Funzione endocrina del testicolo

Testicolo

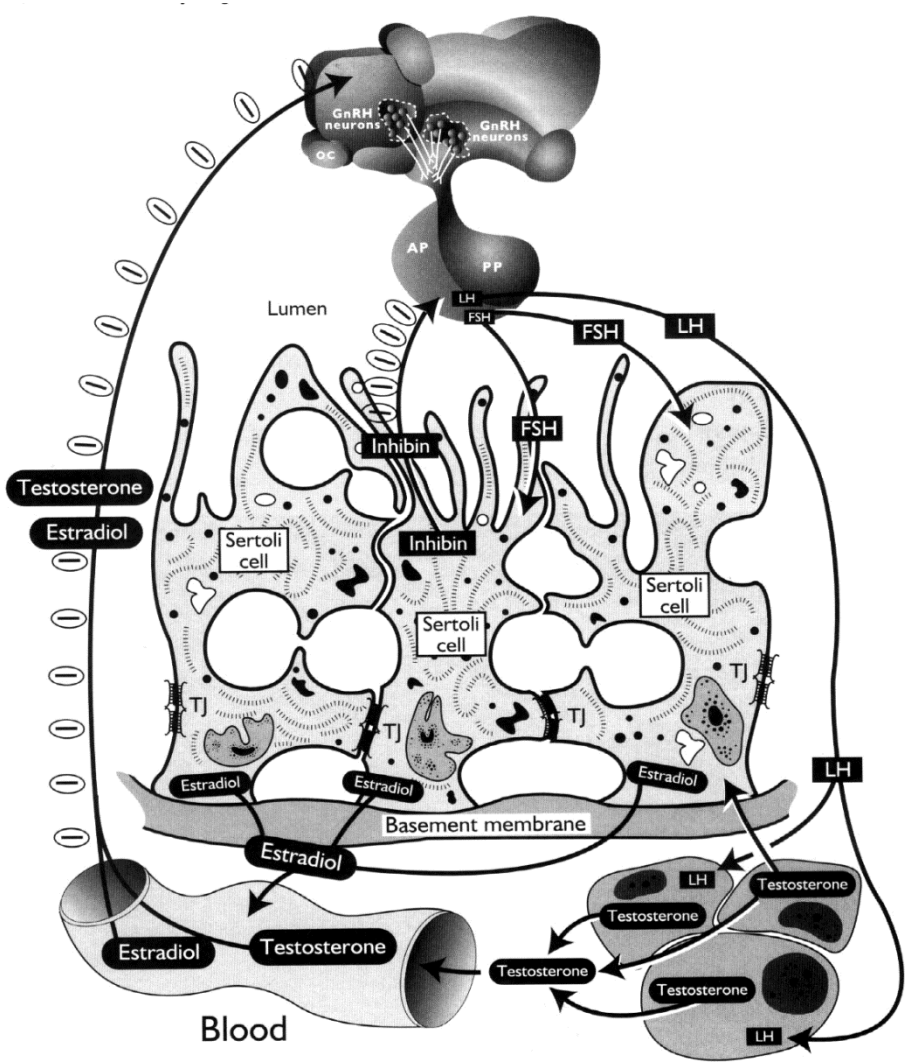
- Funzione gametogenetica
- Funzione endocrina

Controllo gonadotropine

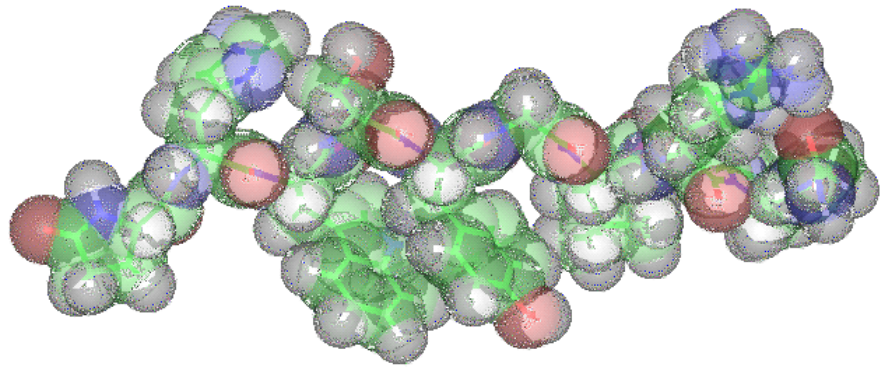
Controllo endocrino

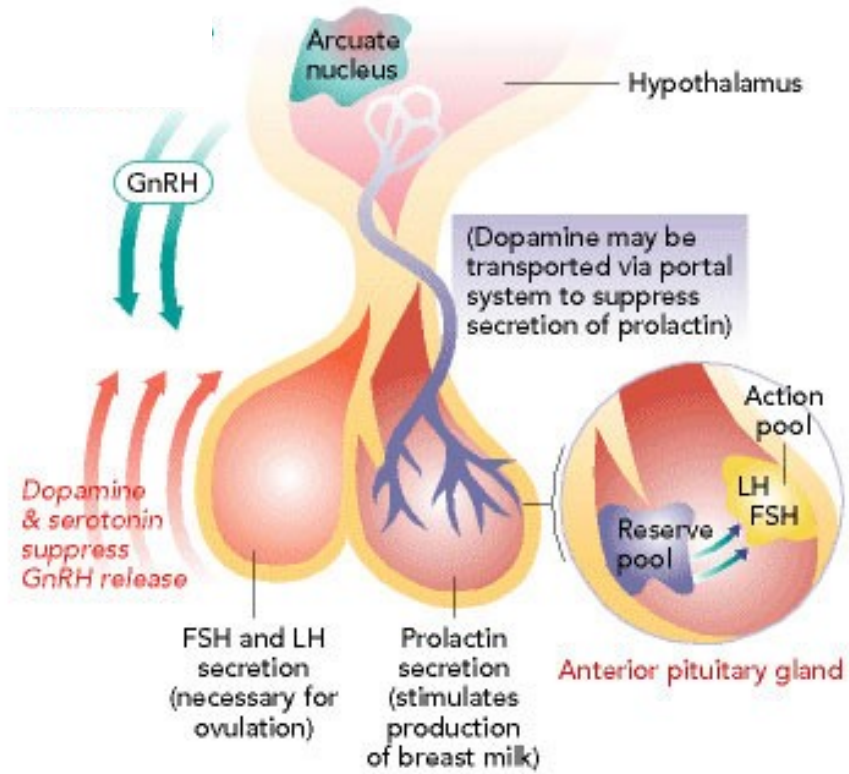
- Ablazione chirurgica ipofisi (tecnicamente difficile, effetti aspecifici).
- Anticorpi anti gonadotropine (complesso per metaboliti, differenze specie specifiche).
- Somministrazione di ormoni per terapie di rimpiazzo (idem).
- Animali konk-out

Asse ipotalamo/ipofisi/testicolo



GNRH

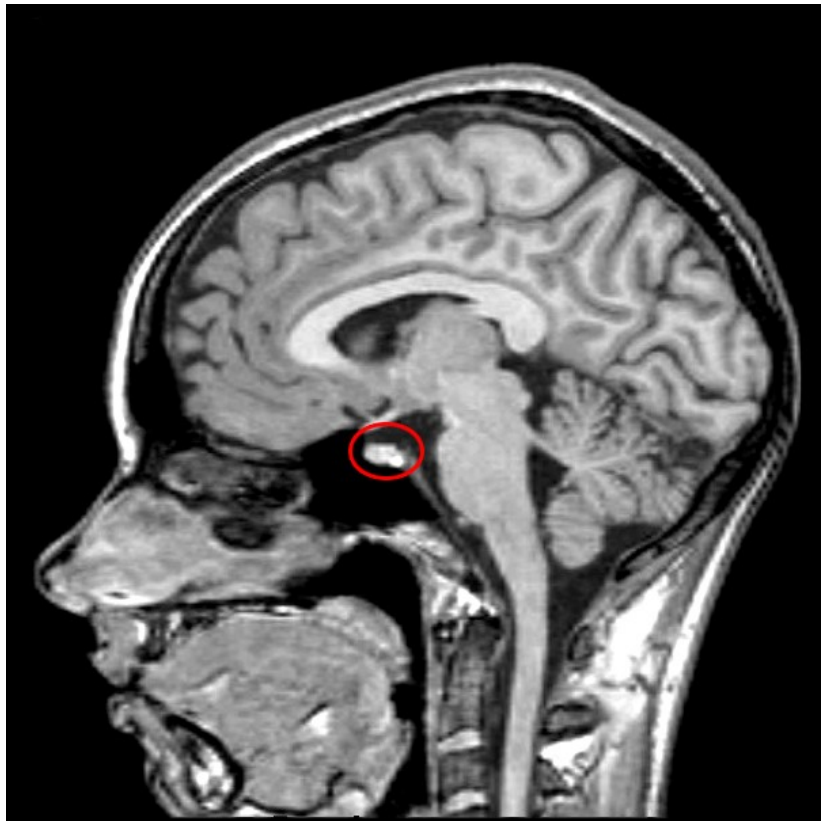



Arcuate nucleus
Hypothalamus
GnRH
(Dopamine may be transported via portal system to suppress secretion of prolactin)
Action pool
LH
FSH
Reserve pool
Dopamine & serotonin suppress GnRH release
FSH and LH secretion (necessary for ovulation)
Prolactin secretion (stimulates production of breast milk)
Anterior pituitary gland

- Decapeptide.

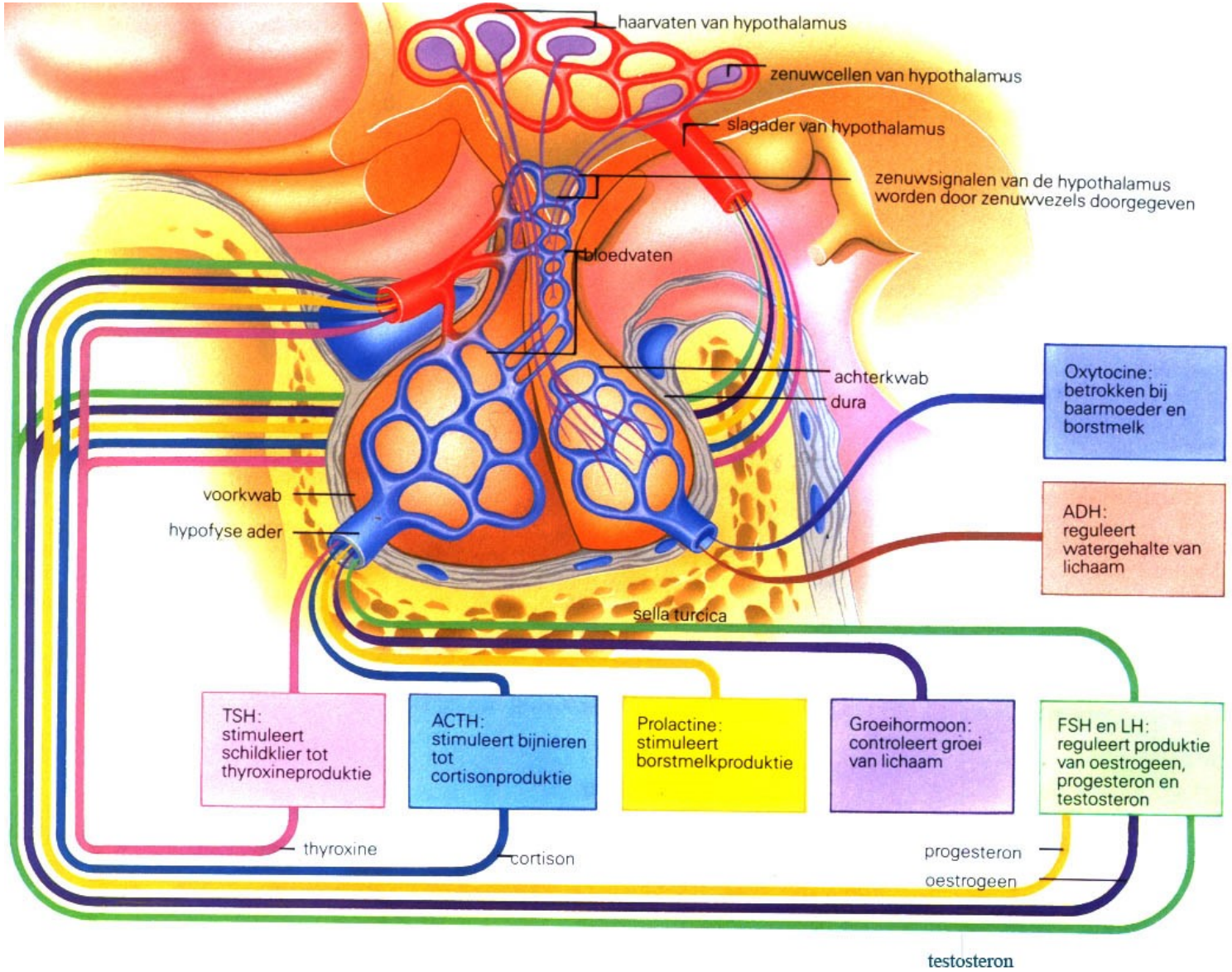
- La secrezione differenziata di LH e FSH dipenderebbe da frequenza ed ampiezza dei pulses

IPOFISI



- Localizzata nella sella turcica dello sfenoide.
- Connessa all’ipotalamo dall’infundibolo.
- Si distinguono ADENOIPIFISI e NEUROIPOFISI.

- Connessa all'ipotalamo dal circolo portale ipotalamo-ipofisario.



GONADOTROPINE

- Glicoproteine, PM circa 28.000 Da, due subunità codificate da due geni su comosomi differenti:

  α: uguale in FSH, LH, TSH.

  β: attività biologica

  entrambe devono essere presenti nell'ormone attivo.

SINTESI E SECREZIONE GONADOTROPINE

mRNA → pro-ormone

↓

Incorporazione e rimodellamento oligosaccaridi

↓

Folding delle subunità

↓

Legame con il recettore

↓

Formazione del complesso → Attività biogica

La secrezione da parte delle cellule gonadotrope è regolata da:

- GnRH
- estrogeni,
- androgeni,
- P4
- Inibina
- attivina

In particolare il GnRH lega recettori sulla membrana cellulare

↓

PI →IP3 e DAG

↓

Fuoriuscita Ca2+ dai depositi intracellulari

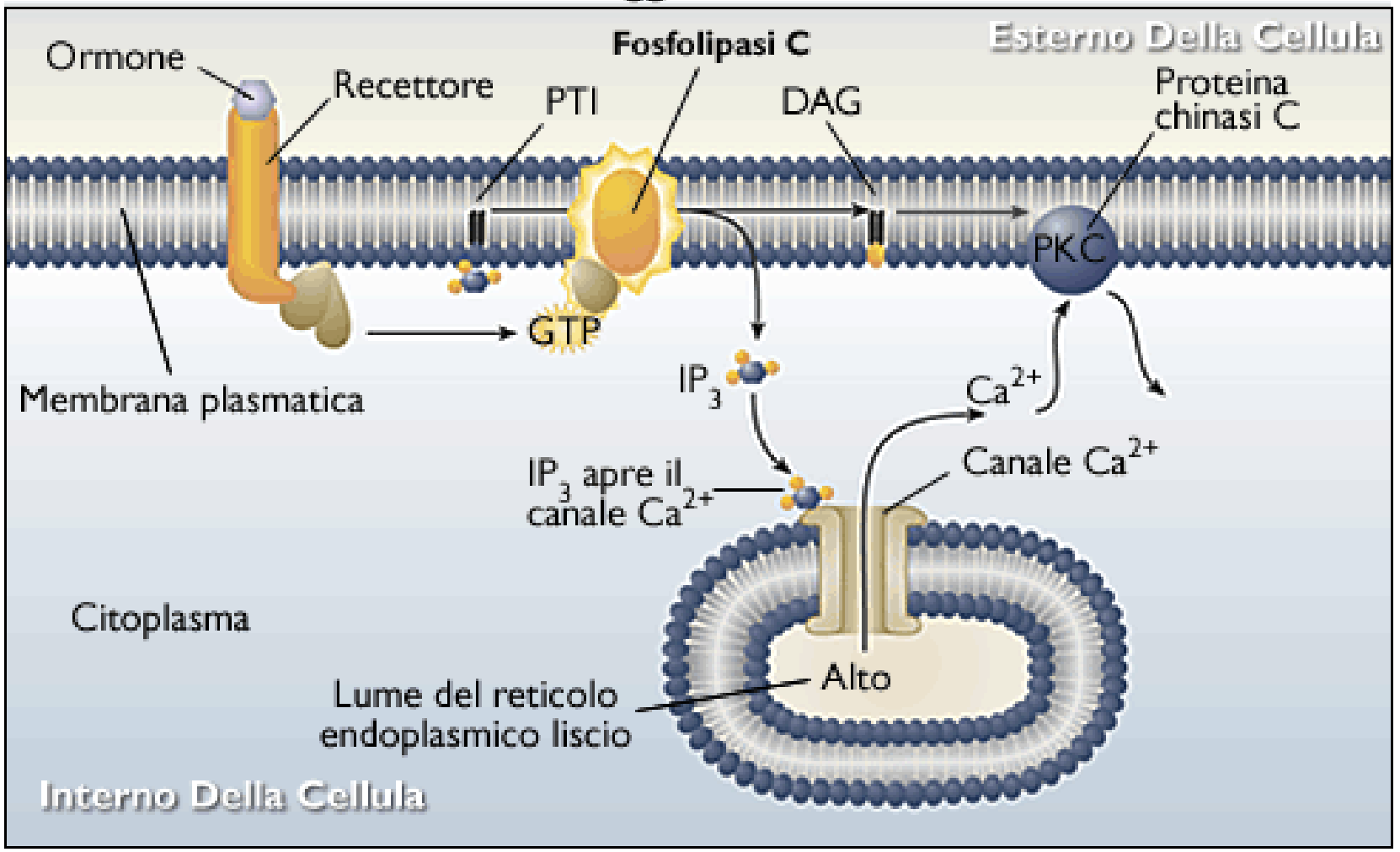
↓

Entrata Ca2+

↓

Rilascio dei granuli di omone vicino alla membrana e fuoriuscita degli stessi - aumento sintesi ormone

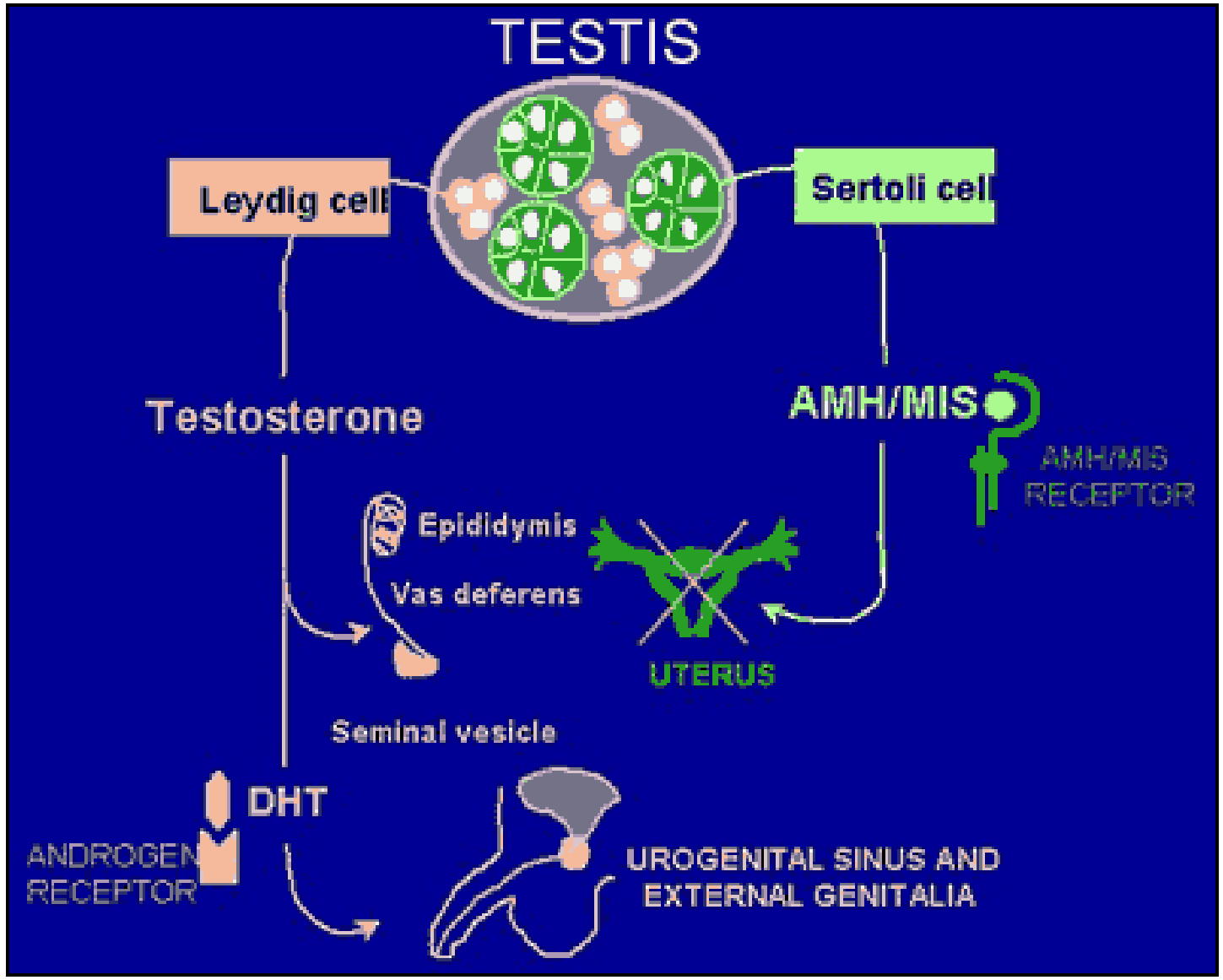
Messaggeri secondari



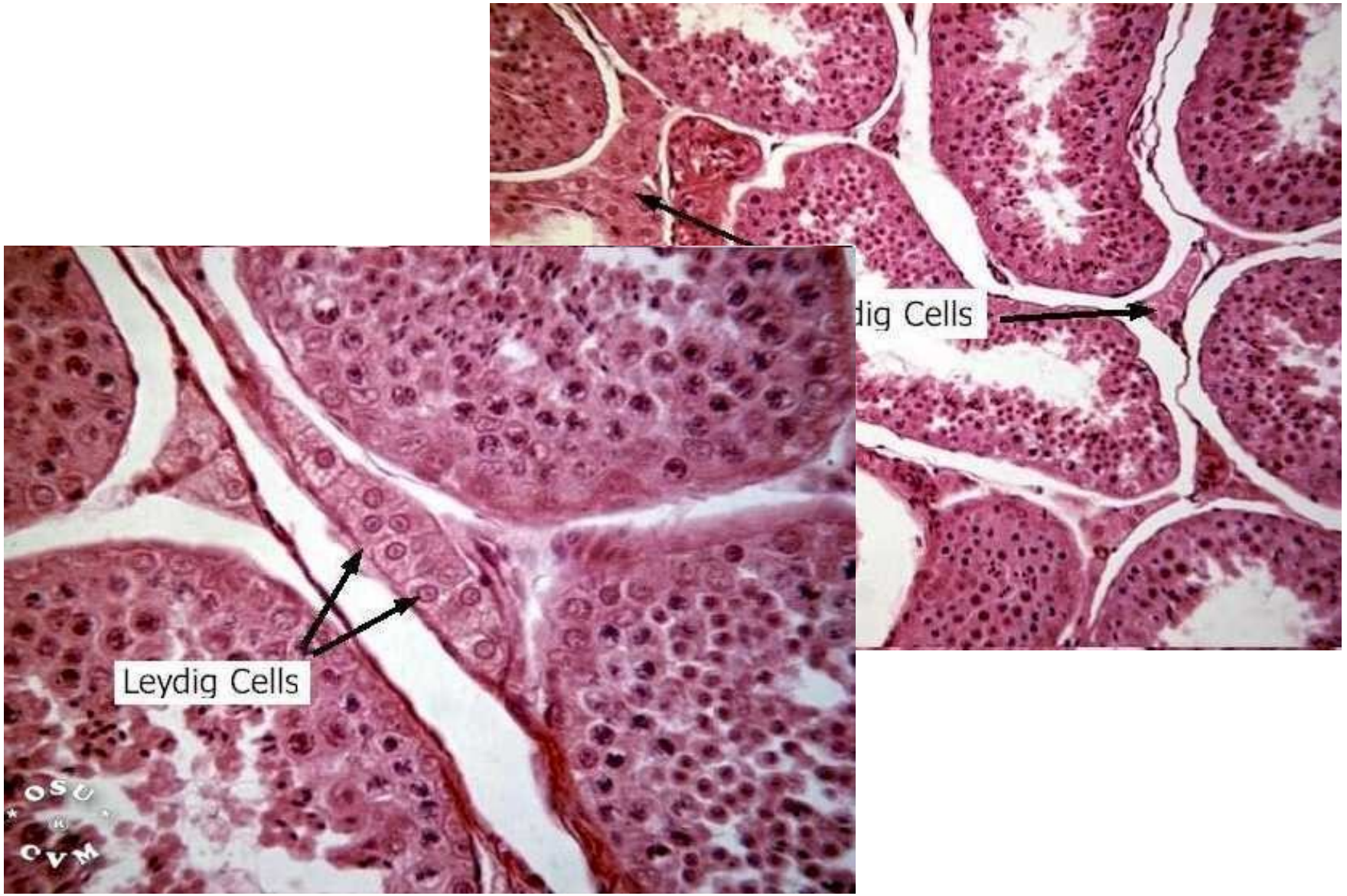
TESTICOLO

- Già durante la vita fetale il testicolo in via di differenziamento e sviluppo secerne androstenedione e testosterone.

- Lo sviluppo dei genitali e dei caratteri sessuali secondari è sotto il controllo degli ormoni testicolari.


TESTIS
Leydig cell
Sertoli cell
Testosterone
AMH/MIS
AMH/MIS RECEPTOR
Epididymis
Vas deferens
UTERUS
Seminal vesicle
DHT
ANDROGEN RECEPTOR
UROGENITAL SINUS AND EXTERNAL GENITALIA

CELLULE DEL LEYDIG



- Producono una classe di ormoni detti steroidi a 19C: testosterone (T), androstenedione e dehydroepiandrosterone
- Rispondono alla stimolazione da parte dell'**LH** che incrementa l'attività della cholesterol desmolase.
- FSH incrementa la risposta all'LH attraverso l'up-regulation dei recettori.
- Le cellule del Leydig si formano tra la 16° e la 20° settimana di gestazione

TABLE 1. Developmental maturation of the rat fetal pituitary–testicular axis

Fetal age (days)	Functions	Number of Leydig cells	LH dependence
13·5	Conversion of progesterone and dehydroepiandrosterone→testosterone	—	Independent
15·5	Testosterone synthesis LHR and LH responses	—	
16	Onset of LH synthesis	$0{\cdot}25 \times 10^5$	
18	Maximal testosterone production	$0{\cdot}60 \times 10^5$	
19	LH detectable in plasma Onset of testosterone decline	—	Dependent
21	Maximum LH in plasma	$1{\cdot}20 \times 10^5$	

TABLE 3. Developmental maturation of the human fetal pituitary–testicular axis

Fetal age (weeks)	Functions	Hormone dependence
2	hCG secretion	
7–8	Onset of LC differentiation Onset of testosterone synthesis	LH/hCG independent
10	Onset of LH synthesis LHR in testis	hCG dependent
11	LH in plasma	
12–15	Maximum hCG in plasma Maximum testosterone synthesis	
22–24	Maximum LH in plasma	LH dependent
24–38	↓ LH and hCG in plasma ↓ LC number ↓ Testosterone in plasma	

LH lega recettore
interiorizzazione complesso OR

↑ AC

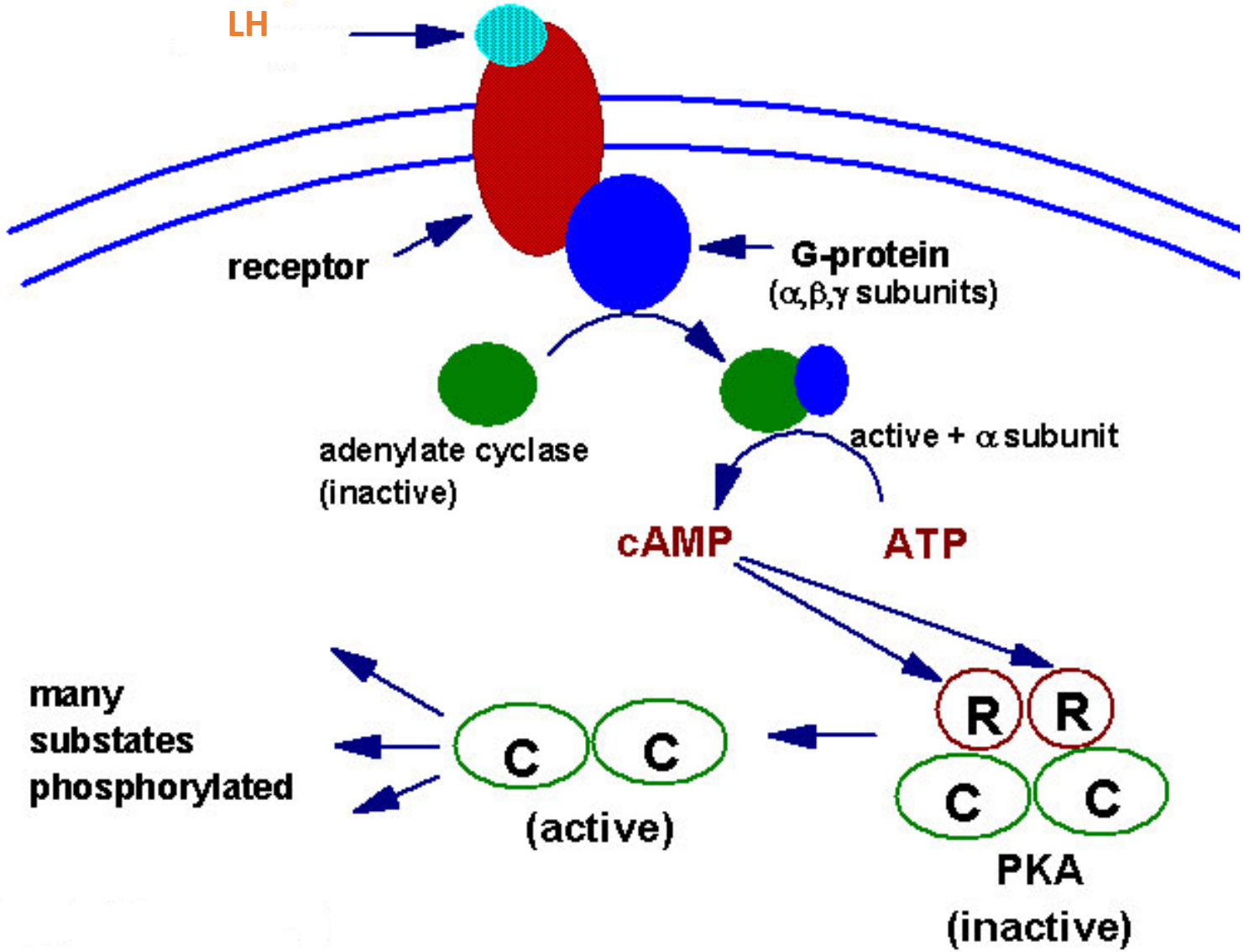
↑ PKA

↑ sintesi RNA

↑ colesterolo -> pregnenolone (mitocondri)

↑ pregnenolone -> T (REL)

Receptor-Mediated Activation of PKA



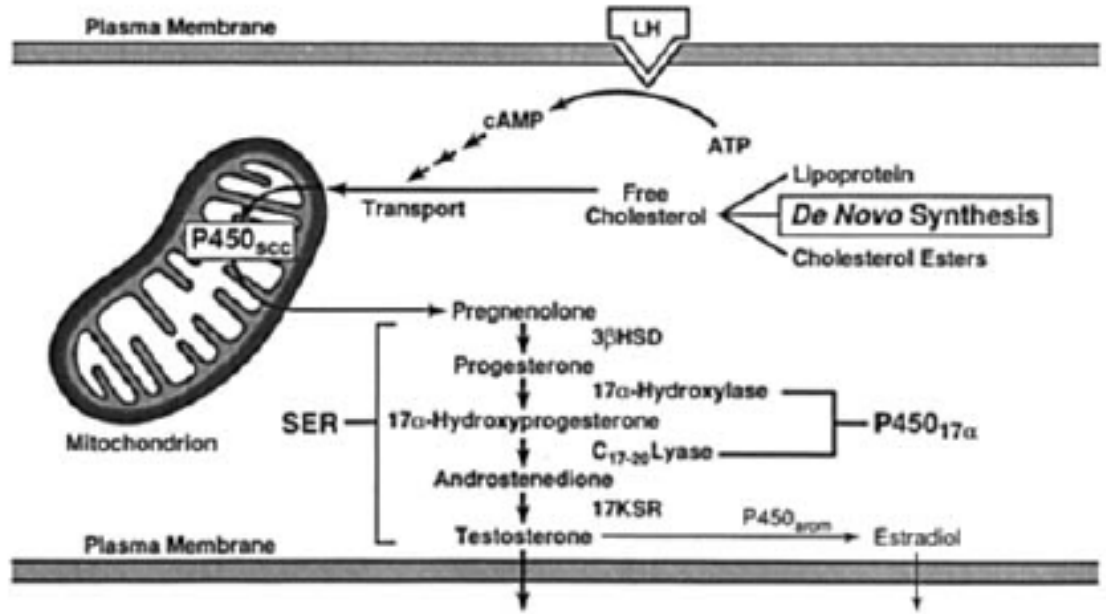


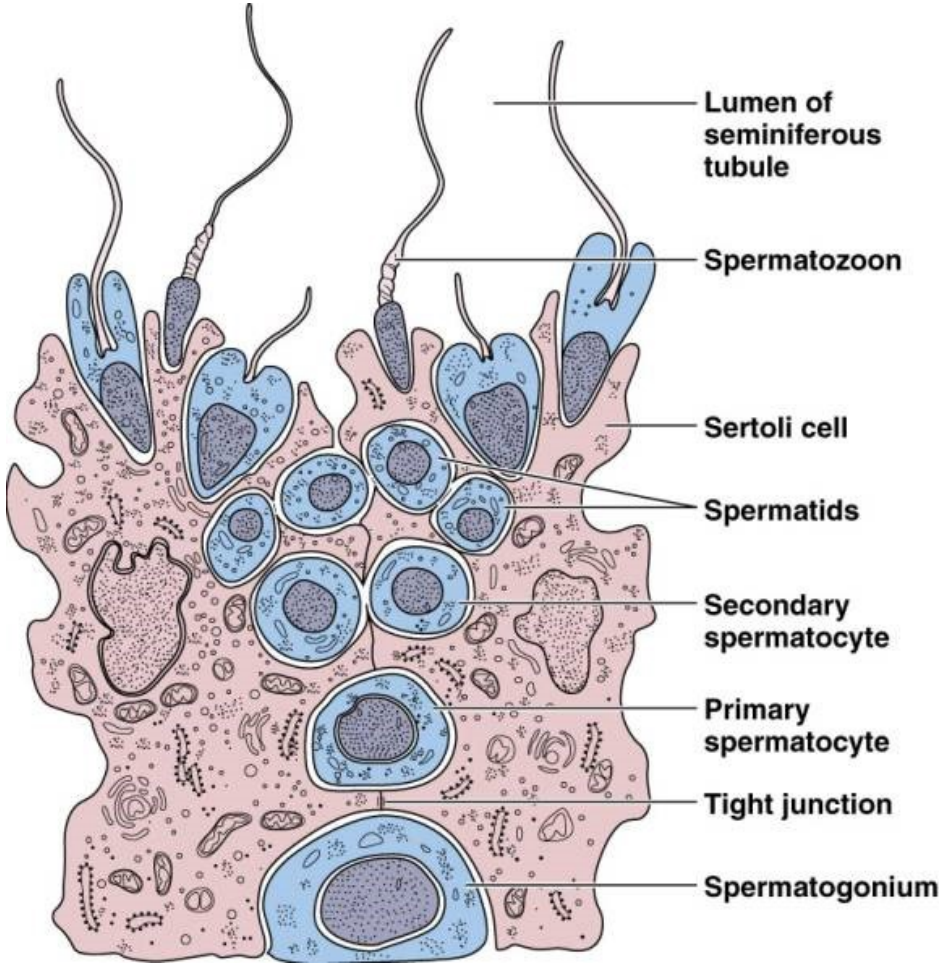
FIG. 1. LH or cAMP-stimulated testosterone production in Leydig cells. 17KSR, 17-ketosteroid reductase; SER, smooth endoplasmic reticulum.

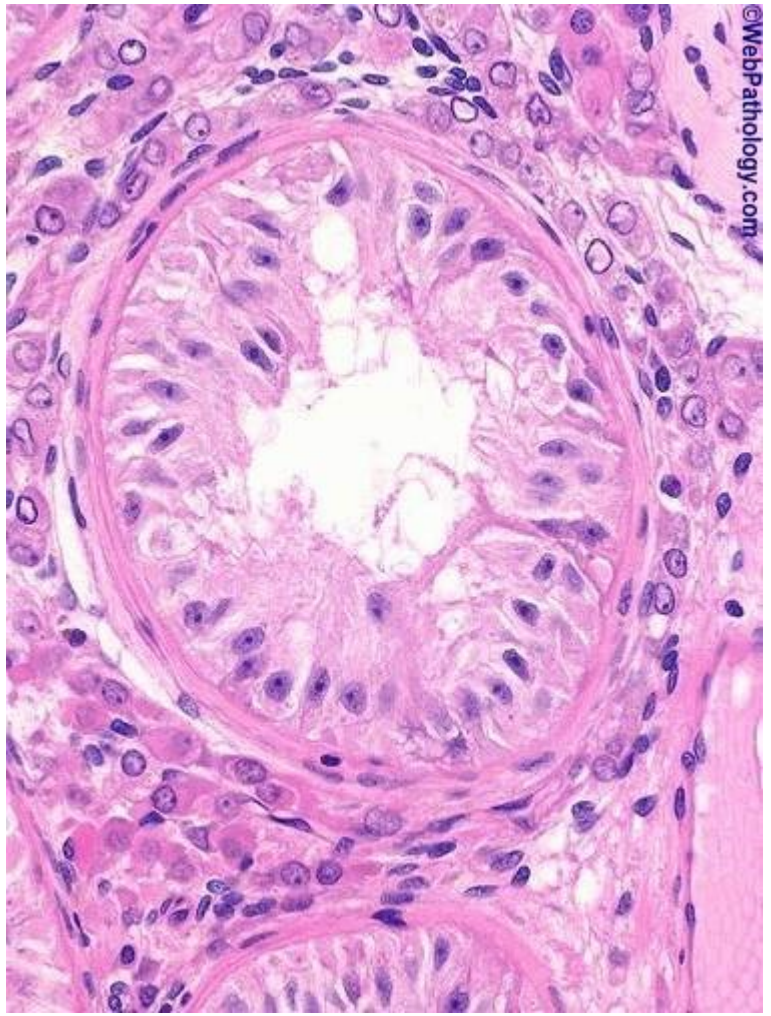
Factor	Site of production	Evidence	Regulation	Leydig cells: Receptor	Leydig cells: Effects
Steroidogenic stimulatory factor	SC	Protein	↑ FSH	ND	↑ Steroidogenesis
Steroidogenic inhibitory factor(s)	SC	Protein		ND	↓ Differentiated functions
Mitogenic factor(s)	SC	Protein	FSH ↑	–	↑ Leydig cell progenitor proliferation
IGF-I	LC, SC	mRNA, protein	FSH ↑ in SC hCG ↑ in LC	+	↑ differentiated functions
TGFβs	LC, SC, PC	mRNA, protein	FSH ↓ in SC	+	↓ Differentiated functions
EGF/TGFα	LC, SC, GC, PC	mRNA, protein	?	+	↑ Steroidogenesis ↓ Differentiated functions
FGF	LC, SC, GC, PC	mRNA, protein	FSH ↑ in SC	+	↓ Differentiated functions
PDGF	LC	Protein	↑ hCG in LC	+	↓ Differentiated functions
Inhibin/activin	LC, SC	mRNA, protein	FSH ↑ in SC hCG ↑ in LC	ND	Inhibin ↑ steroidogenesis Activin ↓ steroidogenesis
Interleukin-1	LC, SC, M	mRNA, protein	LPS ↑ in SC hCG and LPS ↑ in LC	+	↓ Differentiated functions
Interleukin-2	L	mRNA, protein	?	+	↓ Differentiated functions
Interferon (α, γ)	L	mRNA, protein	?	?	↓ Differentiated functions
TNF-α	GC	mRNA	?	ND	Stimulatory: rat Inhibitory: pig, mouse
LHRH	SC	LHRH-like protein	?	+	Acute ↑ steroidogenesis only in rat
GHRH	LC, GC	mRNA, protein	hCG ↑ in LC	ND	Stimulatory or no effect
CRF	LC	mRNA, protein	hCG ↑ in LC	+	↓ LH-stimulated steroidogenesis in rat ↑ LH-stimulated steroidogenesis in mouse
AVP	LC, SC	mRNA, protein	—	+	Acute ↑ steroidogenesis ↓ Differentiated functions
Oxytocin	LC, SC	mRNA, protein	LH ↑ LC	+	↓ Differentiated functions
ANF	Testis	mRNA, protein	?	+	Stimulatory mouse, inhibitory MA-10 cells No effect rat, human
CNF	LC	mRNA, protein	?	+	?
A-II	LC	Protein		+	↓ Inhibitory
Endothelin	SC	mRNA, protein	FSH ↓ in SC	+	↑ Steroidogenesis
NO	M	—	—	–	↓ LH-stimulated steroidogenesis
NRY	LC, SC	mRNA, protein	FSH ↑ SC, LH ↑ LC	?	?

ANF: atrial natriuretic factor; AVP: vasopressin; CNF: C-type natriuretic factor; CRF: corticotropin-releasing factor; EGF: epidermal growth factor; FGF: fibroblast growth factor; GC: germ cell; GHRH: growth hormone releasing factor; IGF-I: insulin-like growth factor I; L: lymphocyte; LC: Leydig cell; M: macrophage; ND: not determined; NO: nitric oxide; PC: peritubular cell; PDGF: platelet-derived growth factor; SC: Sertoli cell; TGFβ or α: transforming growth factor β or α; TNF-α: tumor necrosis factor α; NRY: neuropeptide Y.

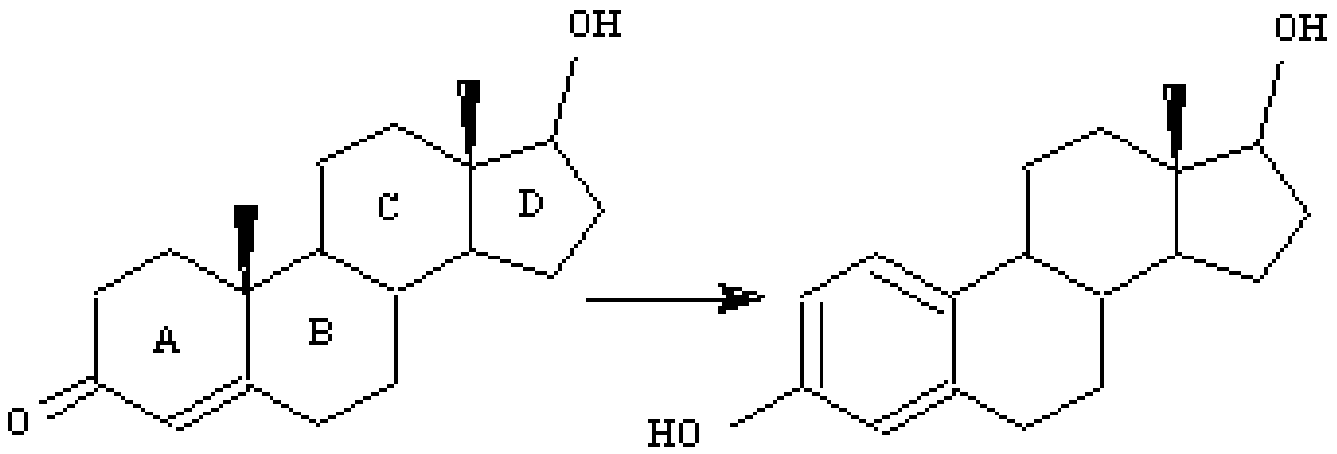
References for Table 6: References before 1994 can be found in Ackland *et al.* 1992, Hales 1996, Lin 1996, Saez 1994, Saez & Lejeune 1996. More recent references concern the effects of nitric oxide (Del Punta *et al.* 1996), C-type natriuretic factor (Middendorff *et al.* 1996), CRF (Huang *et al.* 1995) expression and regulation of neuropeptide Y (Kanzaki *et al.* 1996) and expression of EGF, TGFα and their receptors during testicular development (Caussanel *et al.* 1996).

CELLULE DEL SERTOLI





Sotto lo stimolo dell'FSH convertono il T in E2.
L'E2 regola cellule Leydig (hanno recettori).



Testosterone Estradiol

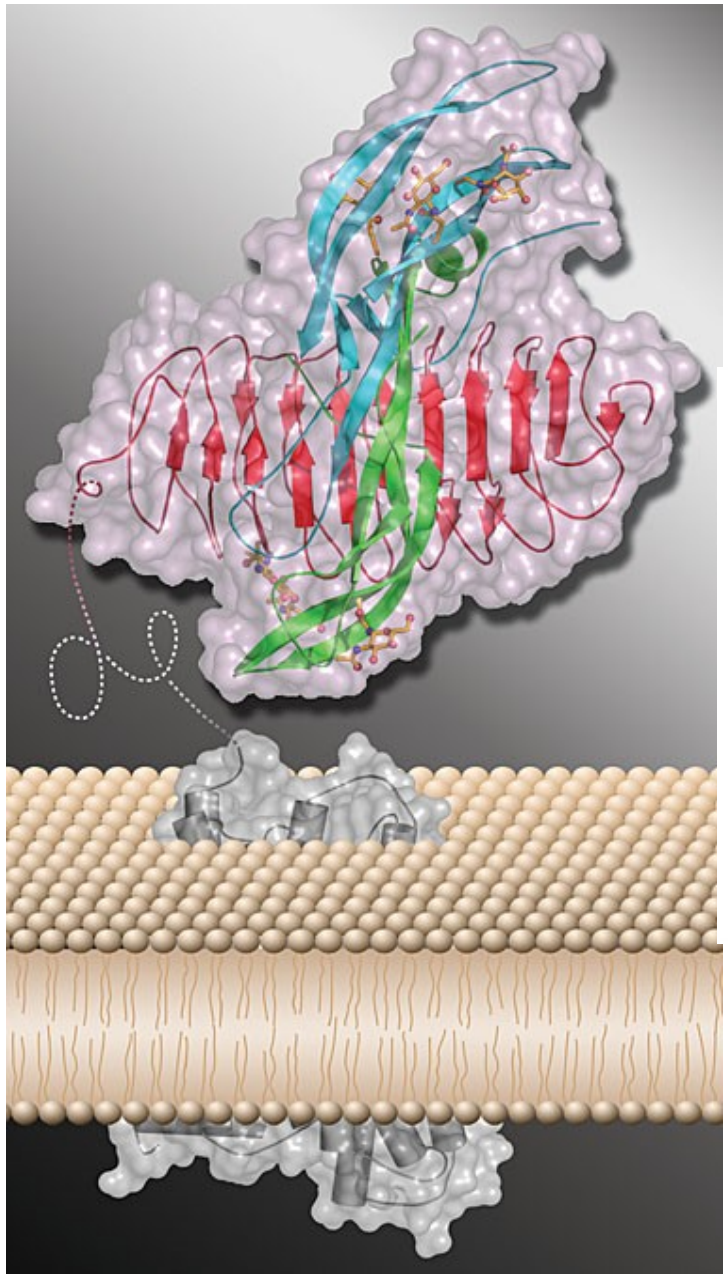
Scheme 1. Aromatase converts androgens to estrogens

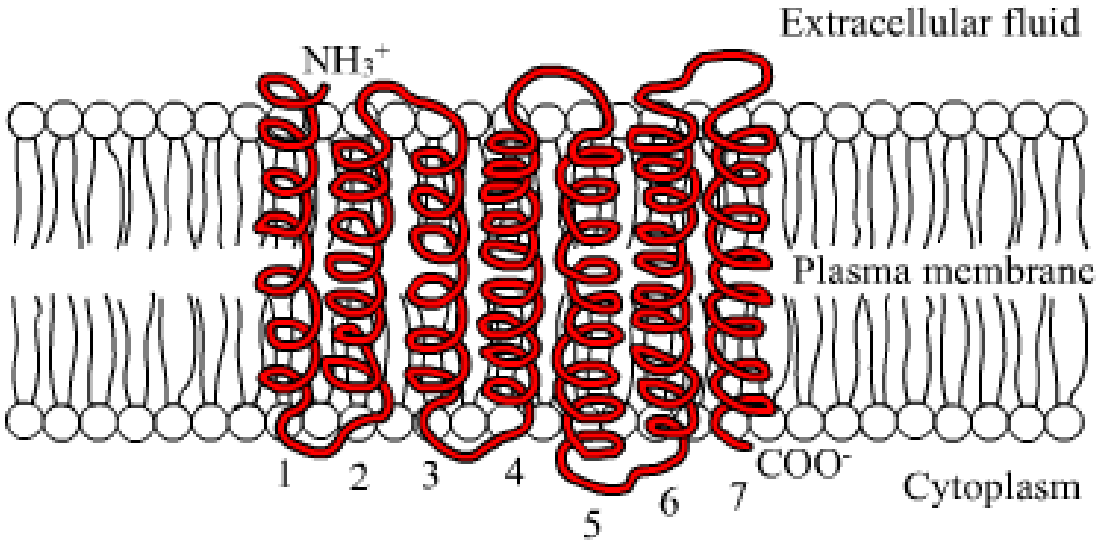
Inoltre producono

- anti-Müllerian hormone (AMH) – prodotto durante le prime fasi della vita fetale
- inibina e attivina – dopo la pubertà, concorrono alla regolazione della produzione di FSH
- Androgen binding protein (ABP)– lega gli androgeni mantenendone alta la concentrazione nel lume tubulare
- glial cell line-derived neurotrophic factor (GDNF) - stem cell self-renewal
- Ets related molecule (ERM transcription factor) – mantenimento cellule spermatogoniali
- transferrina
- IGF-I: crescita e diff. c. germinali, interazione con c. Leydig.
- IGF-II: ?
- Nutrienti, enzimi vari: omeostasi tubulare

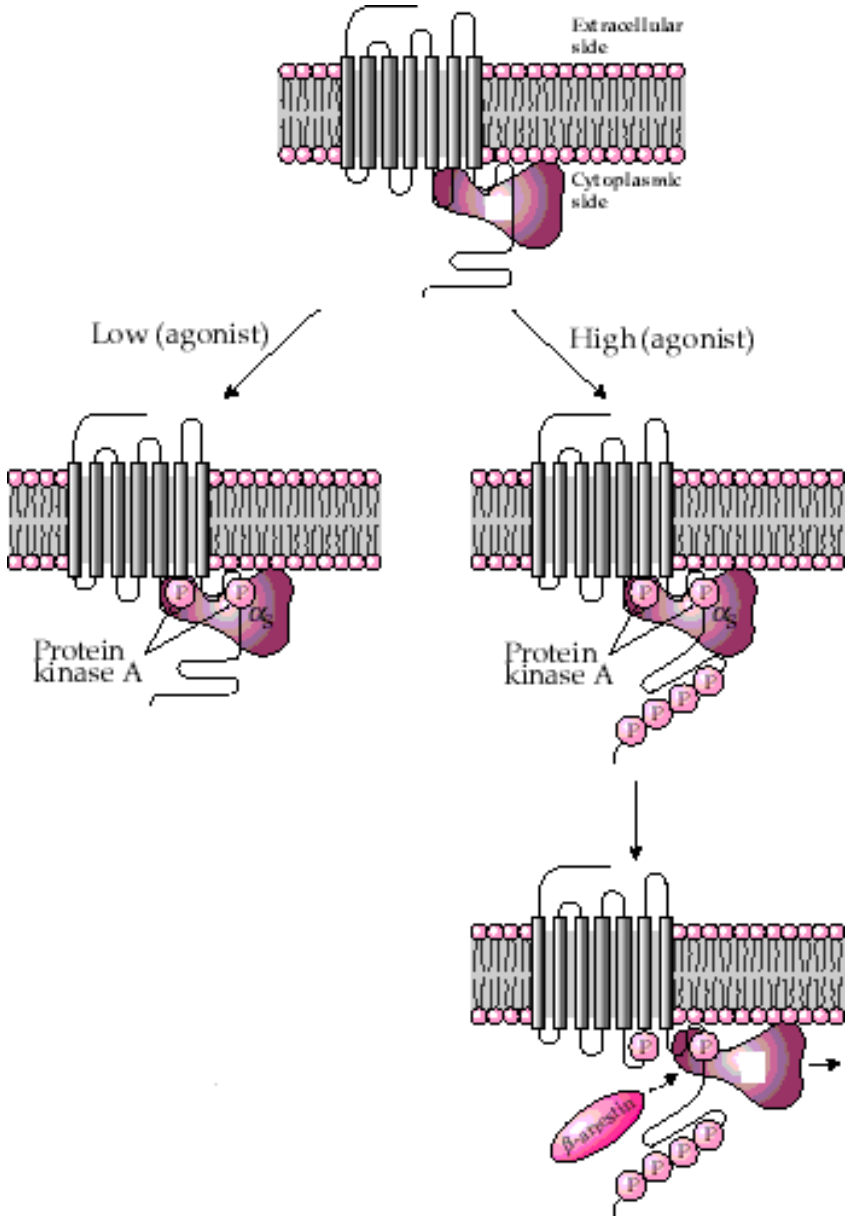
FSH Sertoli

- Lega FSH-R (75kDa, 675 aa), 7 α-eliche transmembrana.
- 10 esoni (9 per i domini extracellulari, 1 per la porzione transmembrana).
- Gs protein-coupled receptor.
- L’espresiione di FSH-R varia in maniera ciclica, coerentemente con ciclicità di spermatogenesi.




Extracellular fluid
NH_3^+
Plasma membrane
COO^-
Cytoplasm
1
2
3
4
5
6
7

Desensibilizzazione del recettore



- cAMP-PKA pathway.
- MAP pathway.
- Ca2+ pathway: via cAMP, coinvolge la liberazione di Ca2+ da depositi intracellulari.
- PLA2 pathway.

Geni regolati da FSH

- FSH-R
- Dmrt: fattore di trascrizione correlato con determinazione del sesso e sviluppo testicolare.
- Transferrina: apporta ferro agli spermatozoi
- ABP
- VEGF
- GDNF: proliferazione cellule germinali staminali.
- LDH-A: lattato fonte energetica per spermatozoi.
- Stem cell factor
- IGF-I

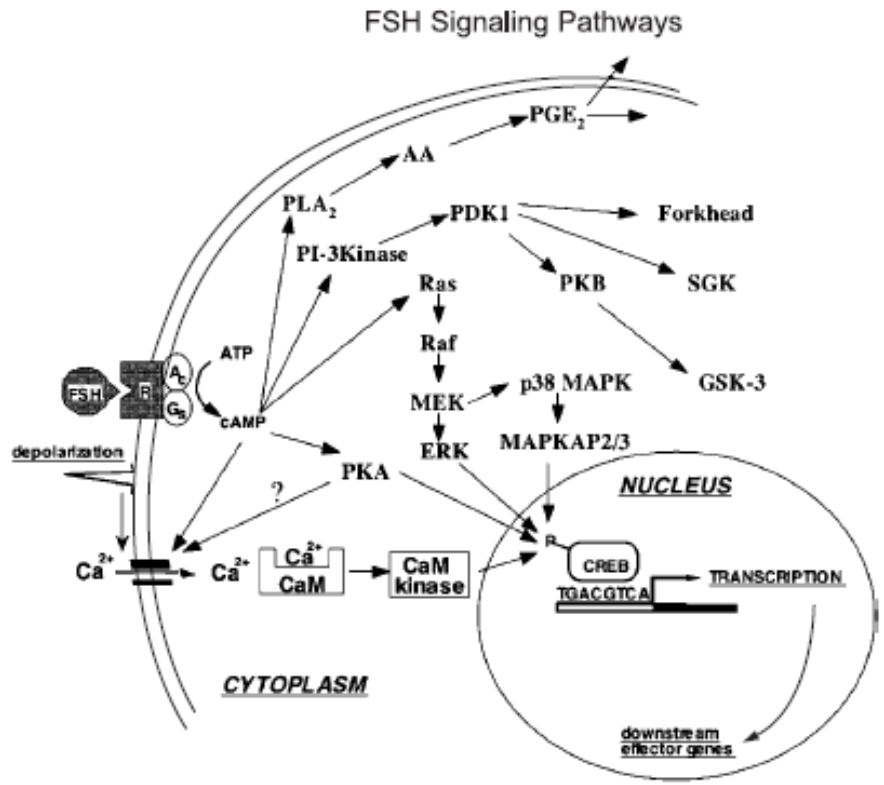


Figure 1 Signaling pathways activated by FSH are displayed. Initially FSH binding to the FSH receptor causes receptor coupled G proteins to activate adenylate cyclase (AC) and increase intracellular cAMP levels. Multiple factors can be activated by cAMP in Sertoli cells including PKA that can phosphorylate a number of proteins in the cell and also regulate the expression and activity of numerous transcription factors including CREB. FSH also causes Ca^{2+} influx into Sertoli cells that is mediated by cAMP and perhaps PKA modification of surface Ca^{2+} channels. Depolarization of the cell is also involved in Ca^{2+} influx. Elevated Ca^{2+} levels can activate calmodulin and CaM kinases that have multiple potential downstream effects including the phosphorylation of CREB. During puberty, FSH activates the MAP kinase cascade and ERK kinase in Sertoli cells most likely via cAMP interactions with guanine nucleotide exchange factors (GEFs) and activation of Ras-like G proteins. ERK is capable of activating transcription factors including SRF, c-jun and CREB. In granulosa cells, FSH also activates the p38 MAP kinase. FSH and cAMP also likely act through GEFs to activate PI3-K and then phosphoinositide dependant protein kinase (PDK1) and PKB in Sertoli cells. Studies of granulosa cells identified Forkhead transcription factor (Forkhead), SGK (glucocorticoid-induced kinase) and GSK-3 (glycogen synthase kinase-3) as additional downstream targets of the PI3-K pathway. FSH also mediates the induction of PLA_2 and the subsequent release of arachadonic acid (AA) and the activation of eicosanoids such as PGE_2 that may act as intracellular or extracellular signaling agents.

Testosterone in Sertoli

- DHT → sviluppo tratto genitale maschile.
- T → concorre alla regolazione della spermatogenesi.

- Le concentrazioni intratesticolari sono circa 50 – 100 vv quelle sieriche. Si lega al recettore per gli androgeni (AR).
- AR espresso in cellule Leydig e Sertoli. No da cellule germinali.
- Signaling classico degli ormoni steroidei (30-45 min fino a qualche ora).

Table 1 Androgen-regulated genes. Three classes of androgen-responsive genes are listed: Androgen-regulated genes in which there is no proof of AR-DNA interactions, Androgen-regulated genes with known AR-DNA interactions and Androgen-regulated genes with known AR-DNA interactions that are expressed in Sertoli cells. The transcriptional response to androgen, activation (A) or repression (R) is noted.

Gene	Antrogen response	Reference
Antrogen-regulated genes		
HMAK	A	Xia *et al.* 2002
SPAK	A	Qi *et al.* 2001
FSH-β	A	Spady *et al.* 2004
Fibroblast growth factor 2[1]	A	Rosini *et al.* 2002
CDK2, CDK4	A	Lu *et al.* 1997
p16	R	Lu *et al.* 1997
p27	A	Chen *et al.* 1996
AlbZIP	A	Qi *et al.* 2002
NKX3.1	A	He *et al.* 1997
PART-1	A	Lin *et al.* 2000
Prostase	A	Nelson *et al.* 1999
Prostein	A	Xu *et al.* 2001
Fatty acid synthase	A	Swinnen *et al.* 1997
c-myc	A	Lim *et al.* 1994
Antrogen-regulated genes with known AR-DNA interactions		
Prostate specific antigen (PSA)	A	Luke & Coffey 1994, Sun *et al.* 1997
Kallikrein 2 (KLK2)	A	Sun *et al.* 1997, Mitchell *et al.* 2000
Probasin	A	Rennie *et al.* 1993, Claessens *et al.* 2001, Zhang *et al.* 2004
Tyrosine aminotransferase	A	Denison *et al.* 1989
p21	A	Lu *et al.* 1999
Neutral endopeptidase 24.11 (NEP)	A	Shen *et al.* 2000
Sex-limited protein (Slp)	A	Verrijdt *et al.* 2000
Ventral prostate C3[2]	A	Tan *et al.* 1992, Claessens *et al.* 1993
Androgen receptor[3]	A	Grad *et al.* 1999
Glycoprotein hormone α sununit[4]	R	Jorgensen & Nilson 2001
Androgen-regulated genes with known AR-DNA interactions on Sertoli cells		
Pem	A	Lindsey & Wilkinson 1996

[1] FGF2 is activated by AR but no binding data is available.
[2] AR binds to the first intron of C3.
[3] AR binds to AREs in exons of the AR gene.
[4] AR down-regulates not by binding DNA but by interacting with c-jun and AP2 to inhibit their binding to DNA.

- la tecnica del microarry ha consentito di individuare almeno 234 geni regolati dal T. Curiosamente molti vengono down-regolati mentre solo pochissimi 1:2 up-regolati.

- *"testosterone paradox"*: interazioni classiche del complesso ormone recettore che attivano trascrizione solo in pochissimi casi. Per il resto vie alternative:

  - necessità di P4

  - il T aumenta la [Ca2+]i attraverso l'influsso di Ca2+ dall'esterno.

  - attivazione delle vie di MAP e CREB.

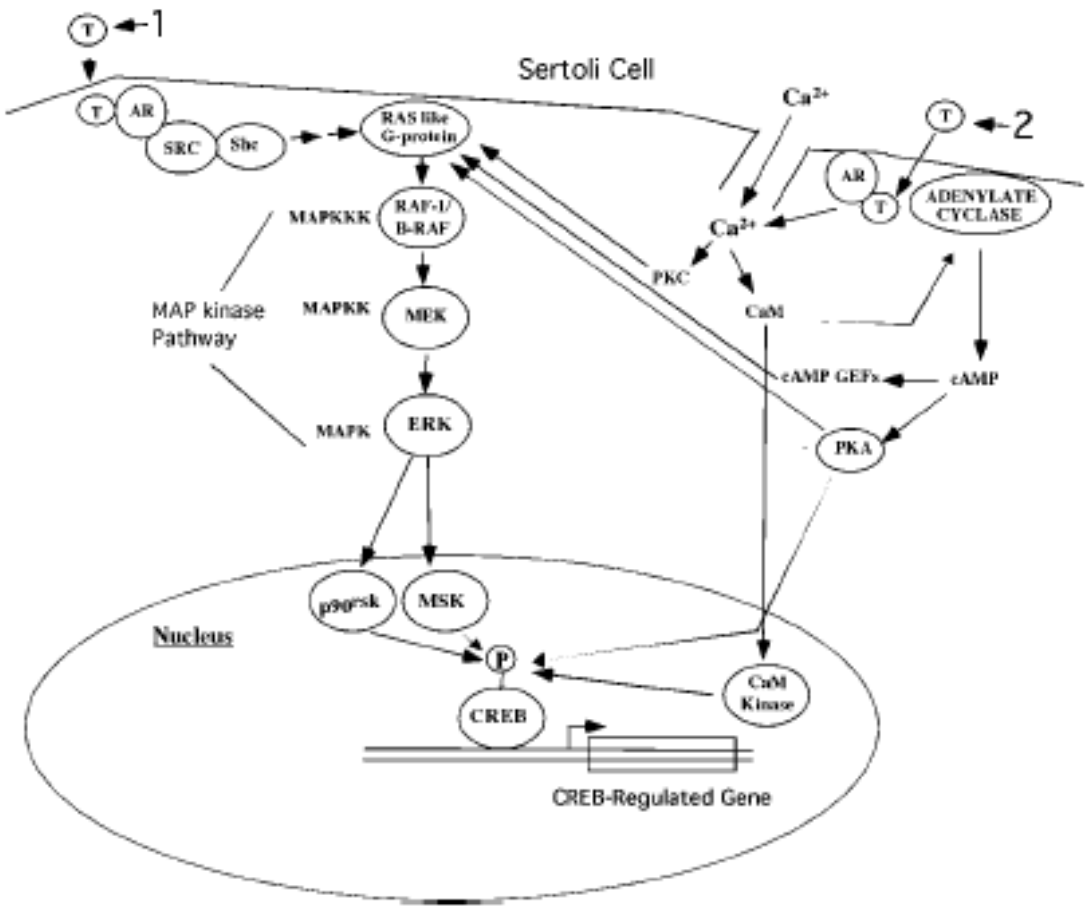


Figure 2 Potential testosterone signaling pathways in Sertoli cells: Two potential pathways are proposed for testosterone-induced CREB phosphorylation. In one pathway (left side, 1), testosterone (T) binding to AR allows AR to bind with and activate Src tyrosine kinase (SRC) resulting in the stimulation Ras and Raf-1 kinase and the activation of the MAP kinase pathway. In the second pathway (right side, 2), testosterone induces Ca^{2+} influx into Sertoli cells that then may cause calmodulin (CaM) to stimulate CaM kinase to translocate to the nucleus and transiently phosphorylate CREB within 1 minute. Ca^{2+} may also stimulate a slower, more persistent pathway in which protein kinase C (PKC), guanine nucleotide exchange factors (GEFs) or PKA stimulate Ras or a Ras like GTP binding protein resulting in the activation of the MAP kinase pathway. Both pathways are capable of inducing CREB phosphorylation and CREB-mediated gene expression.

- L’epitelio geminale necessita di alte concentrazioni locali di T (associazione con cellule del Leydig).
- Il T regola la permeabilità capillare influenzando la secrezione delle cellule del Leydig.
- Le cellule del Sertoli producono la gonadocrinina (peptide GnRH-like) che agisce localmente regolando la secrezione di T aumentando la permeabilità al Ca2+ delle cellule del Leydig.

Estrogeni testicolari

- Prodotti da aromatasi (citocromo p-450 aromatasi; P450arom).
- 55-kDa, 503 aa
- Dal gene CYP 19, appartiene alla famiglia dei cytochrome *P*-450 cui appartengono >481 membri codificati da 74 geni
- Nell'uomo CYP 19 è localizzato nella regione q21.1 nel cromosoma 15. Il gene misura oltre 75 kb e si compone di 18 esoni, 9 dei quali trascritti

- Topi knok-uot per l’aromatasi (ArKO) sviluppano normalmente e sono capaci di accoppiarsi e generare prole. A partire dai 5 mesi iniziano a manifestare decremento dell’attività spermatogenetica, fino ad 1 anno. Poi spermatogenesi anormale.

- Sia cellle del Leydig che del Sertoli producono estrogeni. Queste ultime sotto il controllo delle cellule germinali.

- Sono stati individuati due differenti recettori per gli estrogeni ER-α e ER-β.

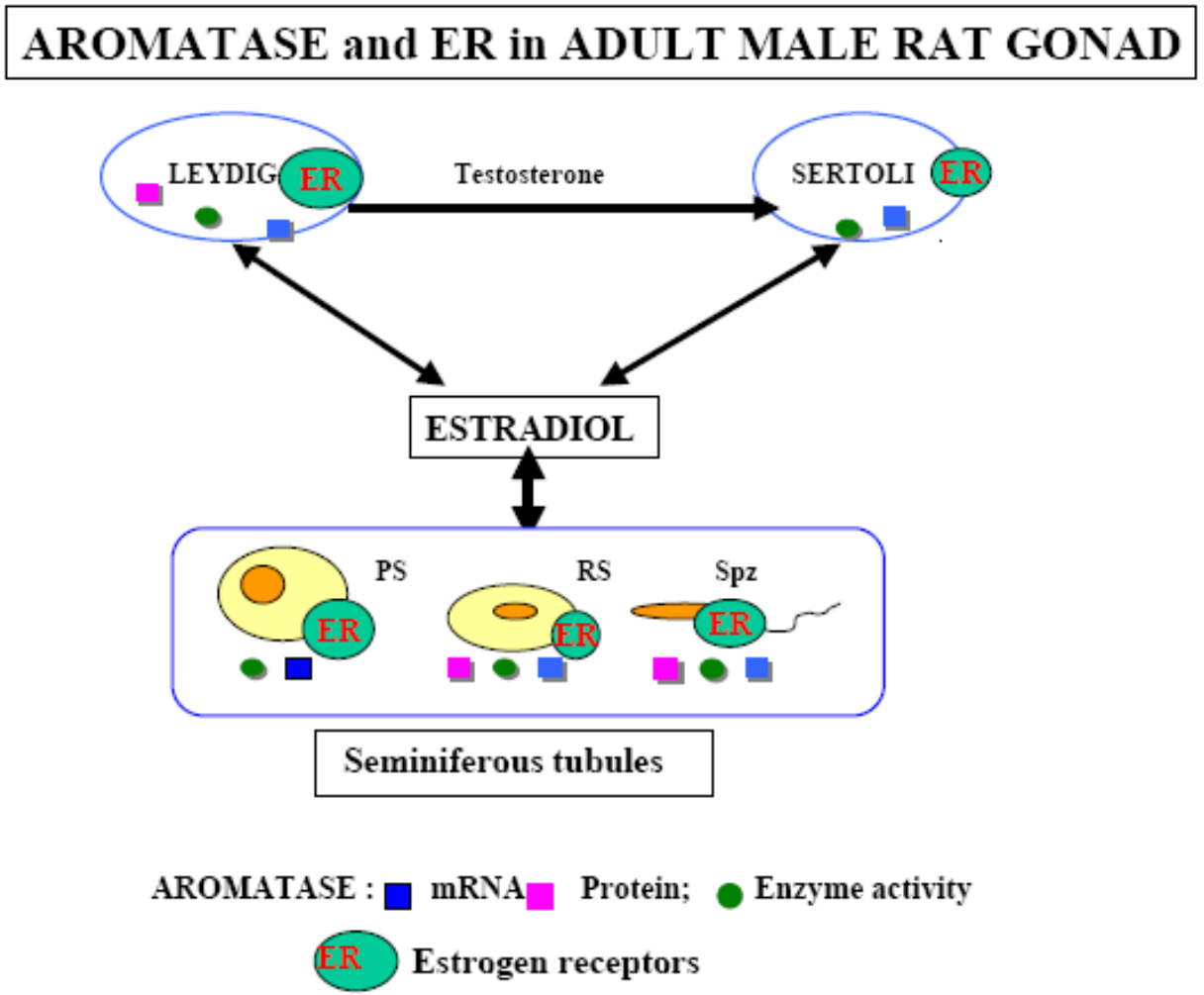


Figure 1

Aromatase and estrogen receptors (ER) in adult male rat gonad. PS: pachytene spermatocytes, RS: round spermatids, Spz: spermatozoa. Aromatase has been demonstrated in terms of mRNA (RT-PCR), protein (Western blots) and enzyme activity (measurements of estradiol output in culture media) in the various testicular cells. ER : estrogen receptors localisation.

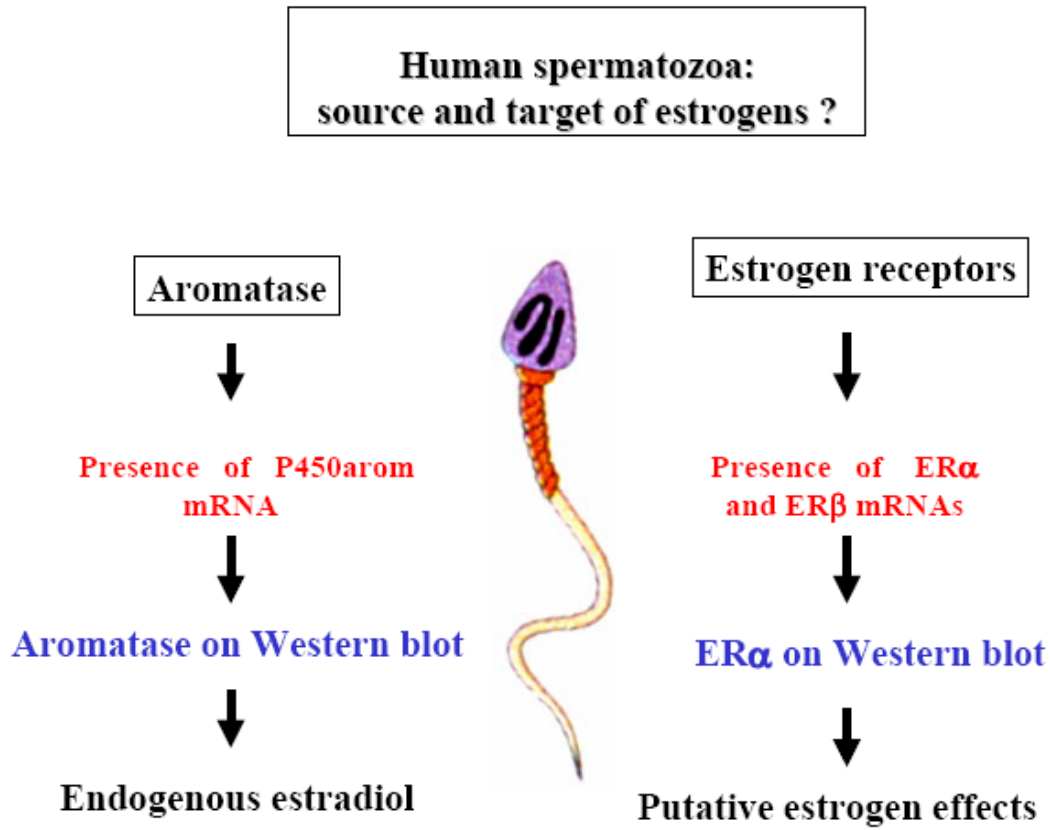


Figure 2
Human spermatozoa : source and targets of estrogens. It has been shown that human ejaculated spermatozoa contain aromatase as revealed by the mRNA, protein on Western blots and the presence of endogenous estradiol. As far as estrogen receptors are concerned mainly ER alpha are present.

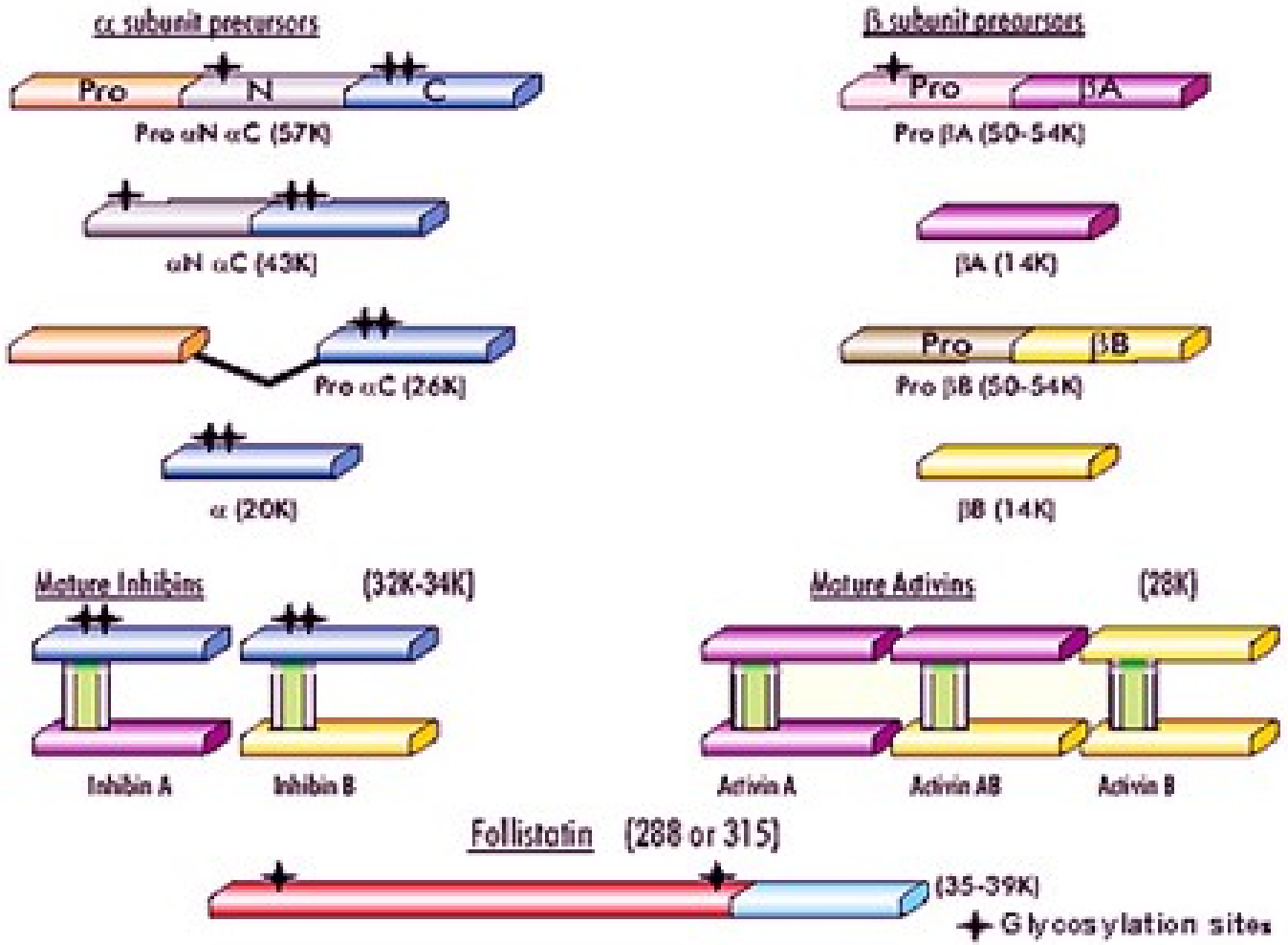
Inibina e Attivina

- L’ inibina è una glicoproteina eterodimerica
- Subunità α e β legate da un ponte disolfuro
- α è costante, β può essere A o B (inibina A o B)
- Possibili anche altre forme e tipi di aggregazione (α momomerica o dimerica, …)

- L’Attivina è omo o eterodimero (A, B, AB)

- Entrambi membri della famiglia TGFβ

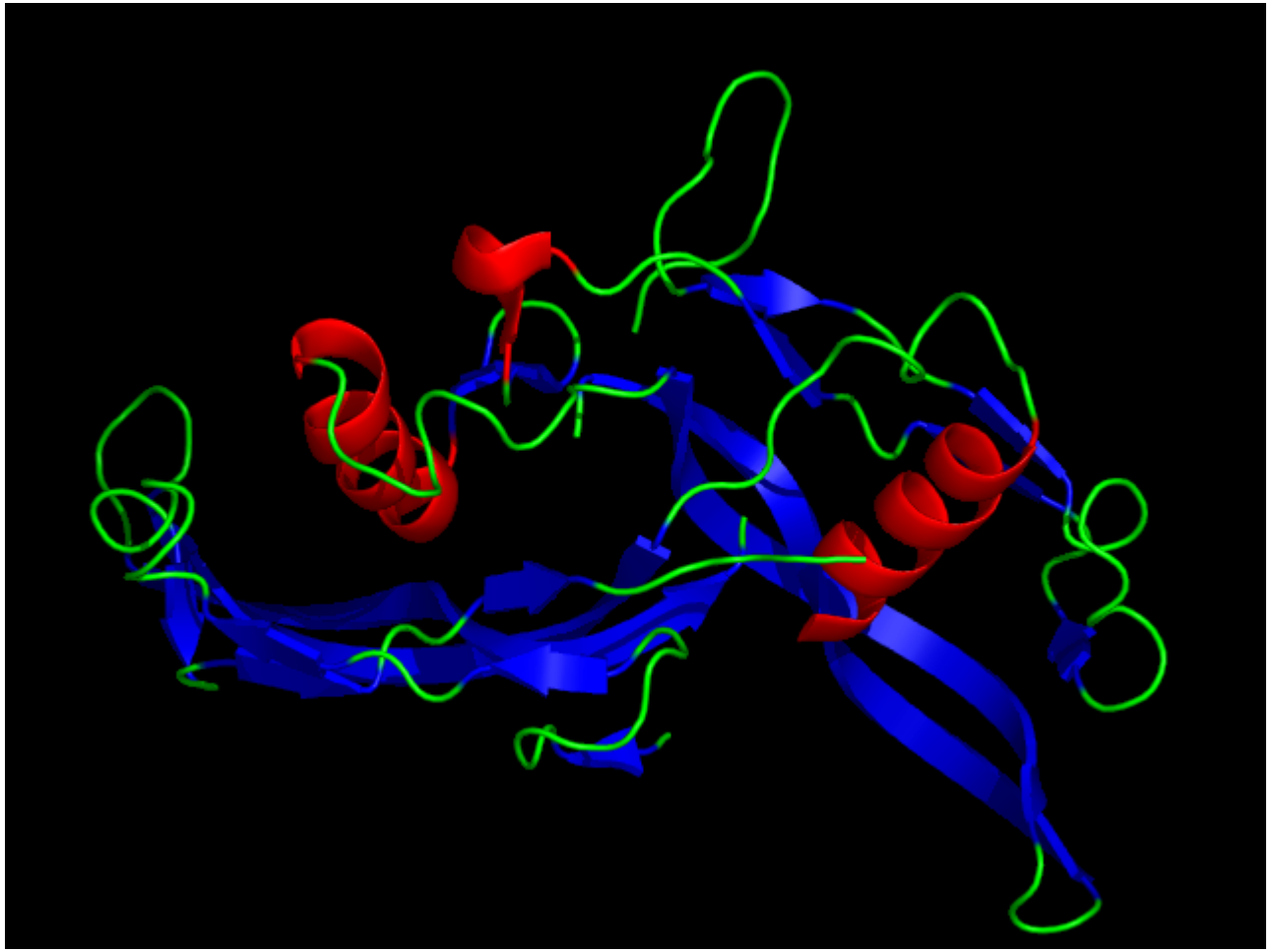
Various molecular forms of inhibins and activins



Adapted from Pangas A. and Woodruff T., Trends in Endocrinology & Metabolism, 11, 309-314 (2000).

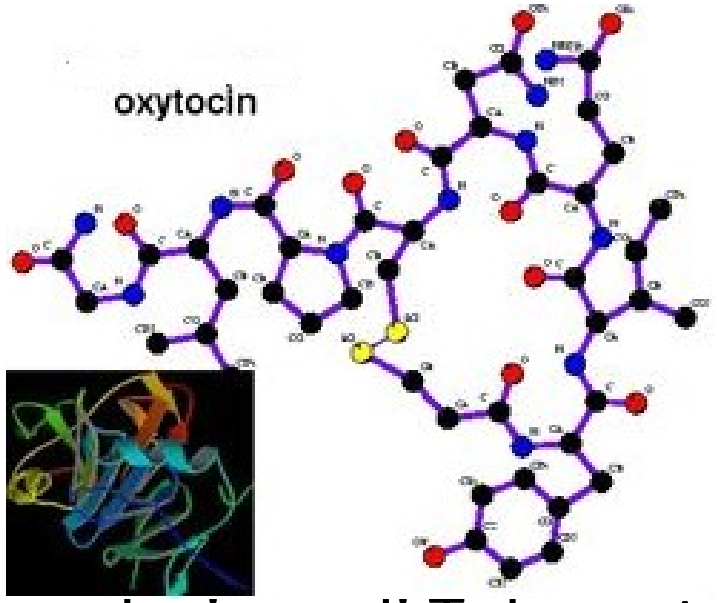
- Inibina: prodotta dalle cellule del Sertoli, sotto lo stimolo dell'FSH, come agente di fb- sulla secrezione dell'FSH stesso. In maschio solo forma B.

- Attivina: prodotta dalle cellule del Sertoli, sotto lo stimolo dell'FSH, come agente di fb+ sulla secrezione dell'FSH stesso.

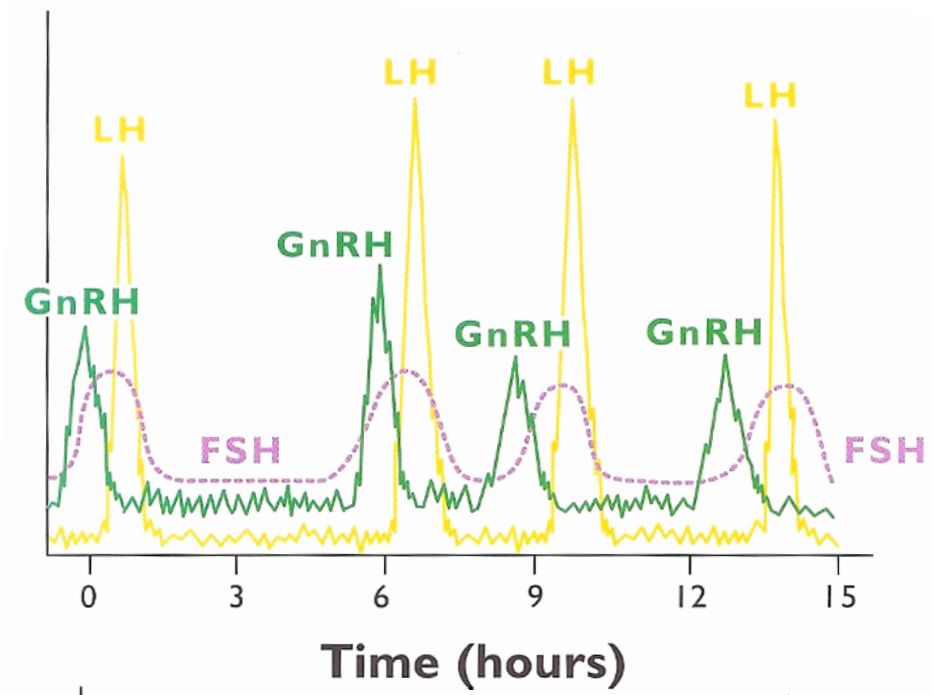


Ossitocina

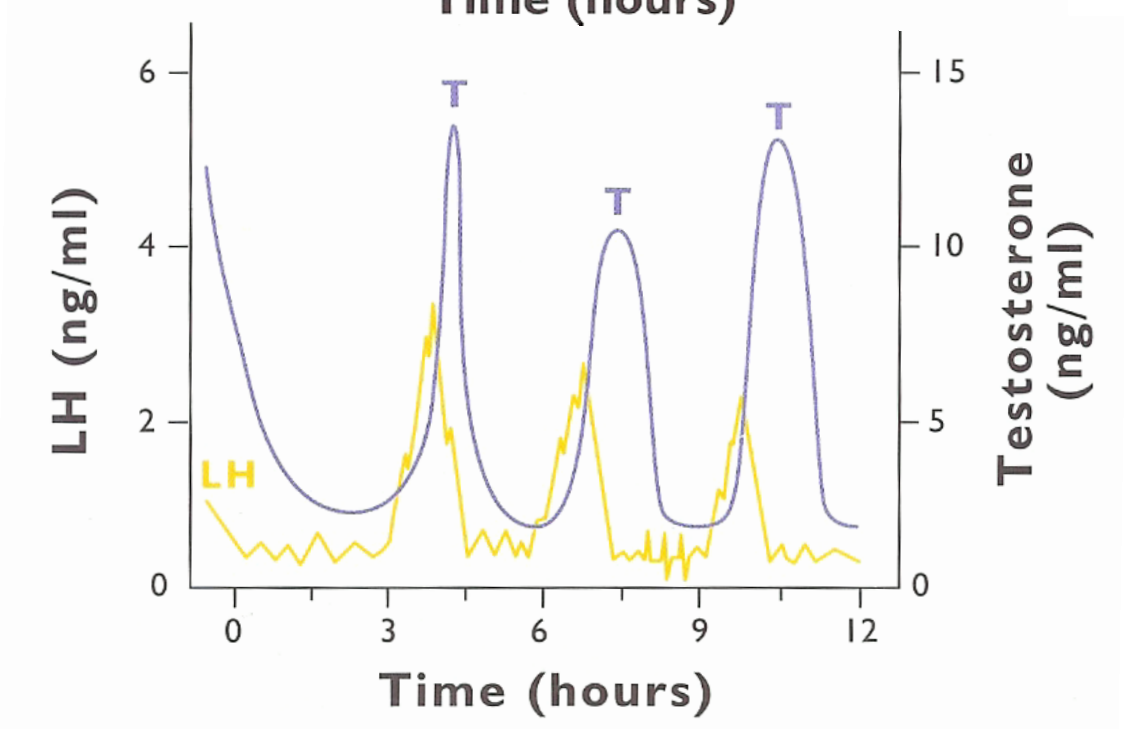
- Prodotta sia cellule del Leydig che da cellule del Sertoli
- 9 aa



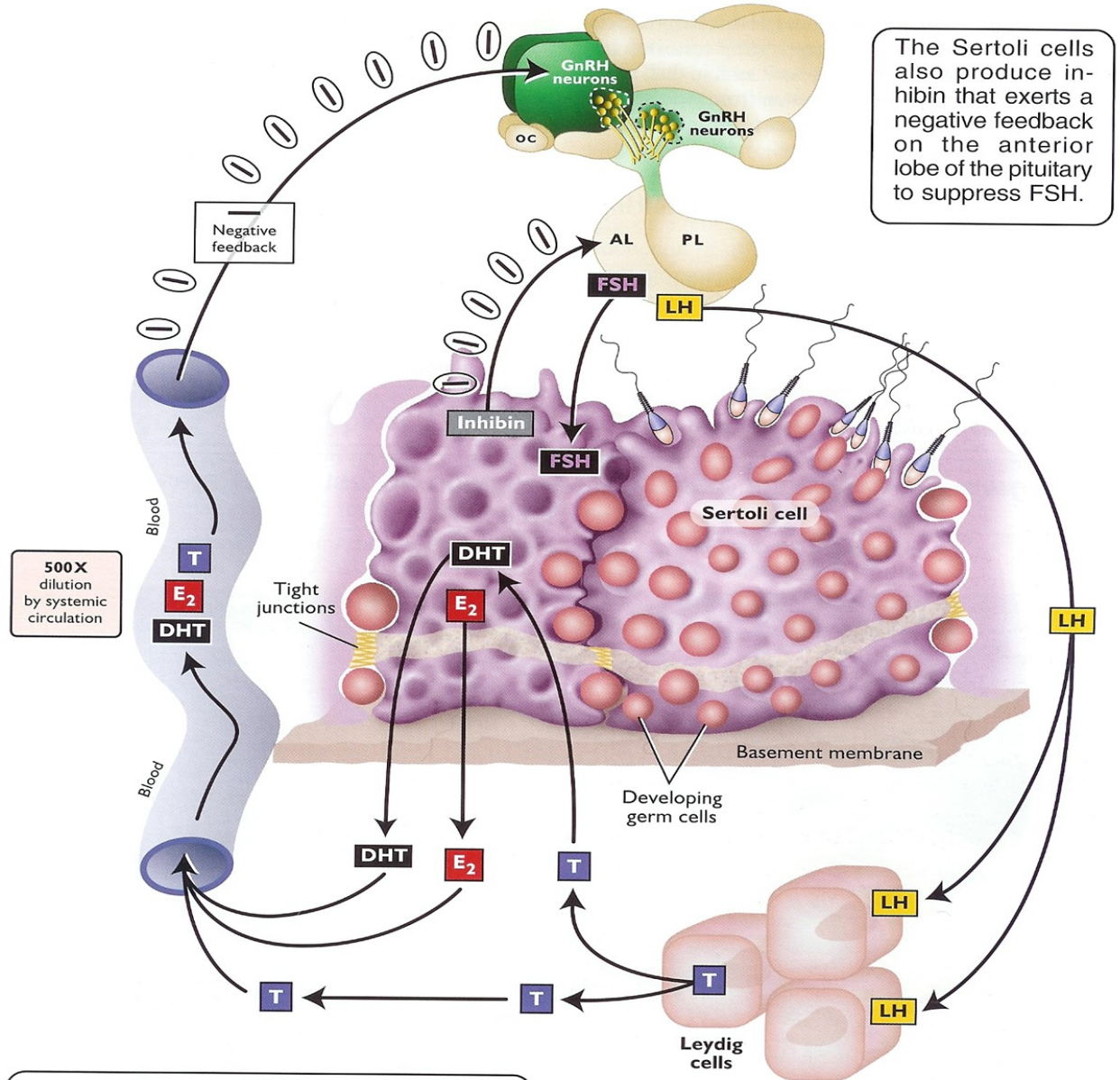
- Incrementa la produzione di T da parte delle cellule del Leydig



GnRH causes the release of LH and FSH. Episodes of all three hormones occur between 4 and 8 times in 24 hours. The lower FSH profile, when compared to LH, is due to inhibin secretion by Sertoli cells. Also, the greater duration of the FSH episode is probably due to its longer half-life (100 min) when compared to LH (30 min).



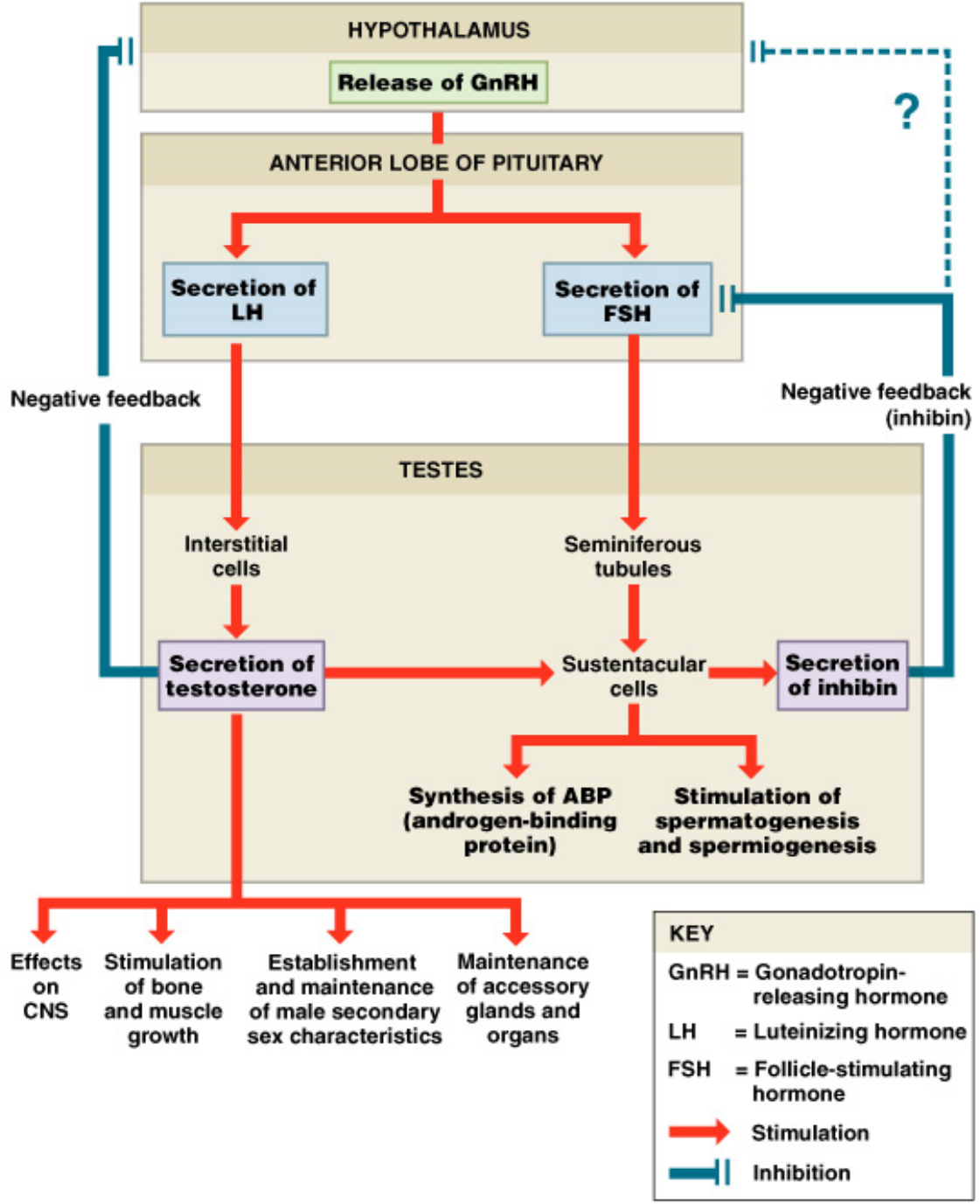
LH is elevated for a period of 0.5 to 1.25 hours, while the subsequent testosterone (T) episode lasts for 0.5 to 1.5 hours.



The Sertoli cells also produce inhibin that exerts a negative feedback on the anterior lobe of the pituitary to suppress FSH.

Testosterone (T) produced by the Leydig cells is transported into the Sertoli cells where it is converted to dihydrotestosterone (DHT) and also estrogen (E_2). Testosterone and E_2 are transported by the blood to the hypothalamus where they exert a negative feedback on the GnRH neurons.

LH binds to receptors in the interstitial cells of Leydig and FSH binds to Sertoli cells. Leydig cells produce testosterone that is transported to the adjacent vasculature and the Sertoli cells where T is converted to DHT.


HYPOTHALAMUS
Release of GnRH
?
ANTERIOR LOBE OF PITUITARY
Secretion of LH
Secretion of FSH
Negative feedback
Negative feedback (inhibin)
TESTES
Interstitial cells
Seminiferous tubules
Secretion of testosterone
Sustentacular cells
Secretion of inhibin
Synthesis of ABP (androgen-binding protein)
Stimulation of spermatogenesis and spermiogenesis
Effects on CNS
Stimulation of bone and muscle growth
Establishment and maintenance of male secondary sex characteristics
Maintenance of accessory glands and organs
KEY
GnRH = Gonadotropin-releasing hormone
LH = Luteinizing hormone
FSH = Follicle-stimulating hormone
Stimulation
Inhibition