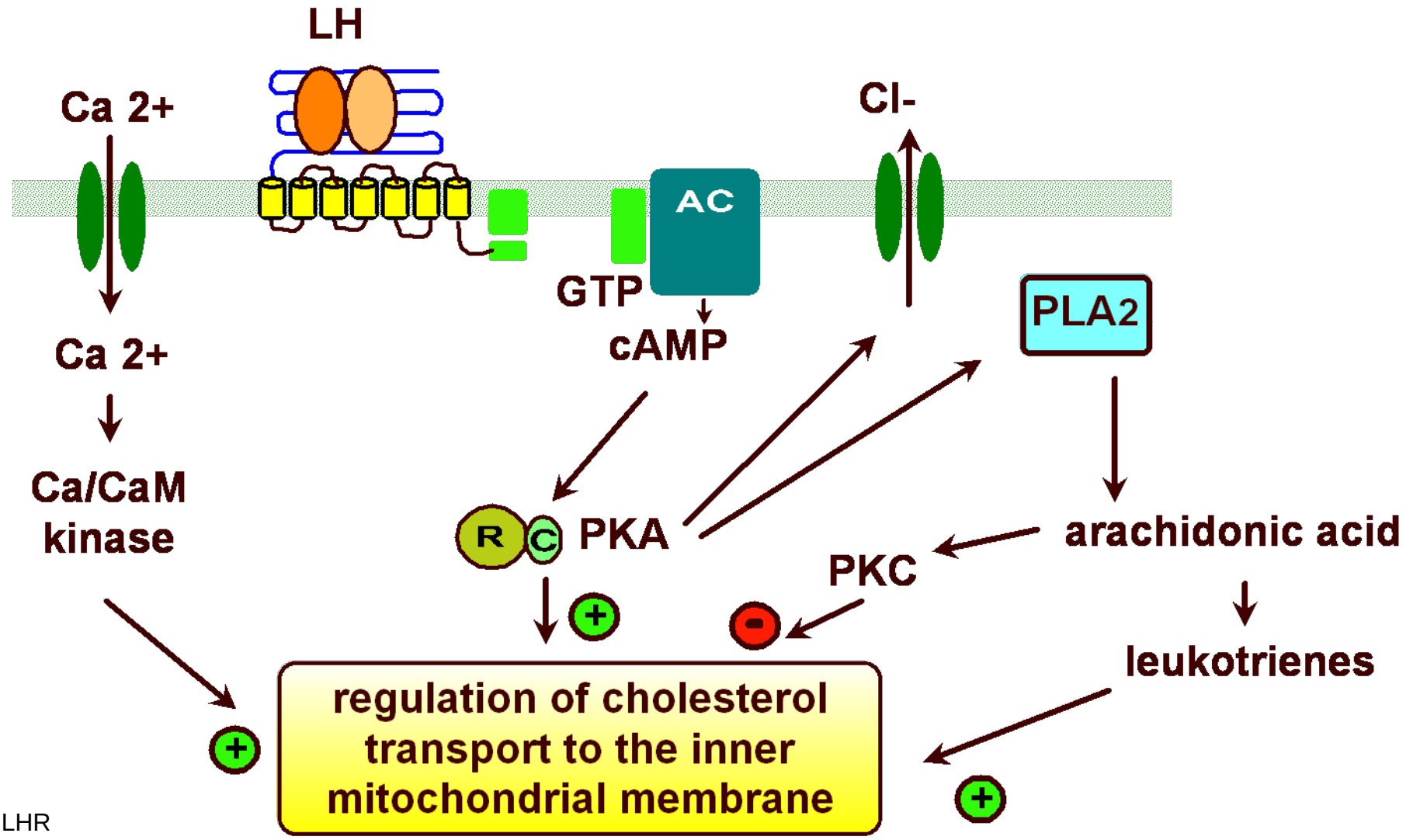


r-hCG


TWO CELL/TWO GONADOTROPHIN HYPOTHESIS
LH
LH receptor
ATP
cAMP
+
cholesterol
androstenedione
theca cells
blood
basement membrane
androstenedione
oestrogen
ATP
cAMP
+
+
aromatase
granulosa cells
FSH receptor
FSH
follicular fluid

genrepro

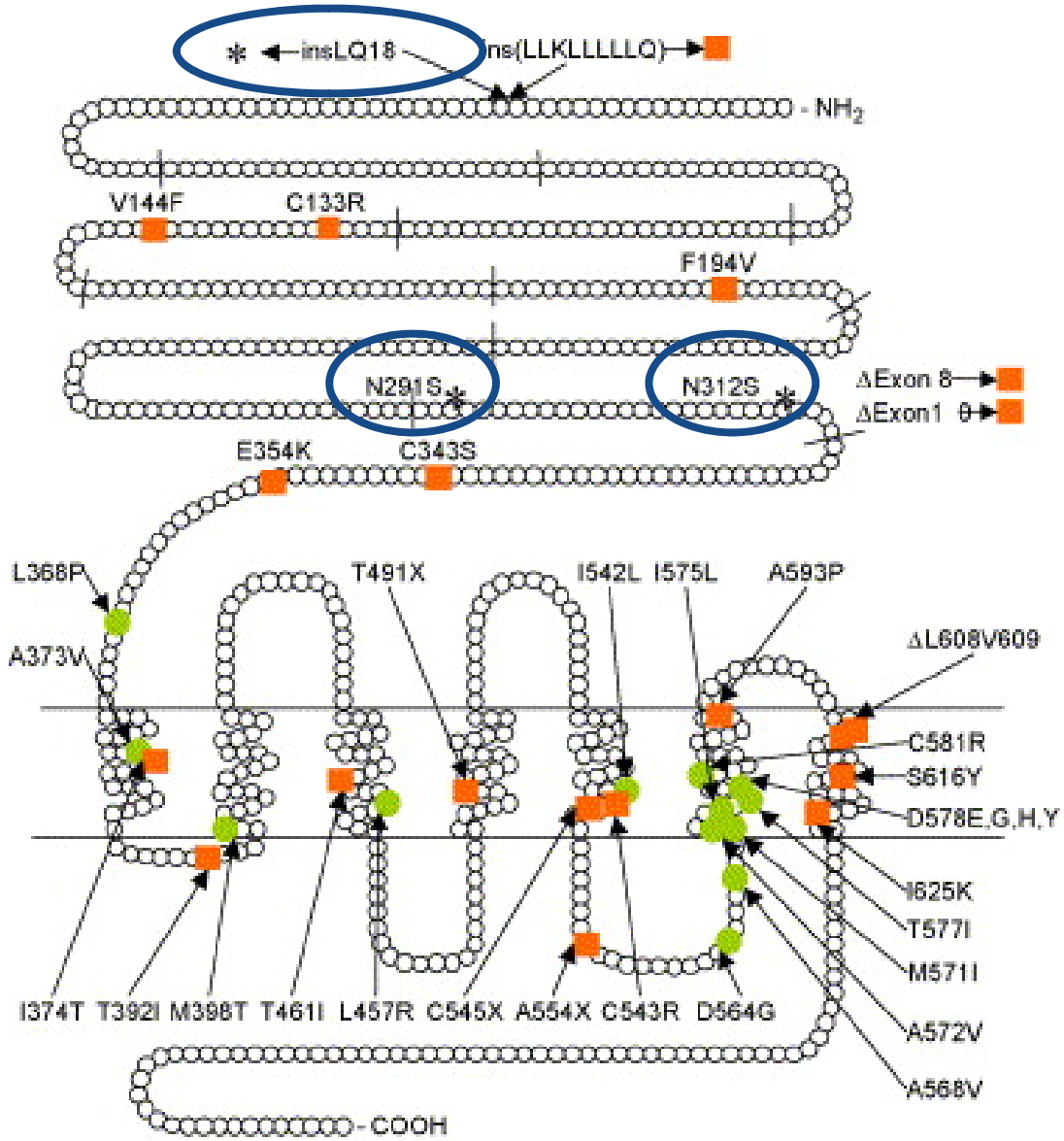
Signal transduction pathway of the LH receptor



LHR

Mutations and polimorphysms of *LHCGR*

- inactivating
- activating
- polimorphysms



Piersma et al., 2007

Mutations of the LH receptor

Inactivating mutations:

primary amenorrhea

Activating mutations:

no phenotype

Inactivating mutations:

Leydig cell hypoplasia

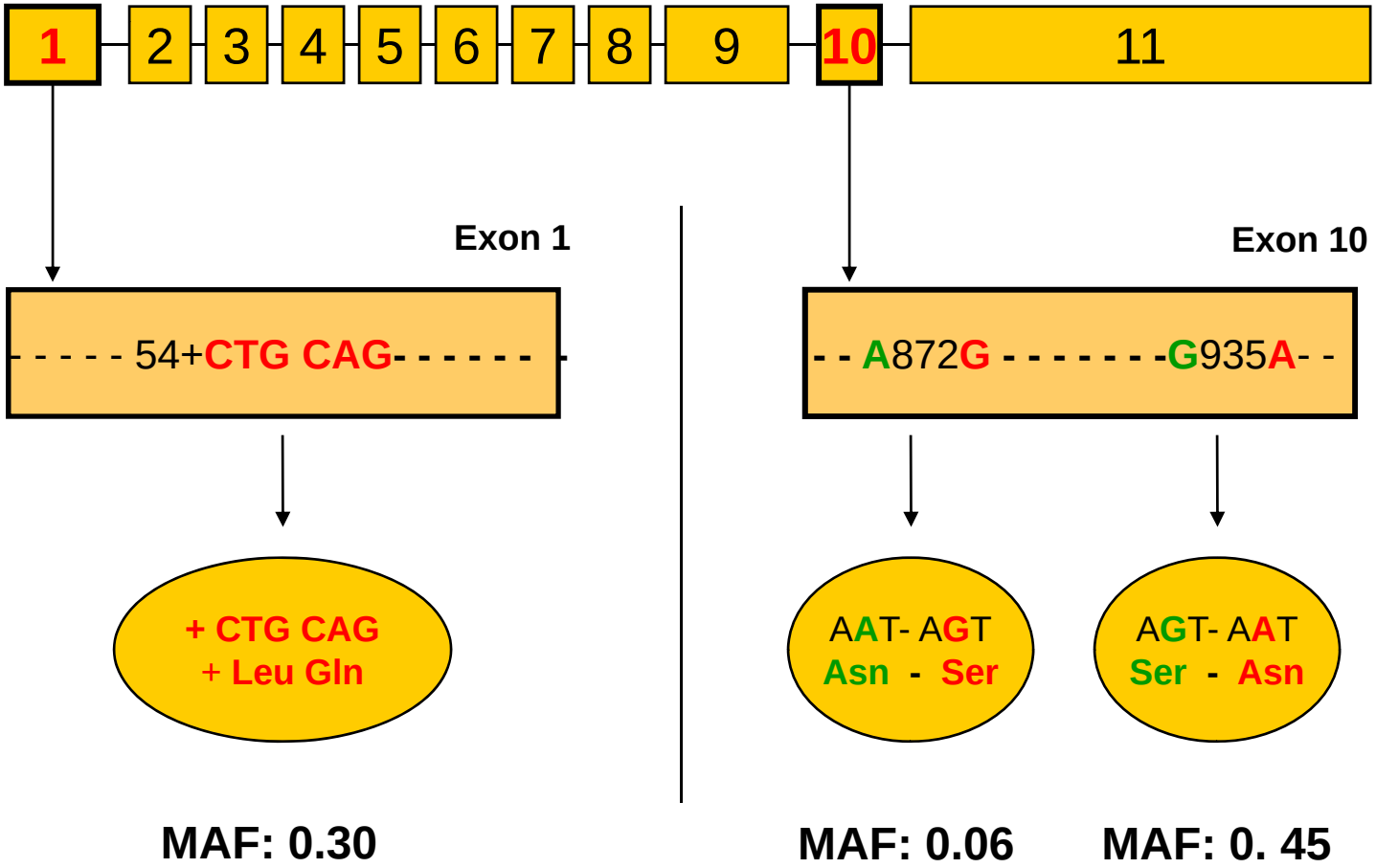
Activating mutations:

male-limited

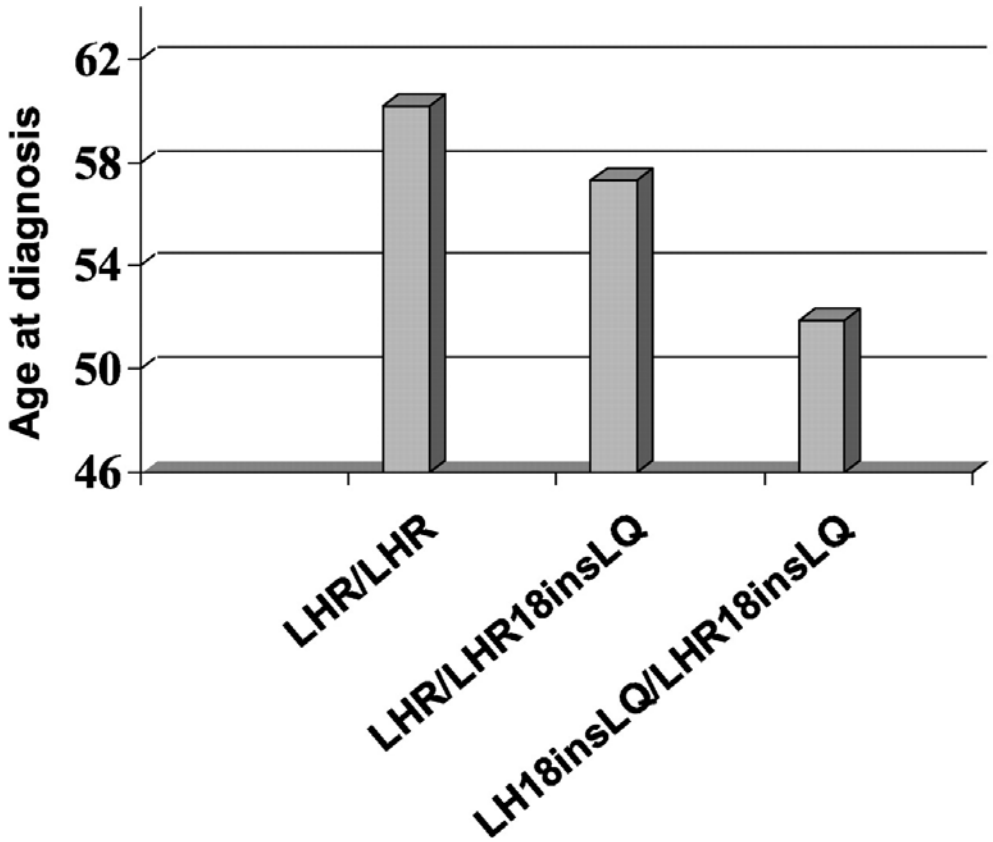
psuedopubertas praecox

LHR

Non-synonymous SNPs in *LH*R



LHR genotype and age at diagnosis of breast cancer



Powell et al. J Clin Endocrinol Metab 2003;88:1653-1657

hCG-induced cAMP response element activation by the insLQ- and non-LQ-LHR variants

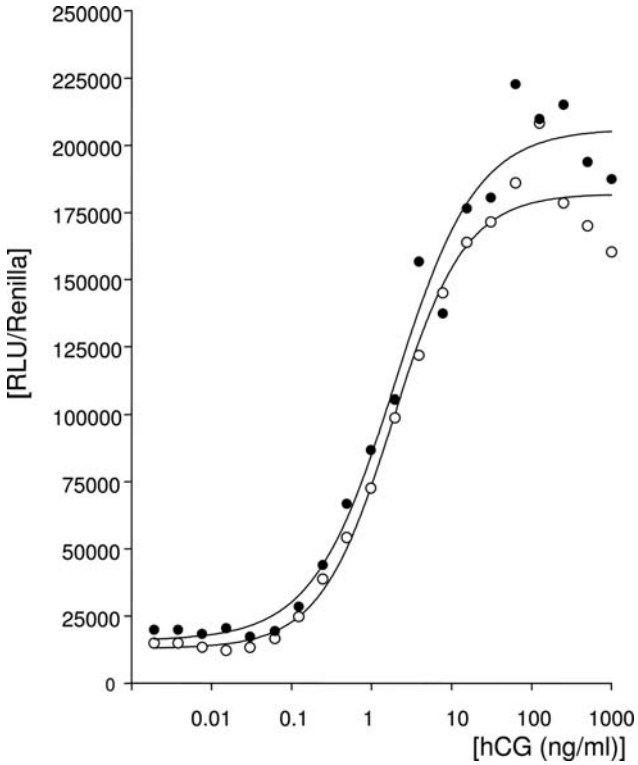


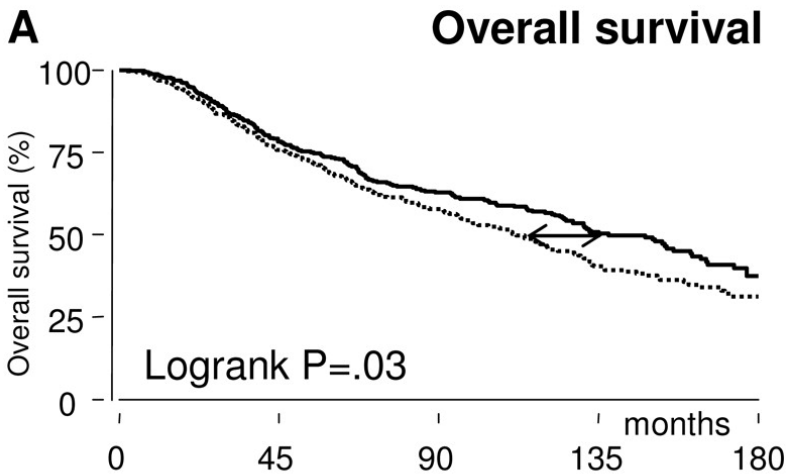
TABLE 2. EC_{50} and B_{max} values for insLQ- and nonLQ-LHR variant

	insLQ-LHR	non-LQ-LHR	n	*P* value[a]
$\log EC_{50}$ (95% CI) (ng/ml)	0.15 (0.05–0.26)	0.29 (0.16–0.41)	7	0.004
B_{max} (95% CI) (fmol/Renilla)	3.38 (2.64–4.12)	2.41 (1.89–2.94)	40	0.0006

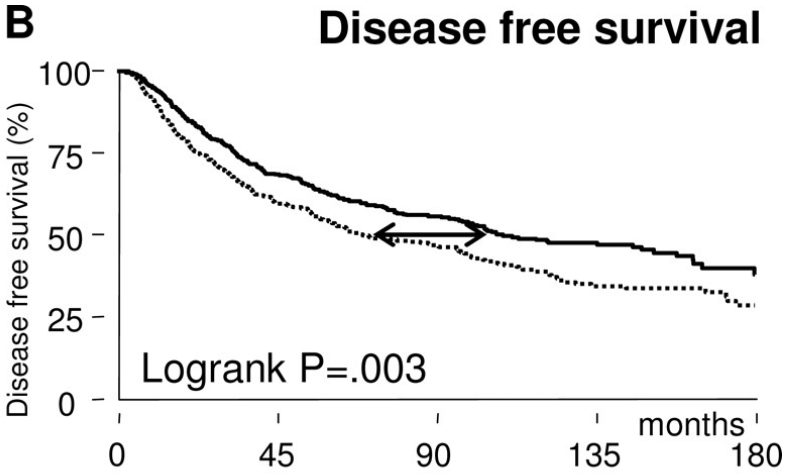
[a] Difference between mean values was tested using Student's *t* test.

Piersma et al., JCEM 2006 91:1470-1476

Patients with breast cancer with the non-LQ allele survive longer than those with the insLQ allele



Obs.	No. at risk:				
191	379	294	212	106	30
218	372	279	191	79	31



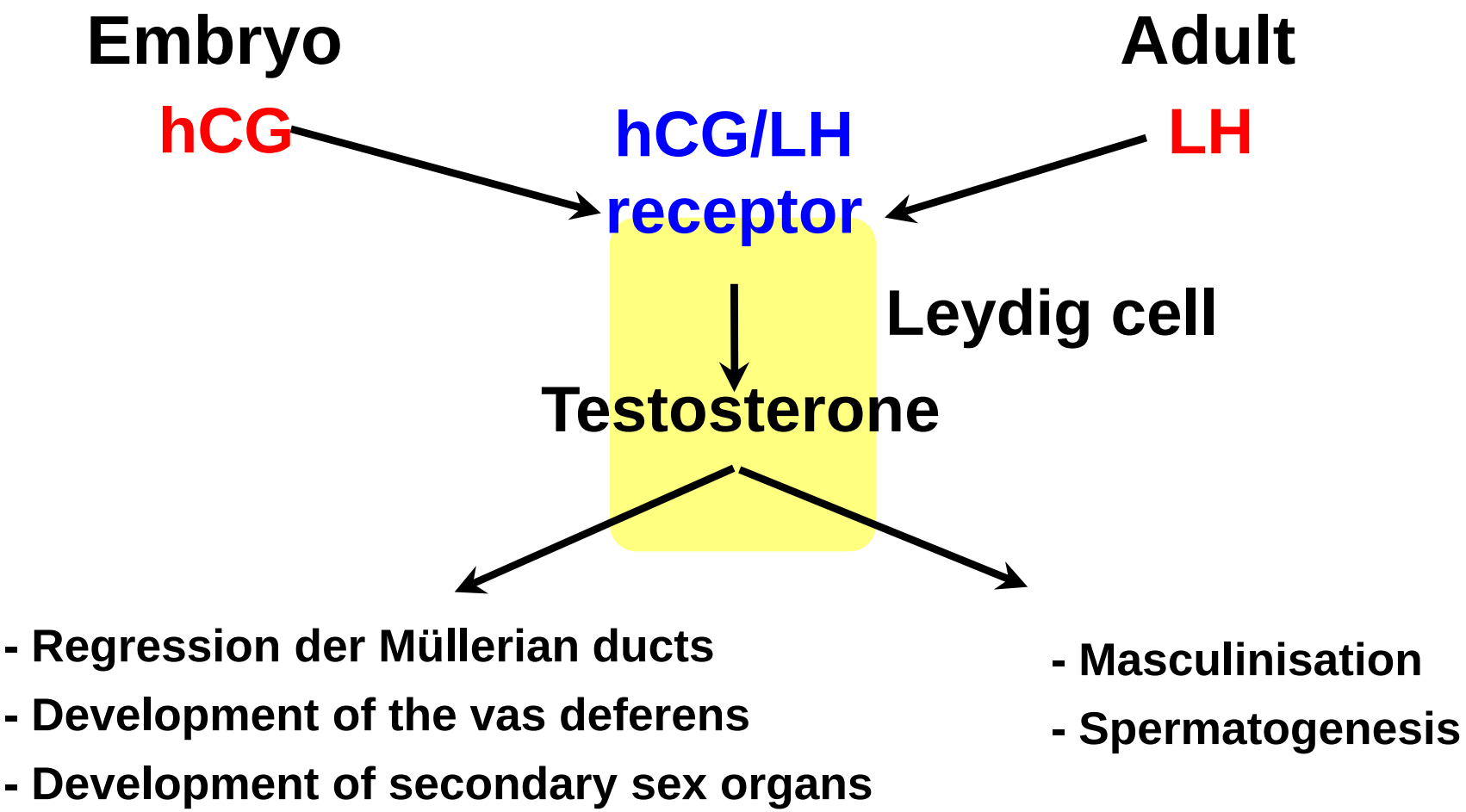
Obs.	No. at risk:				
190	379	243	173	80	20
222	372	206	131	53	21

Piersma et al. JCEM 2006 91:1470-1476

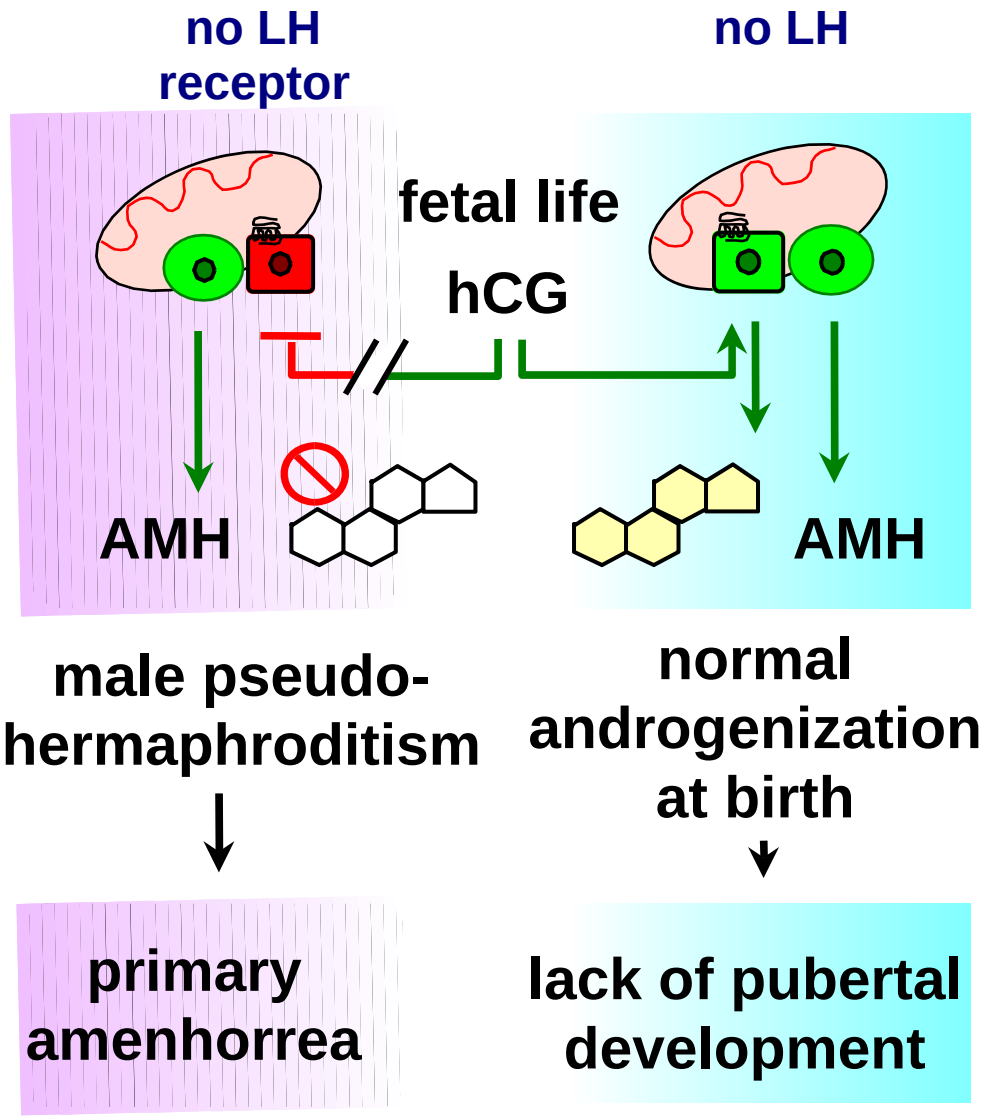
Outline

- Physiology of follicular maturation
- FSH and the *FSHR*
- Mutations and polymorphisms
- Physiological effects of *FSHR* SNP
- LH, hCG and the *LHCGR*
- Mutations and polymorphisms
- **Can the *LHCGR* distinguish between LH and hCG?**

Role of hCG and LH in male sexual differentiation and function

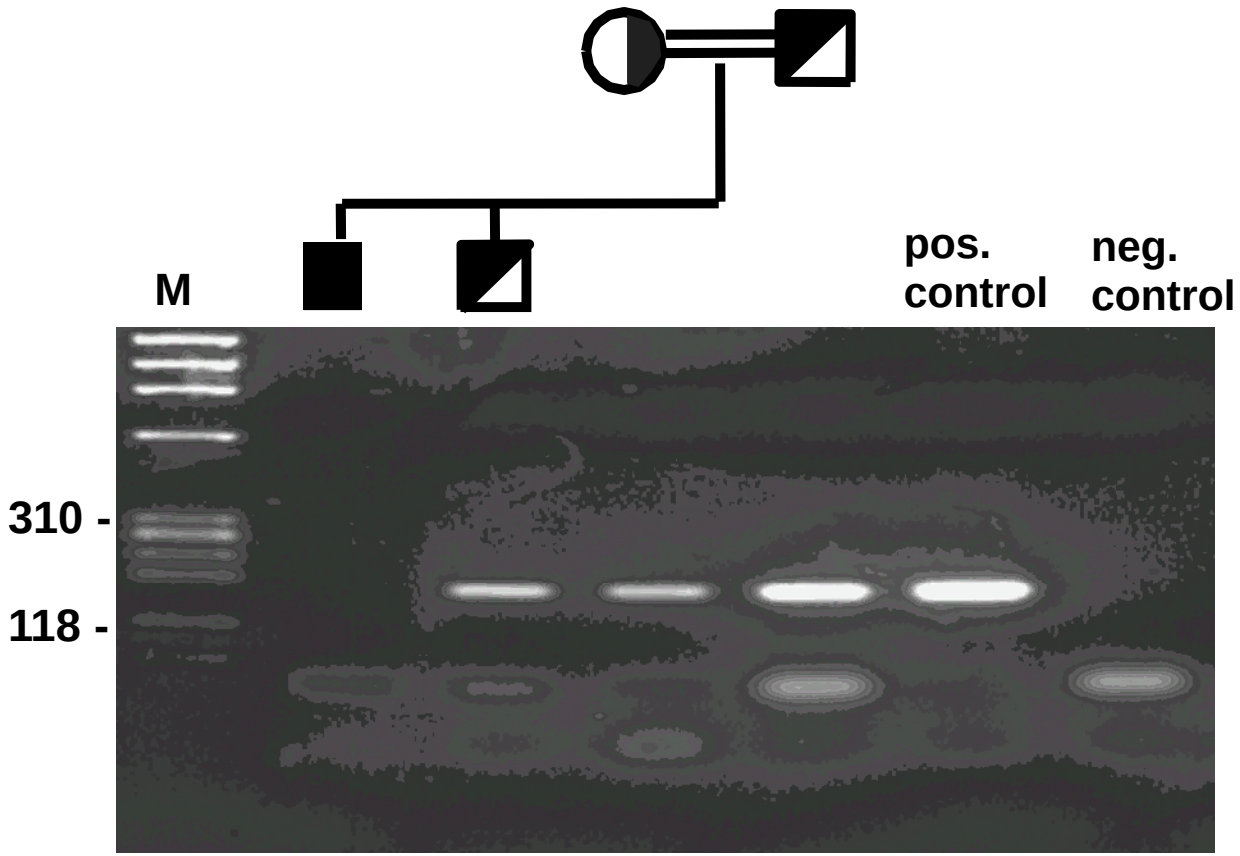


Pathophysiology of inactivating mutations of the LH receptor or LH β subunit in the male

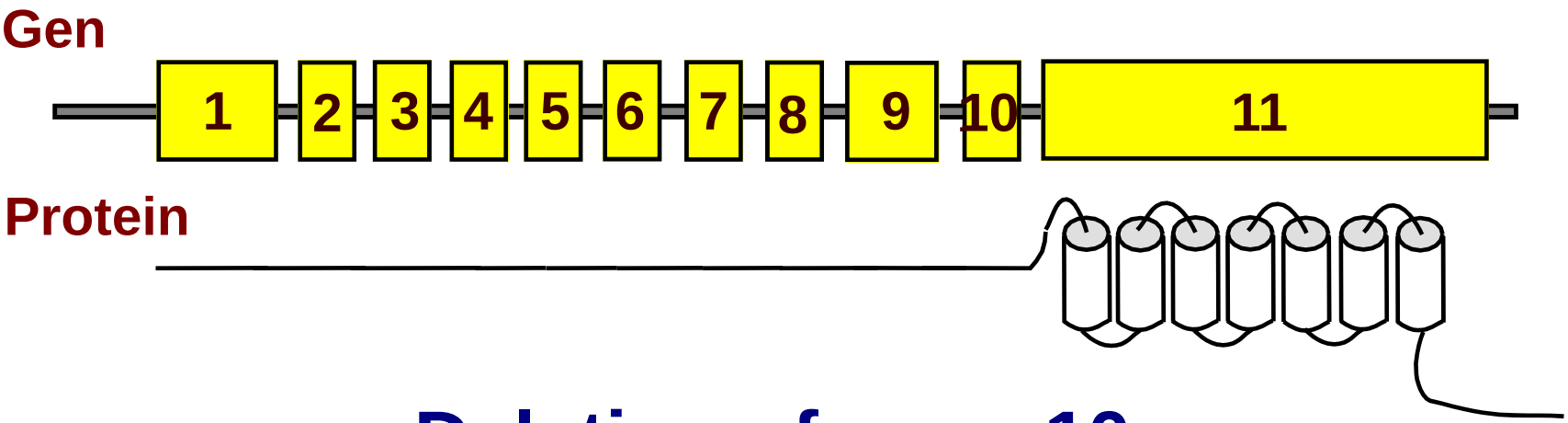


LHR

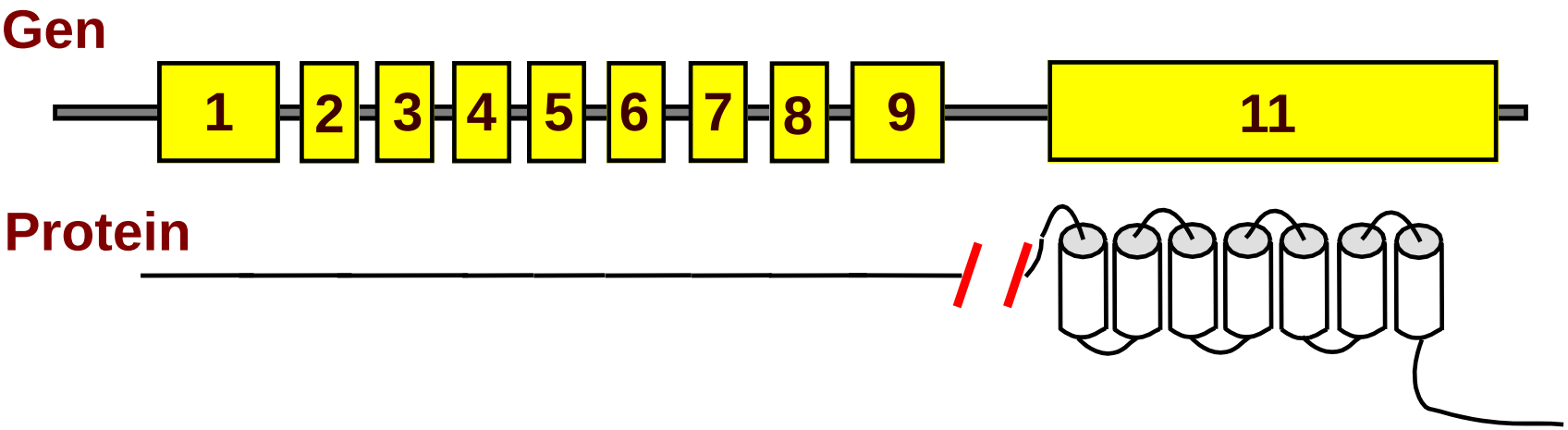
PCR amplification of exon 10 of the *LHCGR* in a male patient with lack of pubertal development



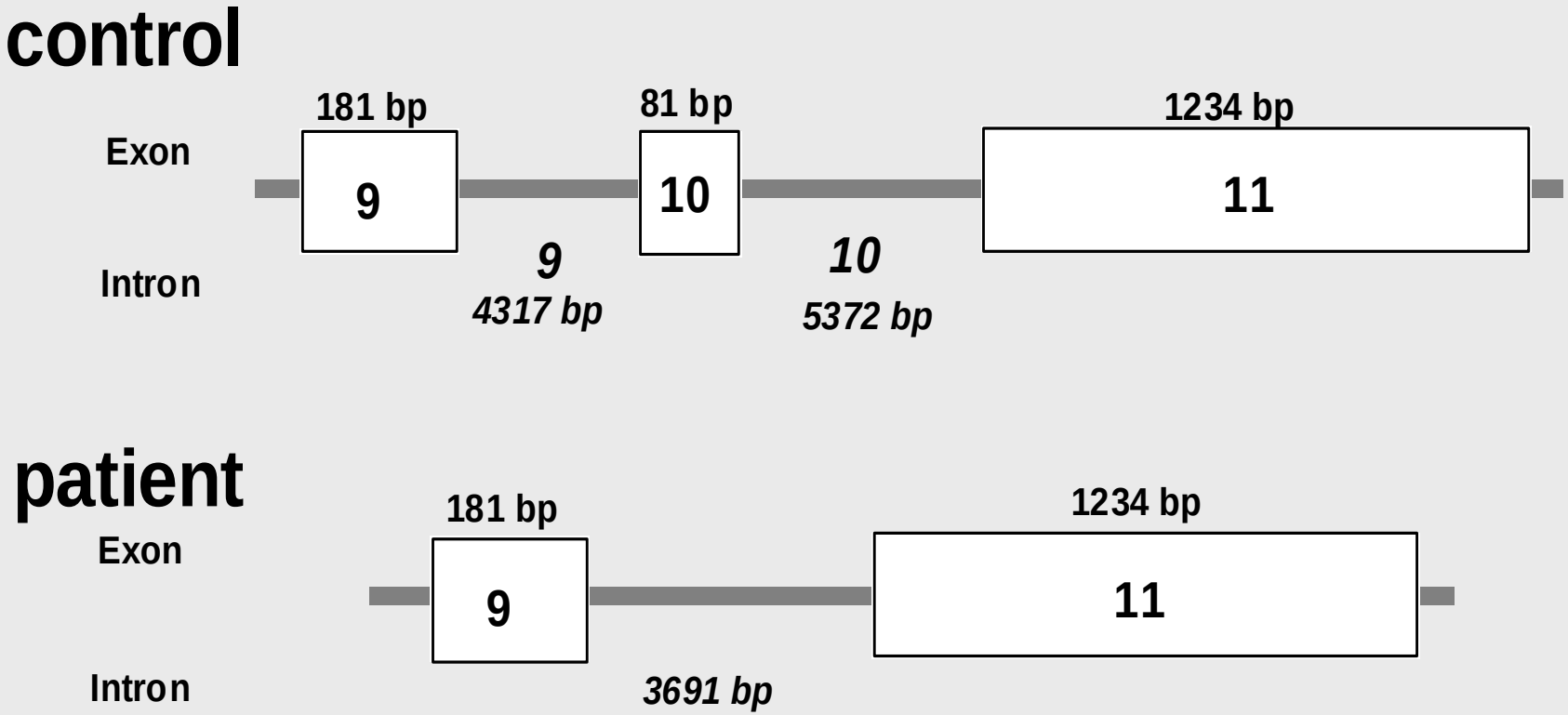
Genomic structure of the *LHCGR*



Deletion of exon 10

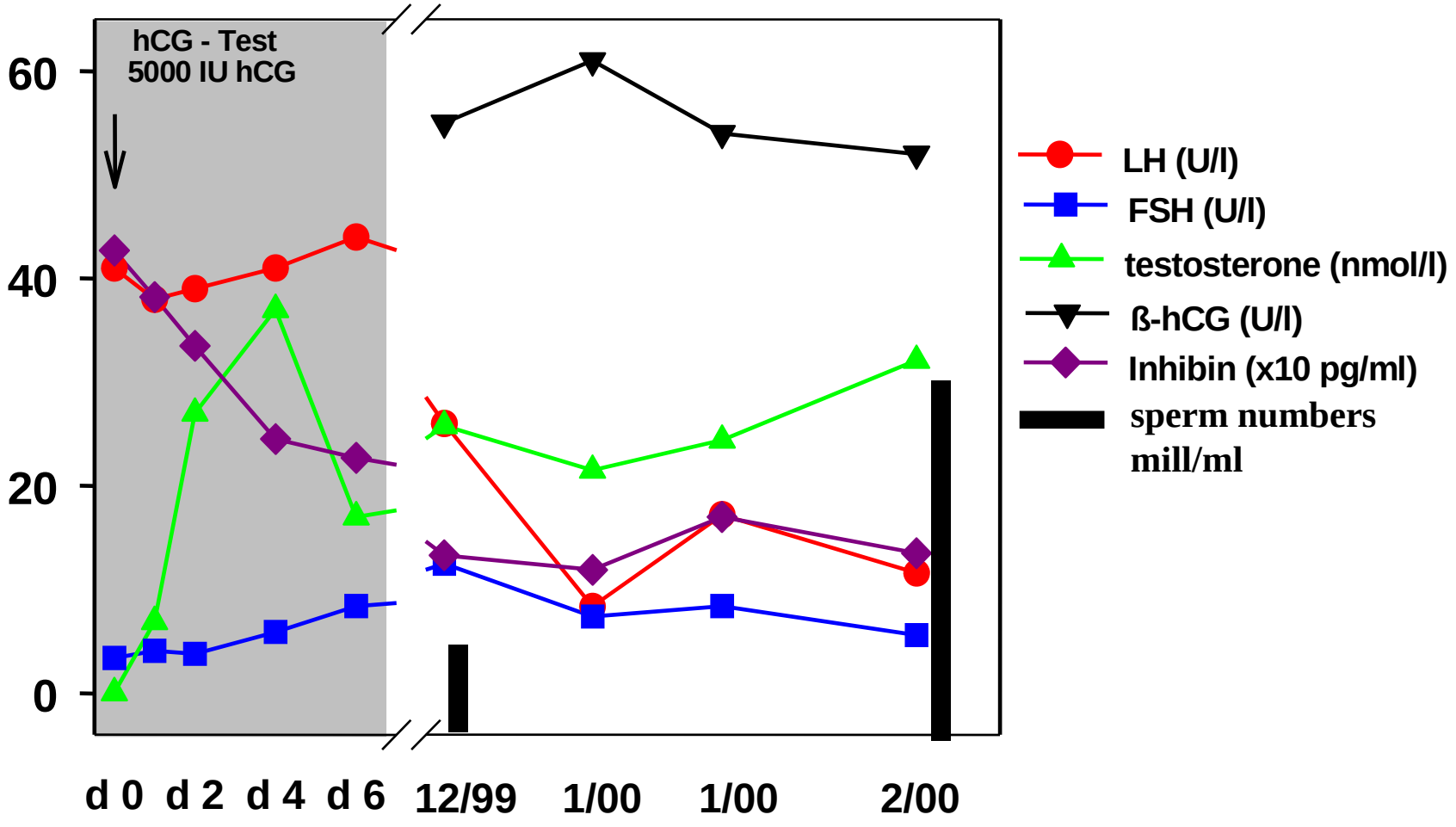


Molecular analysis of the exon 10 deletion of the LH receptor



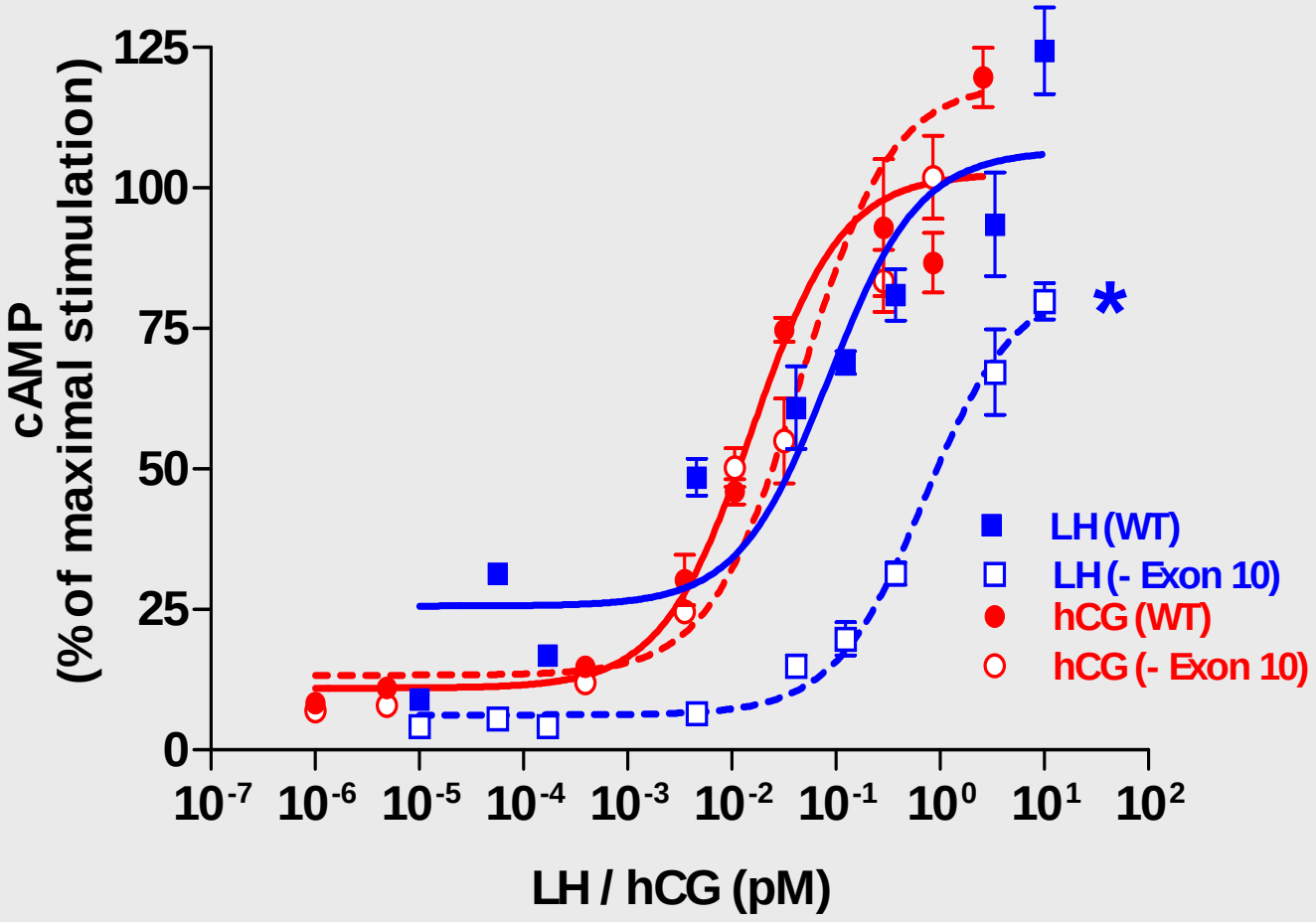
Gromoll et al., JCE&M 85: 2281, 2000

Hormone profiles of a patient with a deletion of exon 10 of the *LHCGR*



Gromoll et al., JCE&M 85: 2281 (2000)

cAMP production in COS-7 cells expressing the WT or the -Exon 10 human *LHCGR*



Müller et al., JCE&M, 2003

The hinge region of the *LHCGR* contains key residues for hormone-induced receptor activation

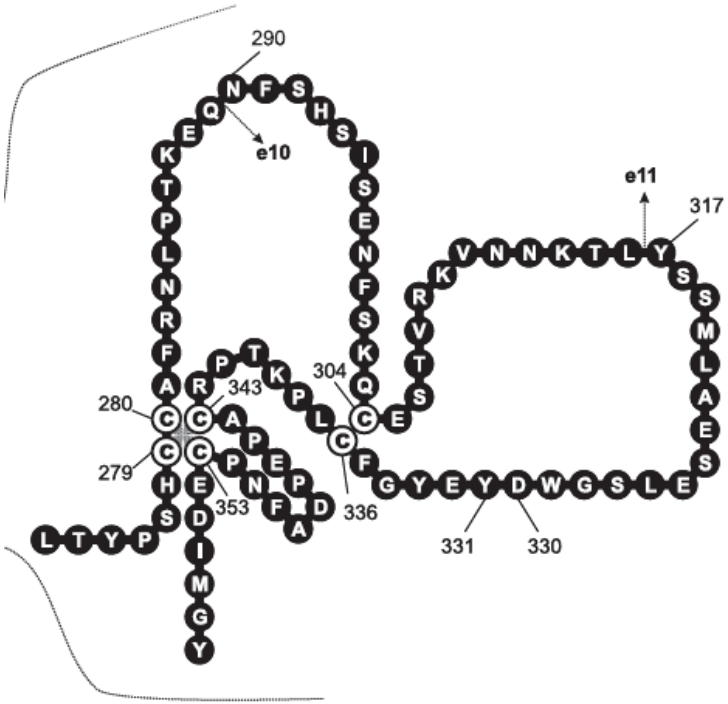


TABLE 2

LH or hCG activation of WT or partial CCR deletion mutants of the LH receptor

Ligand induced activation in HEK 293 cells transiently transfected with WT or mutant LH receptors in which part of the CCR is deleted. EC_{50} values were determined using a CRE-driven reporter gene assay after transfection in HEK293 cells. Numbers in brackets indicate which amino acids of the LH receptor are deleted (Δ). Data are calculated as the mean ± S.E. (*, $p > 0.05$; **, $p > 0.01$; ***, $p > 0.001$ *versus* WT).

	LH		hCG		Org41841	
	EC_{50} (pM)	n	EC_{50} (pM)	n	EC_{50} (nM)	n
WT	31 ± 2	55	15 ± 1	57	238 ± 47	15
Δ(290-316)	490 ± 55***	5	62 ± 9***	5		
Δ(290-303)	87 ± 16***	4	12 ± 1	4		
Δ(305-316)	55 ± 10***	6	14 ± 4	6		
Δ(317-335)	>10^5***	8	>10^5***	7	72 ± 20	4
Δ(317-323)	59 ± 5***	10	19 ± 1	10	137	1

Bruysters et al., JBC 2008

Exon 10 of the *LHCGR* can distinguish between LH and hCG action

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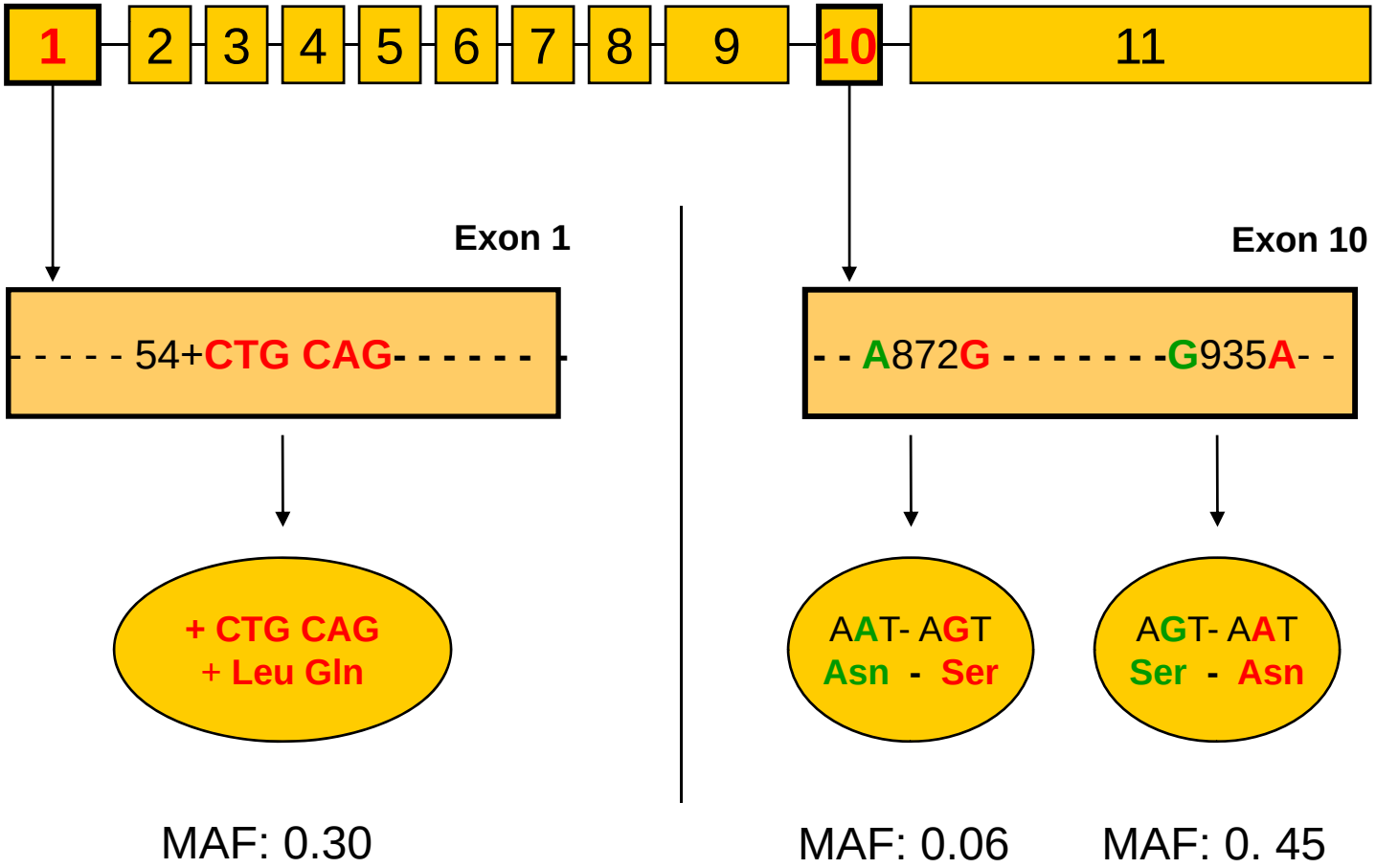
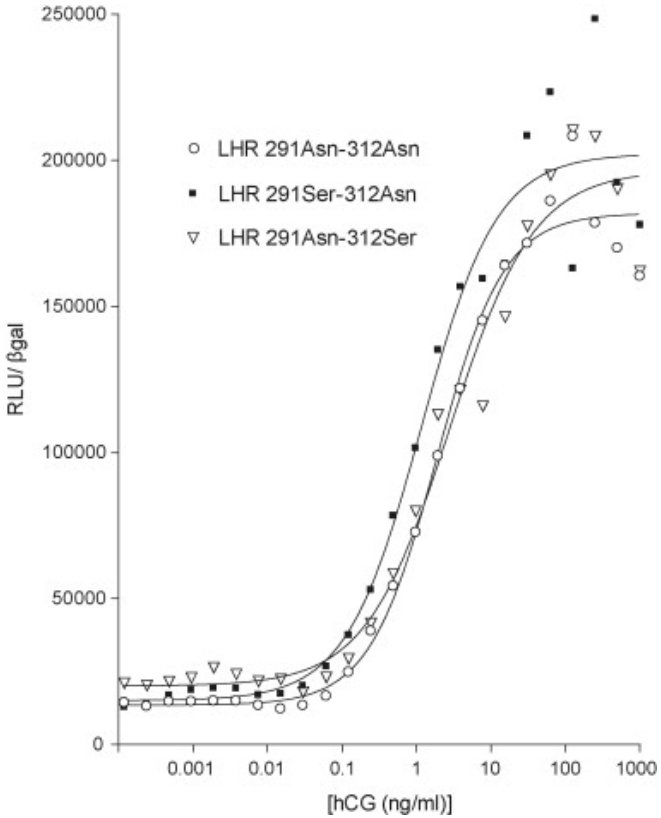
Are polymorphisms in exon 10 influencing LH and hCG activity?

Non-synonymous SNPs in *LH*R



hCG-induced cAMP response element activation by the exon 10 variants



Functional analyses of 291Asn/Ser and 312Ser/Asn LHR variants in HEK293 transfected cells

LHR construct	EC50 (mean ± S.D.) (pM hCG)	n	B_{max} (mean ± S.D.) (fmol/mg protein)	n	K_d ± error (nM)
291Asn-312Asn	61.9 ± 13.6	5	48.2 ± 11.8	10	3.77 ± 0.96
291Asn-312Ser	64.7 ± 9.3	3	56.1 ± 16.4	10	2.16 ± 0.16
291Ser-312Asn	29.3 ± 2.4***	3	59.2 ± 11.6	10	5.67 ± 0.44

EC50 values are determined by CRE receptor assay, B_{max} values by radioligand binding assay and K_d value from one Scatchard analysis; ***$p = 0.001$.

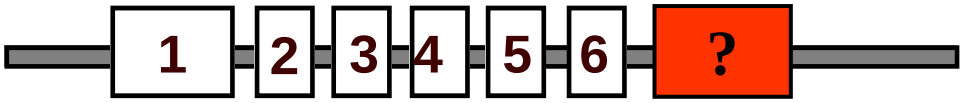
Piersma et al. Mol Cell Endocrinol 2007 July 17 (Epub)

However:

The effects of *LHCGR* polymorphisms on ovarian function remain unknown

Outlook: A novel *LHCGR* mRNA variant

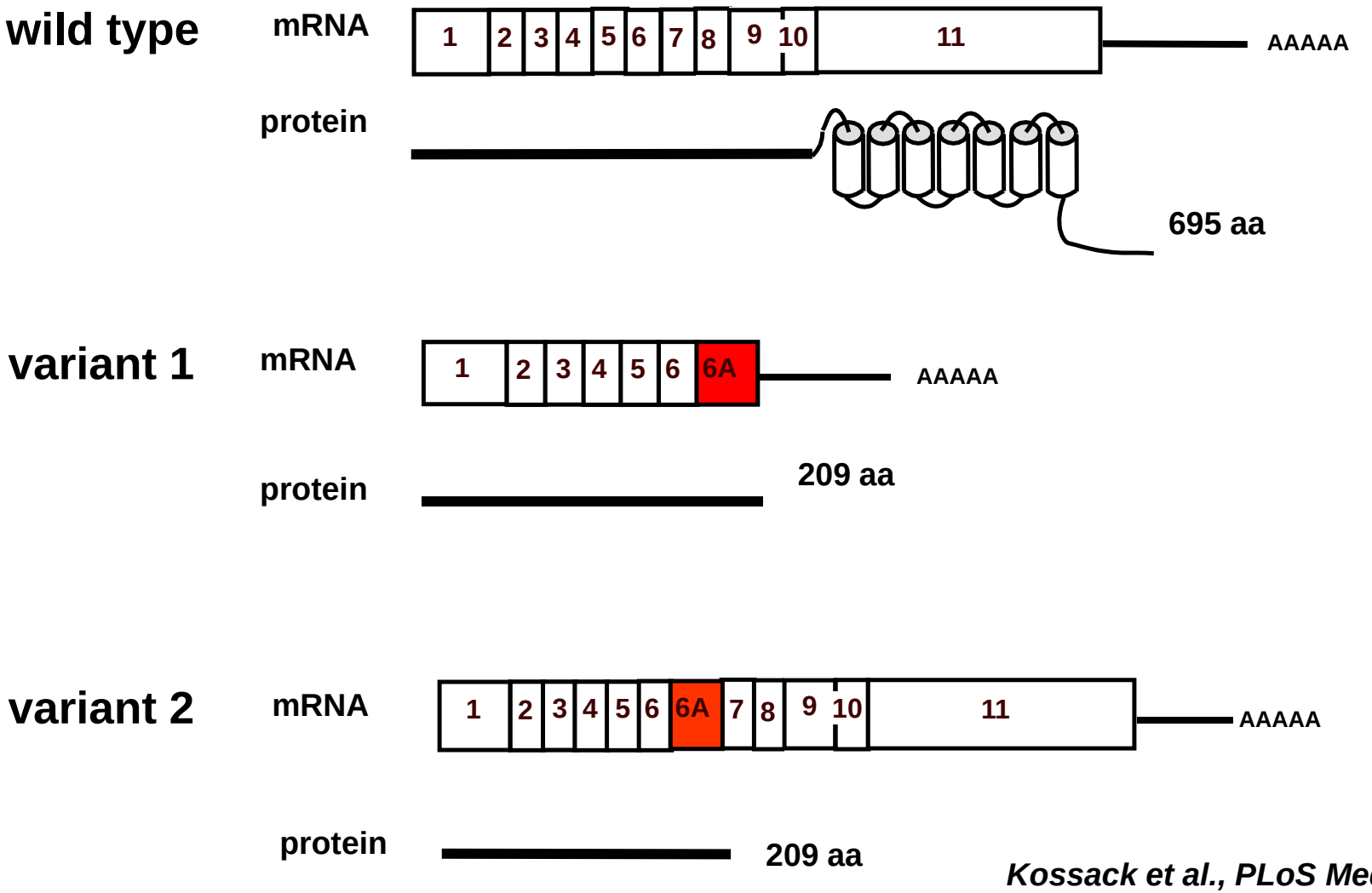
***LHCGR* gene**



- **mRNA with approx. 850 b**
- **element consists of 300 b**
- **encodes 31 aa**
- **no homology to other proteins**
- ***LHCGR* variant encodes protein of 209 aa with 24 kDa**

Kossack et al., PLoS Medicine, 2008

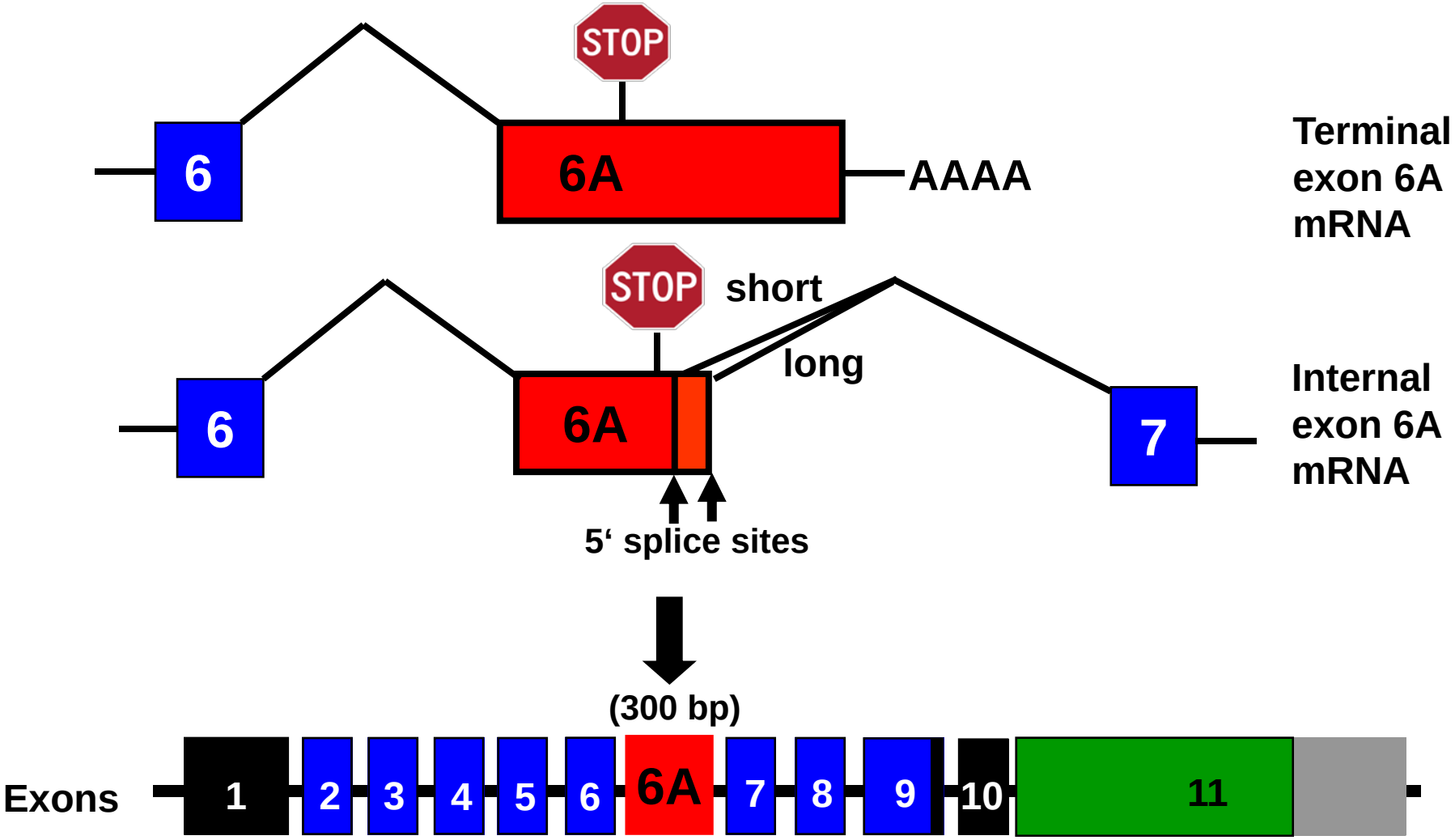
Identification of *LHCGR* exon 6A variants by cDNA library screening and RT-PCR from testis and granulosa cell mRNA



Kossack et al., PLoS Medicine, 2008

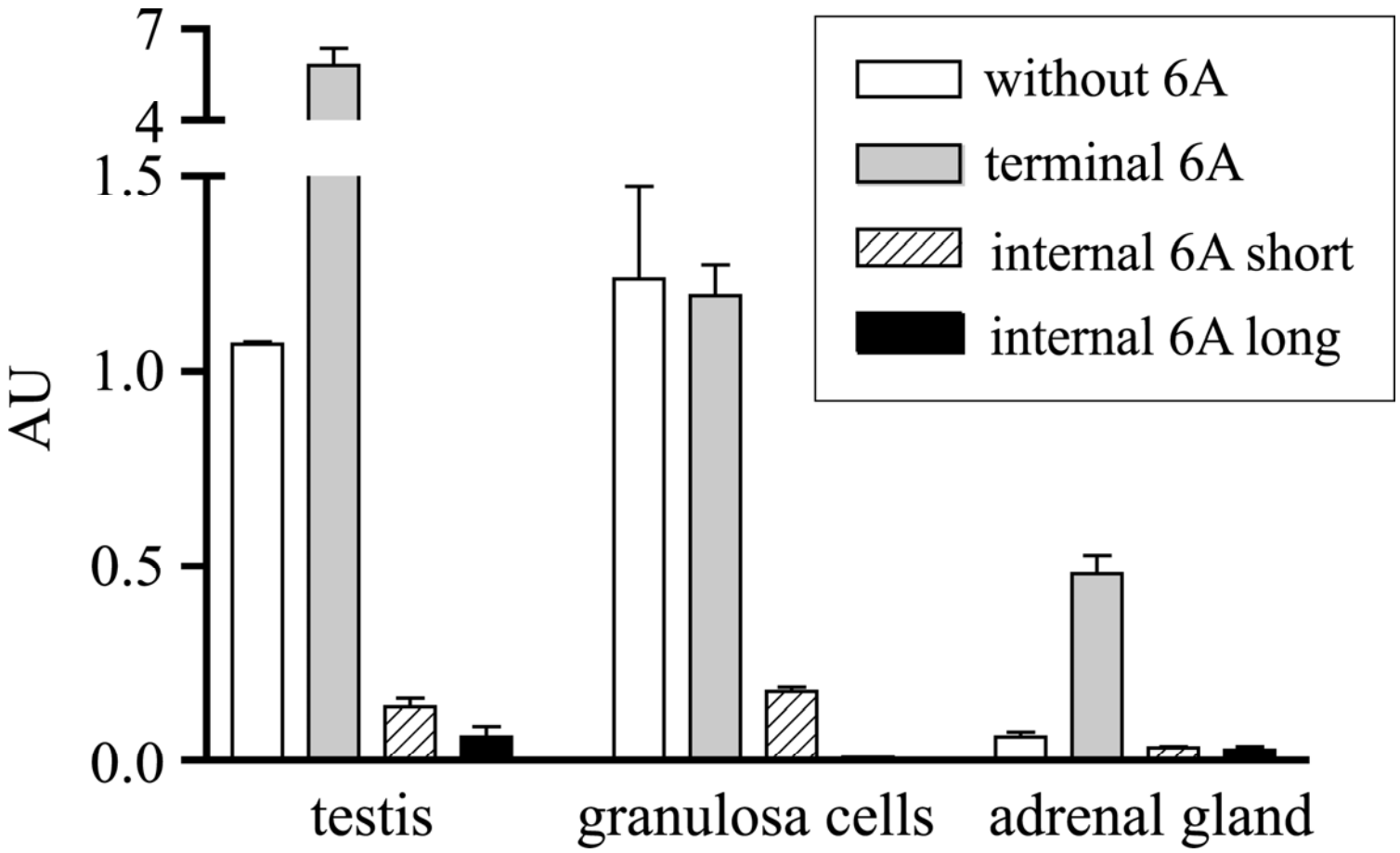
Exon 6A is a *bona fide* novel composite exon of the *LHCGR* gene

Kossack et al., PLoS Medicine, 2008



Revised genomic organization of the *LHCGR* gene

LHCGR mRNA containing exon 6A is highly expressed in the ovary



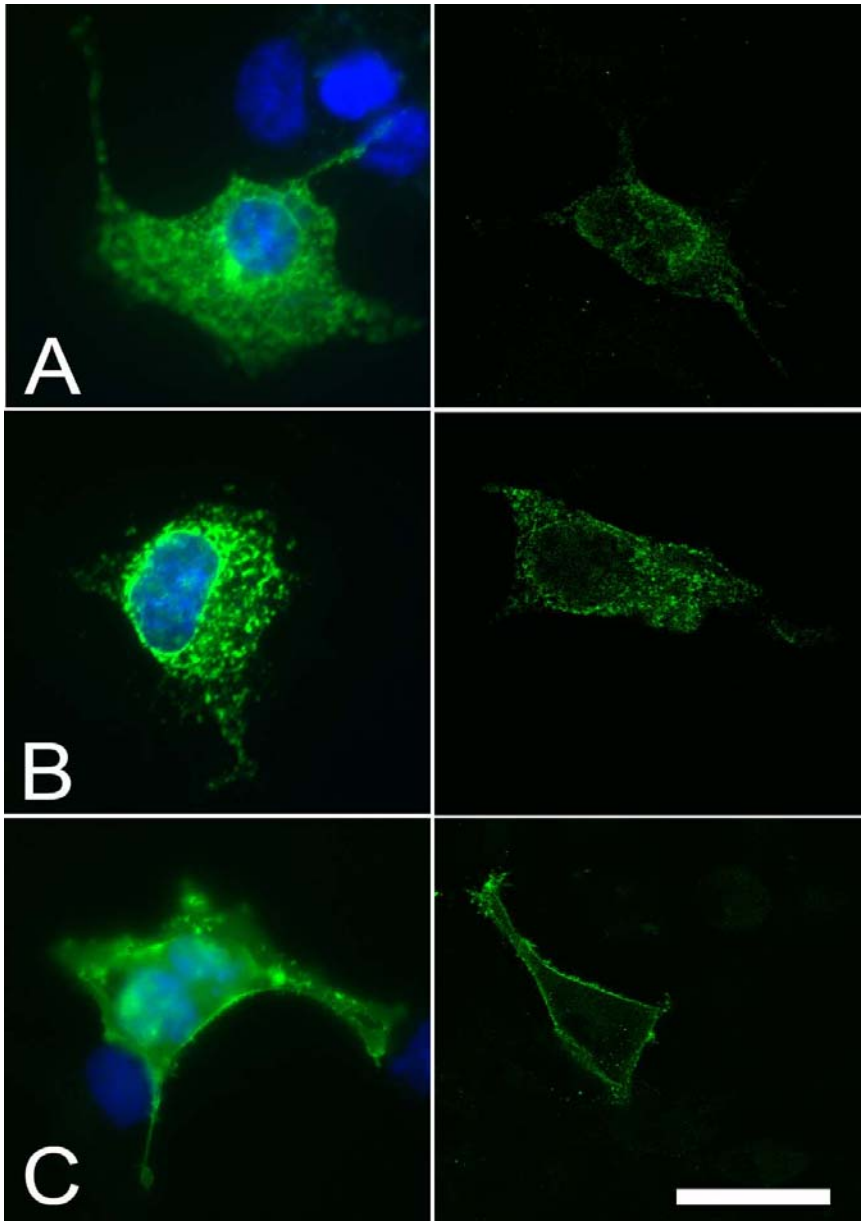
Kossack et al., PLoS Medicine, 2008

Cellular localisation of the *LHCGR* exon 6A terminal variant

wt *LHCGR* exon 6A

***LHCGR* exon 6A mut A557C**

wt *LHCGR*

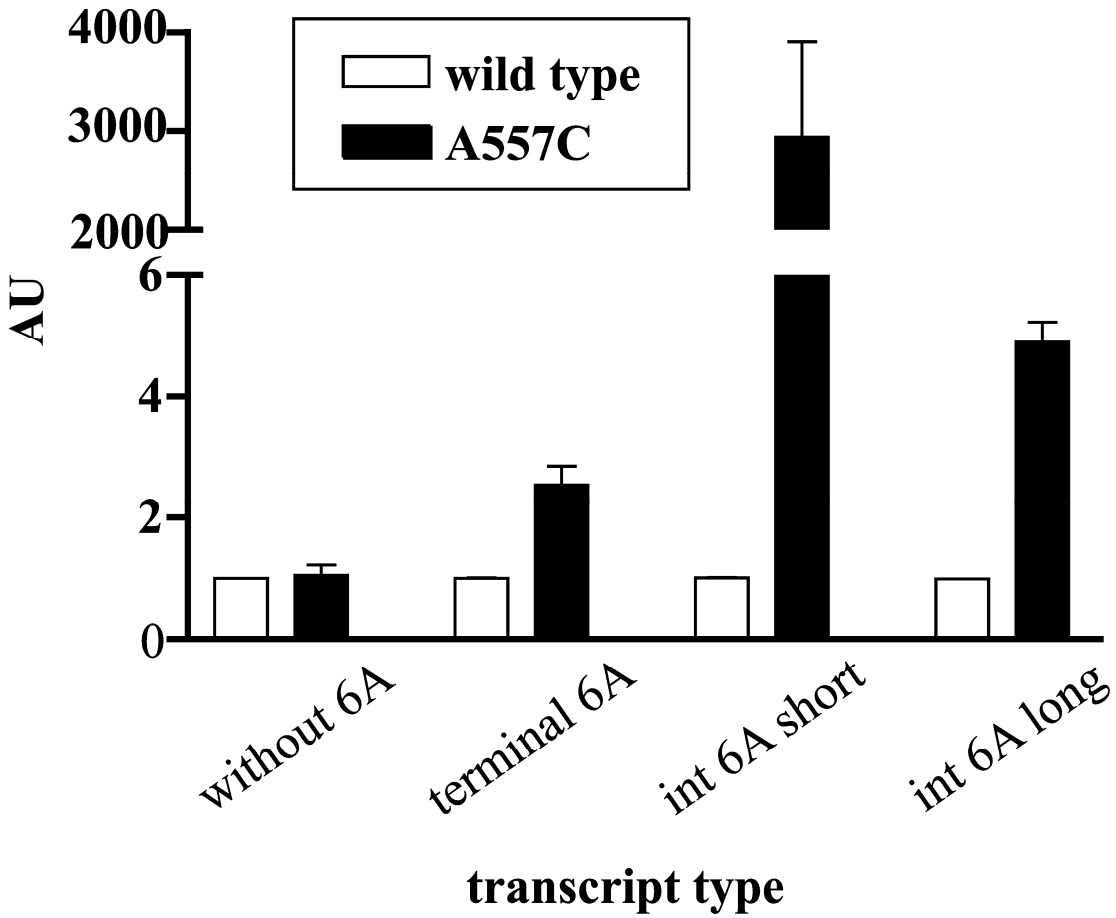


Kossack et al., PLoS Medicine, 2008

Mutations in *LHCGR* exon 6A:

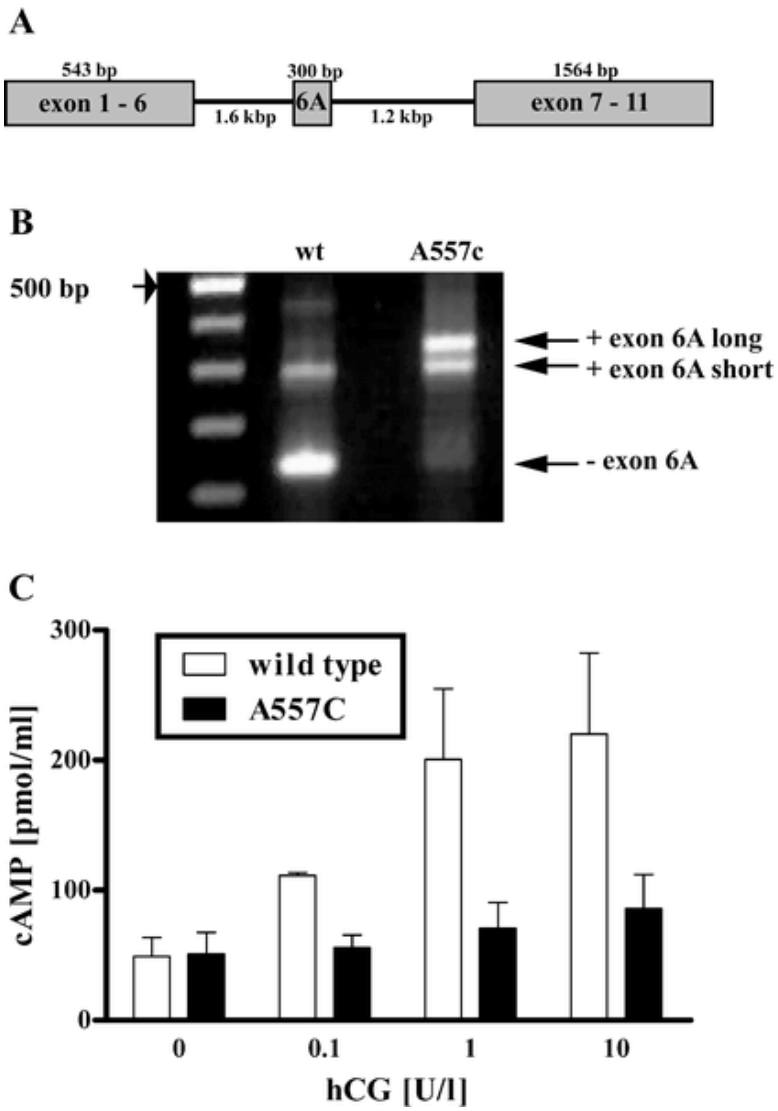
- **Are found in *LHCGR*-negative Leydig cell hypoplasia (resistance to hCG/LH)**
- **Modify the expression level of the full lenght *LHCGR***
- **Alter LHCGR response to hCG**

The A557C Mutation Increases the Expression of the Exon 6A Short Internal Variant



Kossack et al., PLoS Medicine, 2008

Mutations in exon 6A abolish *LHCGR* Response to hCG



Kossack et al., PLoS Medicine, 2008

What is the impact of mutations and SNP in exon 6A on ovarian function?

?

Conclusions

- ***FSHR*** **mutations (inactivating and activating) affect ovarian function**
- ***LHCGR*** **can (possibly) distinguish between LH and hCG**
- **Other genomic elements are involved in modulating *LHCGR* response**
- **Effects of *LHCGR* polymorphisms on ovarian function are not well known**

Thanks to the IRM Münster, DE

E. Nieschlag

Proposed model for the impact of exon 6A on *LHCGR* function

Kossack et al., PLoS Medicine, 2008