Clinical Reproductive Endocrinology

Jacky Chan

Clinical Reproductive Endocrinology

- Reproductive hormones
- Assay methods
- Physiology of reproductive hormones in the female
- Clinical aspects of reproductive endocrinology
- General comments

Reproductive hormones

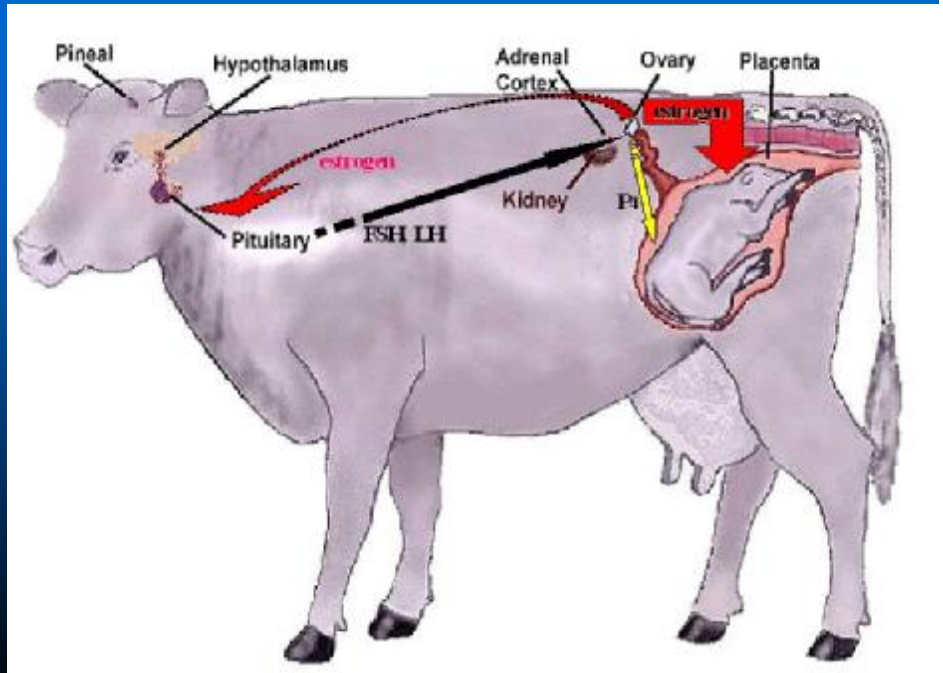
- Definition of hormones
- Chemical classes of reproductive hormones
- Hormone receptors
- Interconversion of steroids in target tissues
- Synthesis and clearance of hormones

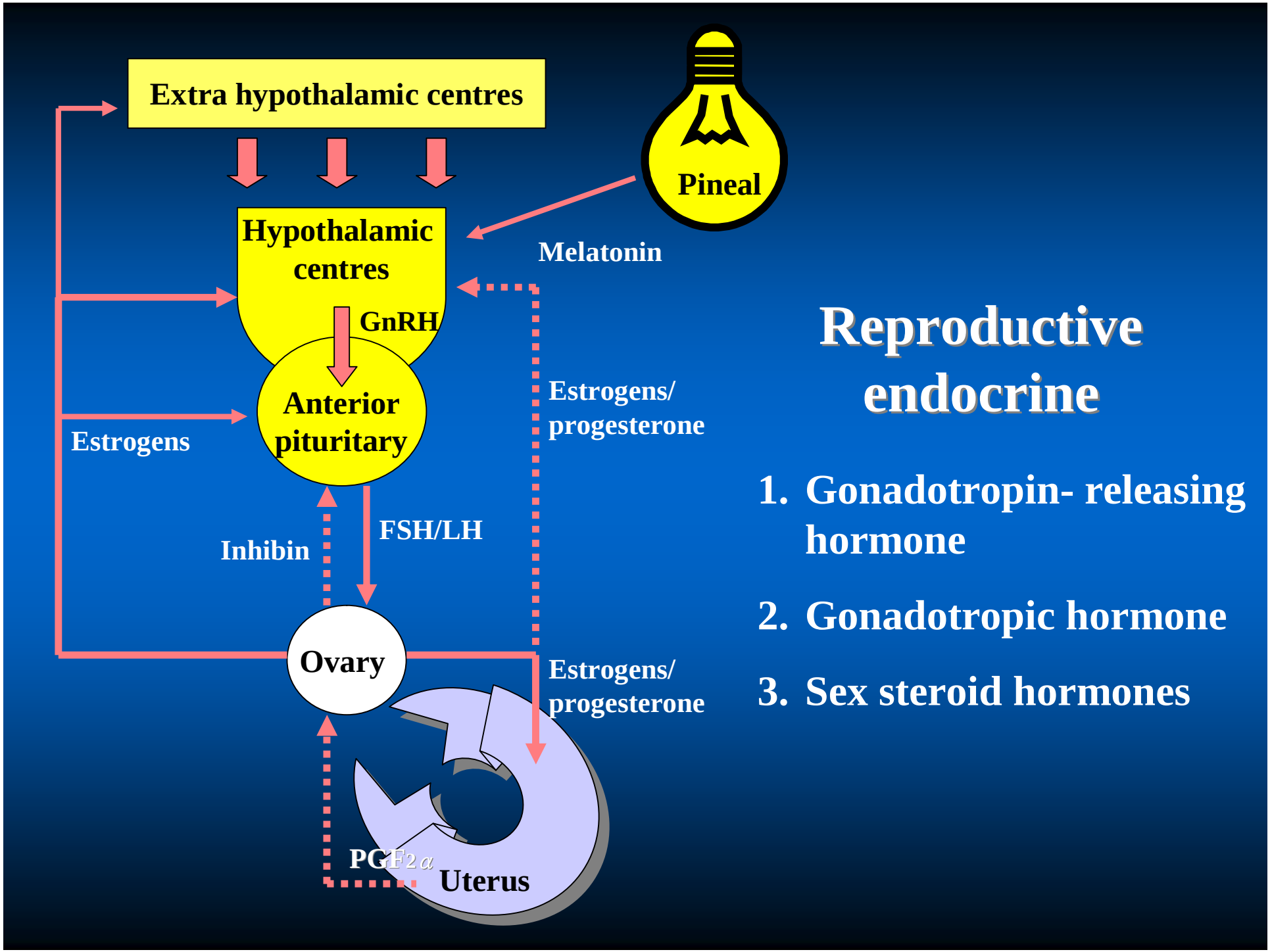
Definition of hormones

- synthesized and secreted by ductless endocrine glands
- minute quantities
- directly into the blood vascular system
- Classic endocrine glands:
  - pituitary, thyroid, parathyroid, adrenal, pancreas, ovary, testis, placenta, pineal gland

Definition of hormones

- **Reproduction**
  - **pituitary, pineal glands, gonads, placenta**
  - **adrenal and thyroid glands**
  - **uterus, hypothalamus**



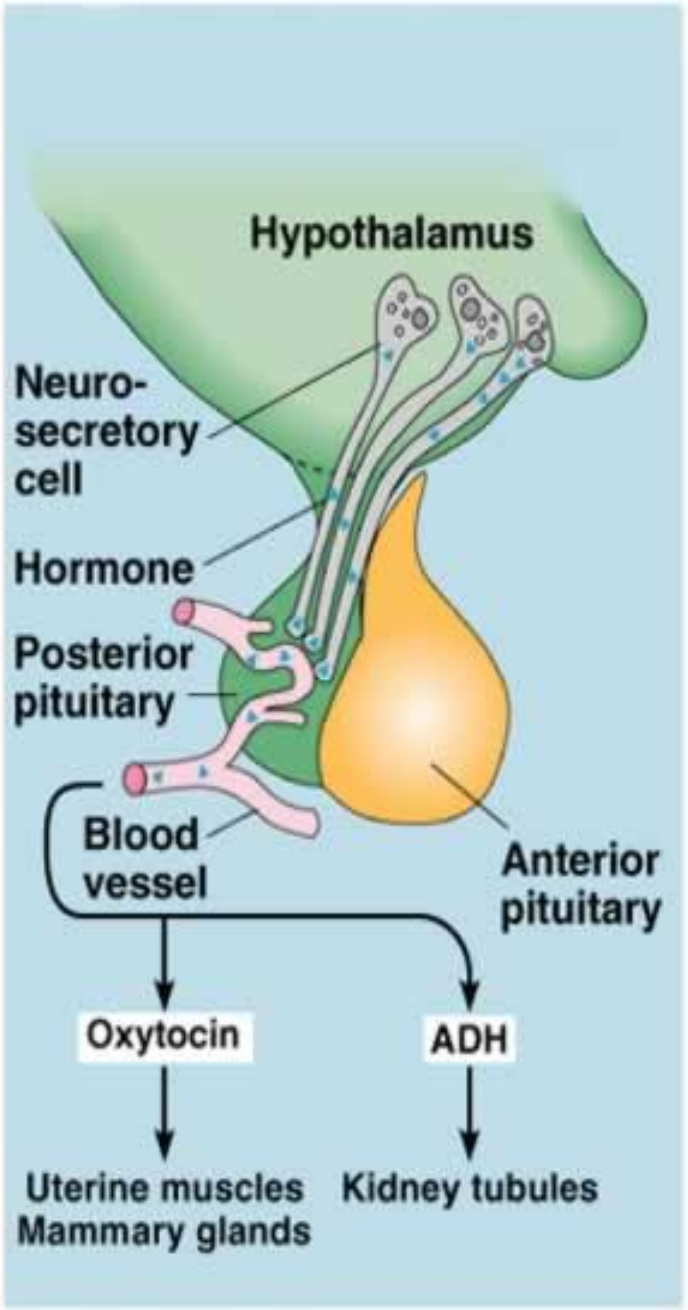


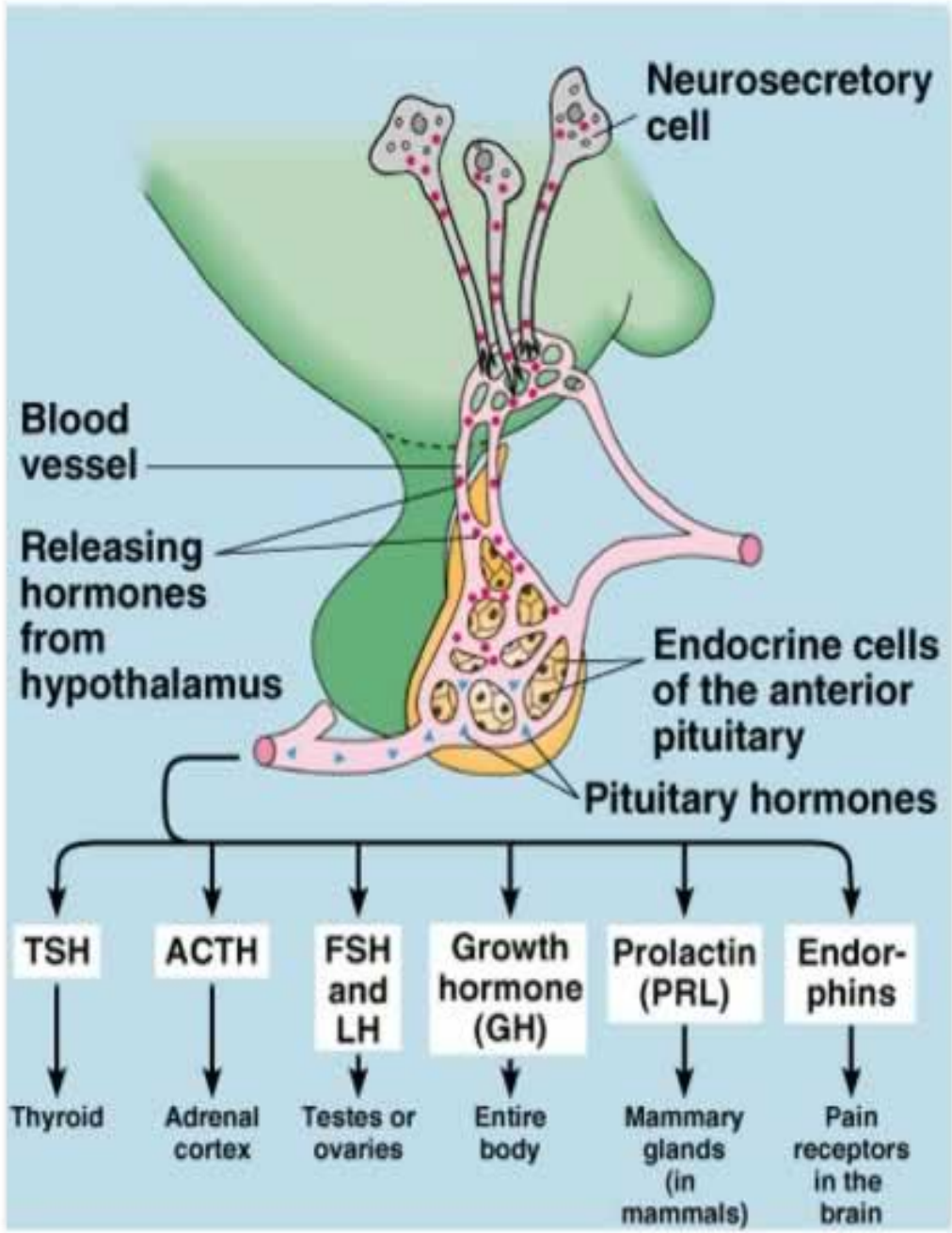
Reproductive endocrine

1. Gonadotropin- releasing hormone
2. Gonadotropic hormone
3. Sex steroid hormones

Chemical classes of reproductive hormones

- Peptide and protein hormones
  - Releasing hormones, Inhibitory hormones
  - Hypophyseal hormones
  - Neurohypophyseal hormones
  - Placental gonadotropins
  - Subunits
- Steroid hormones
- Prostaglandins





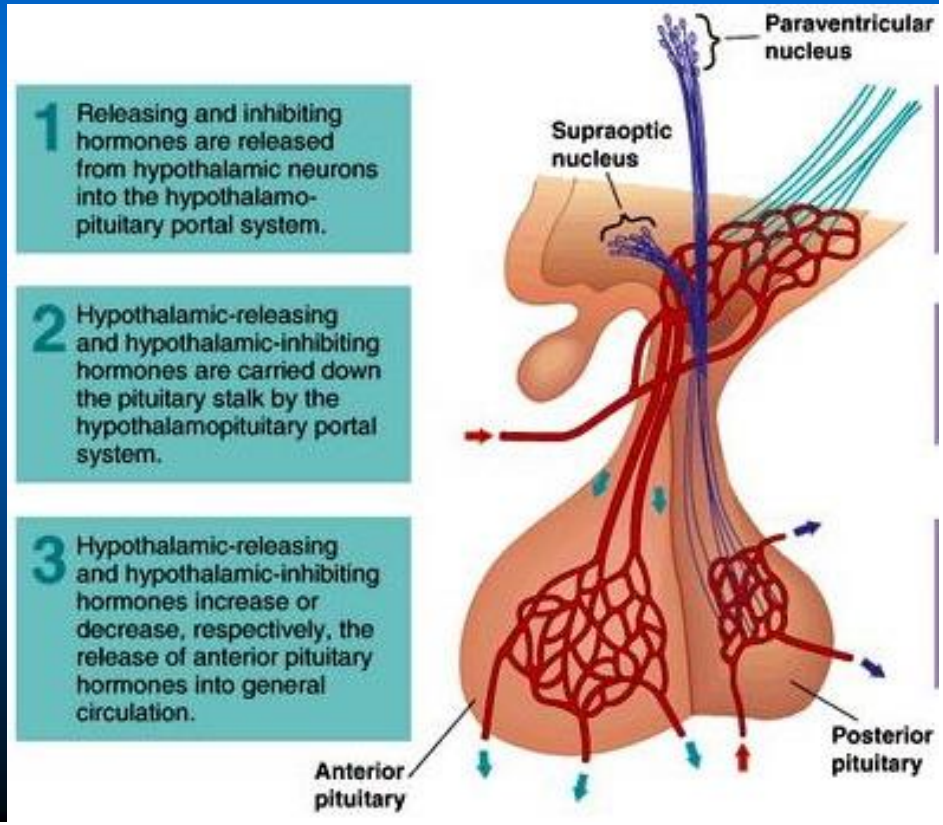
Peptide and protein hormones

- **Releasing hormones, (GnRH, TRH, CRH, PRH, GH-RH)**
  - **Peptide hormones**
  - **produced within the hypothalamus**

portal vein

  - **Adenohypophysis**
- **Inhibitory hormones**
  - **Growth hormone(GIH)**
  - **Prolactin(PIH)**

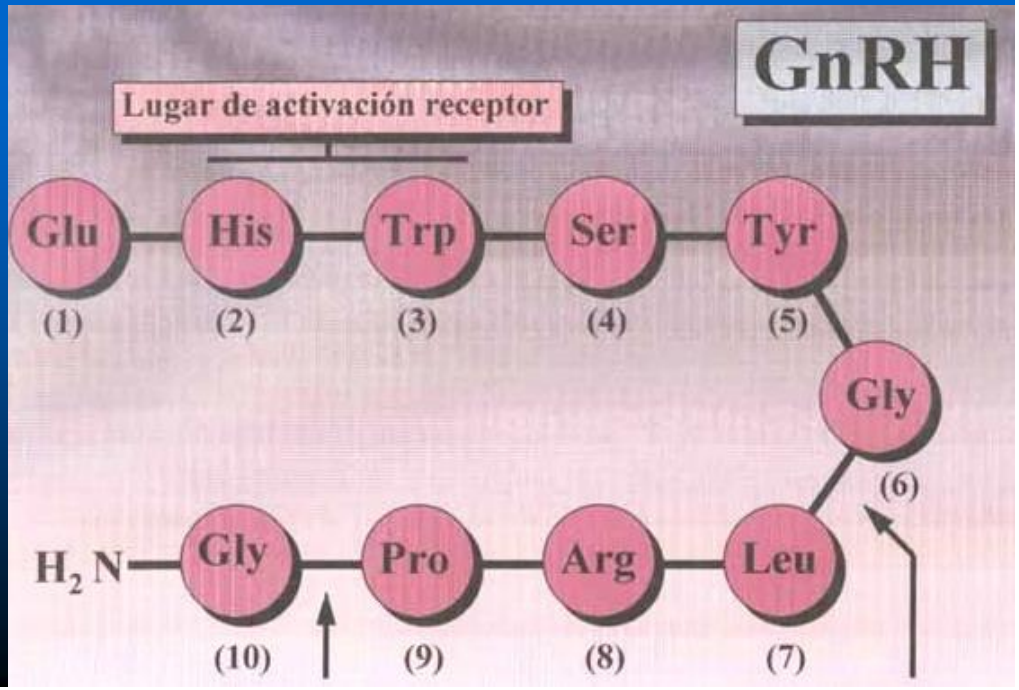
TSH -
Insulin –, glucagon –



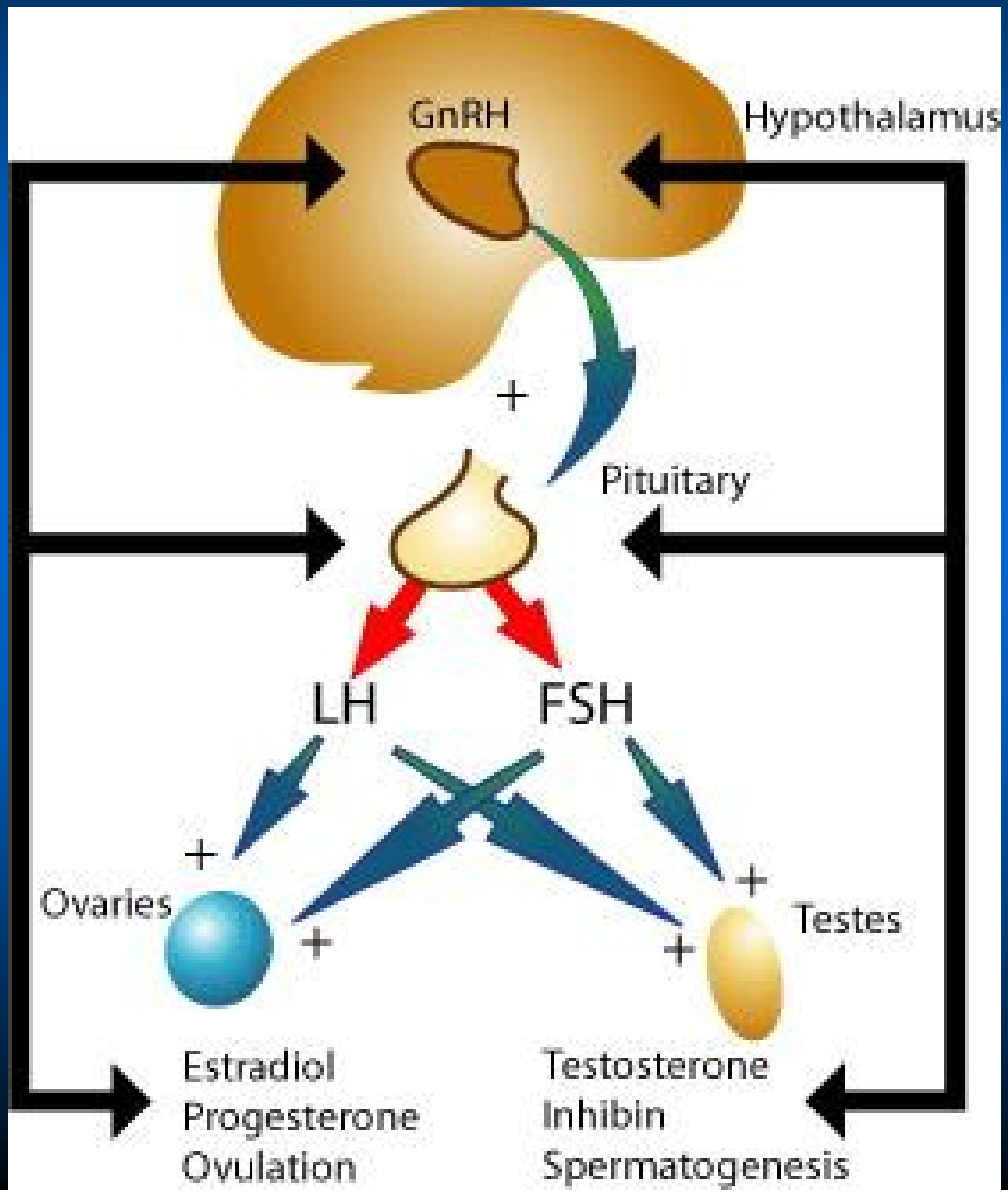
Releasing hormones

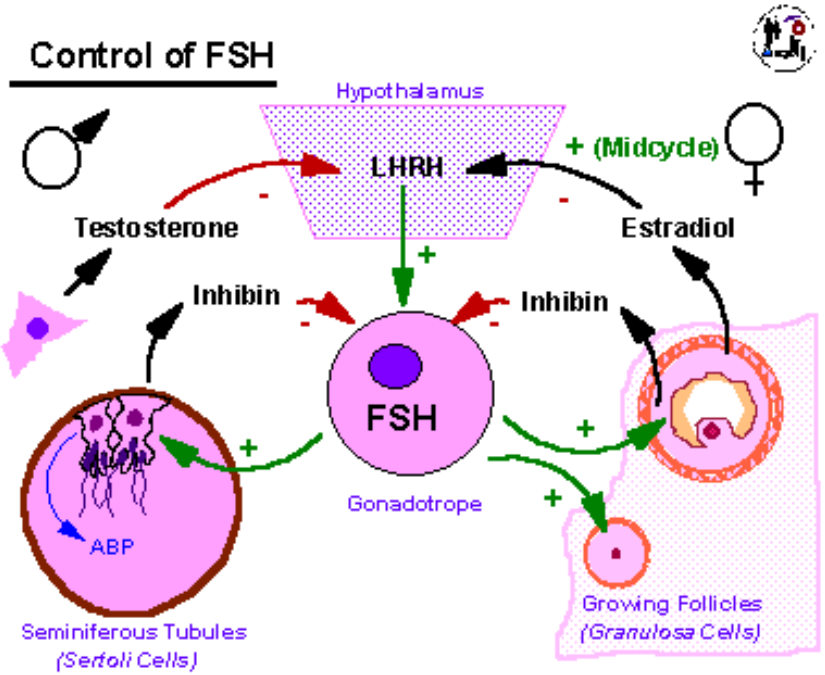
- **Gonadotropin-releasing hormone (GnRH)**
  - **pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2 (MW of GnRH @ 1,182)**

➡ **regulate the release of FSH and LH**



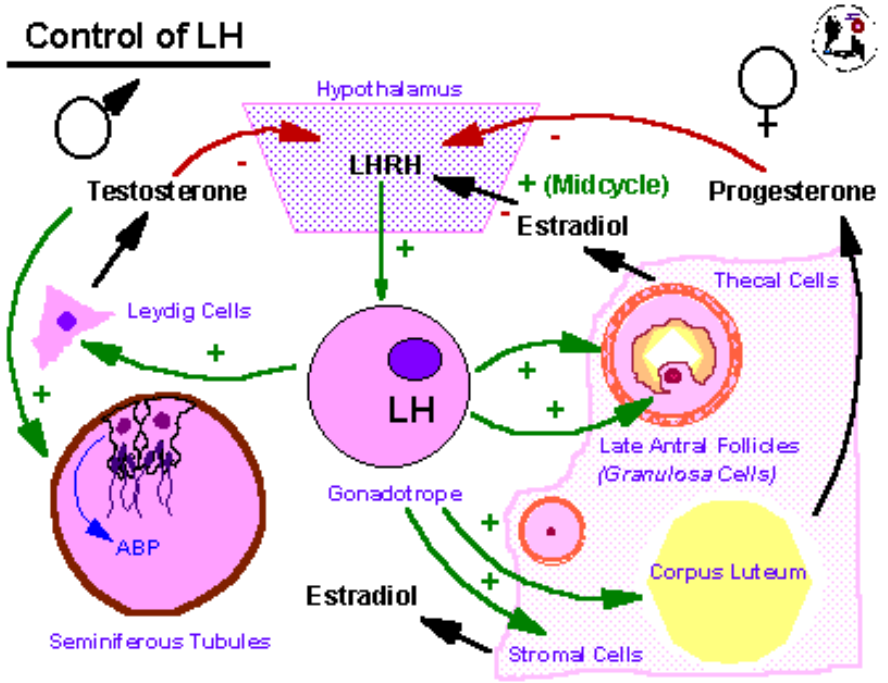
hypothalamic-pituitary-ovarian axis





LH and FSH are released into the systemic circulation, traveling to the target reproductive organs (testes and ovary) where sex steroid hormones are controlled.

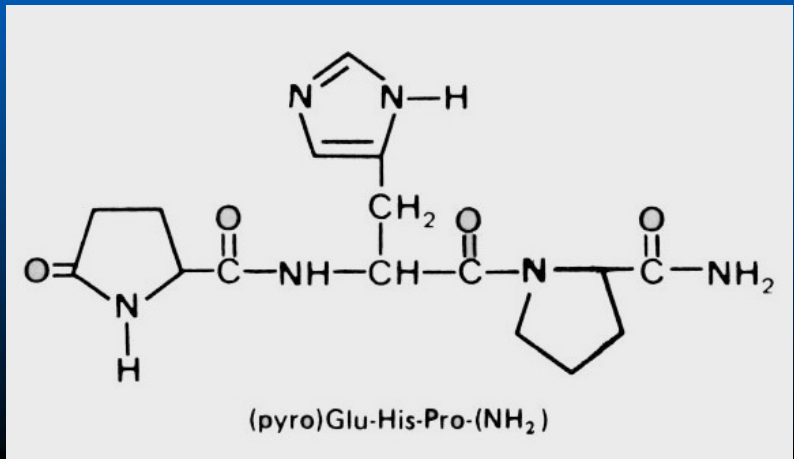
LH sends the same message and results in increased testosterone production by the testis due to LH effect on the Leydig cells of the testis.



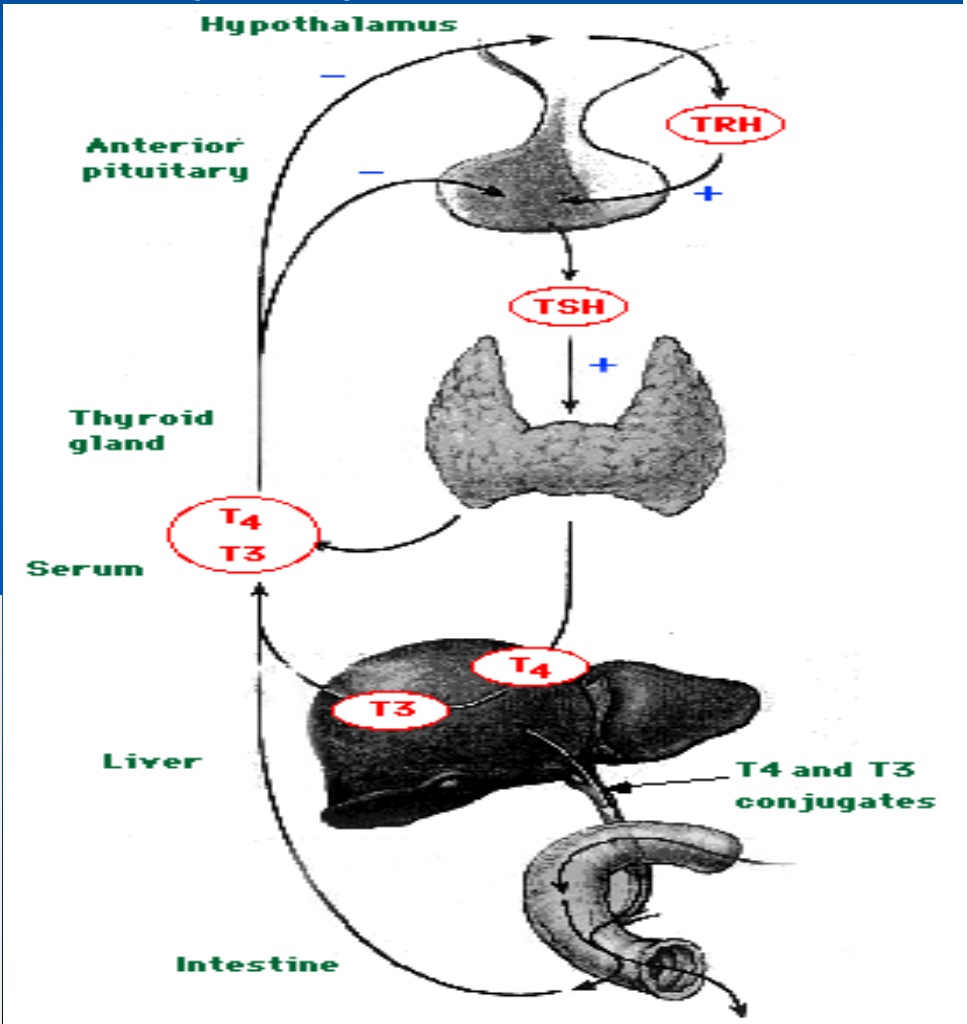
Releasing hormones

- **Thyrotropin-releasing hormone (TRH)**
  - **pGlu-His-Pro-NH2 (MW of TRH @ 362)**

➡ **regulate the release of TSH, GH, and prolactin**



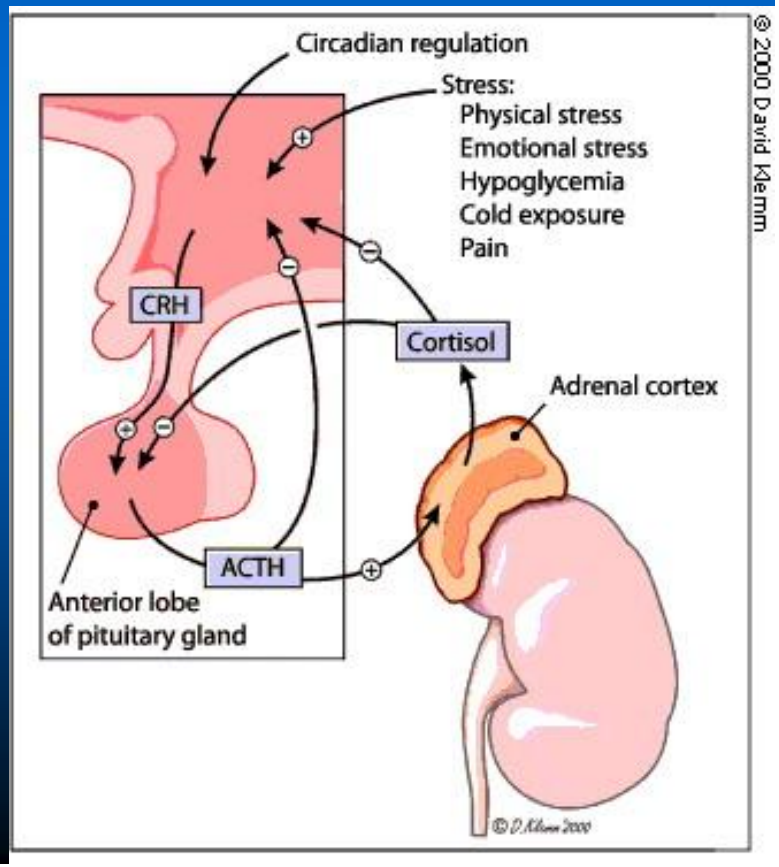
Pathways of thyroid hormone metabolism



Releasing hormones

- **Corticotropin-releasing hormone (CRH)**
  - **CRH is a 41-amino acid peptide**

➡ **regulate the release of ACTH**



Peptide and protein hormones

- **Hypophyseal hormones**
  - **Glycoprotein, FSH and LH, prolactin**
  - **MW of LH @ 30,000 (bovine, ovine, procine, equine)**
  - **MW of FSH @ 32,000 (ovine, equine)**
  - **MW of prolactin @ 23,000 (bovine, ovine, procine)**
- **FSH, LH, TSH, ACTH – produced in basophilic cells**
- **Prolactin– localized in acidophilic cells**

- **Prolactin**
  - **Luteotrophic hormone (LTH): a corpus luteum-stimulating effect in mouse, rat, dog and cat**
  - **198 a.a, MW 23,300 and a 15-minute half-life in sheep**
  - **increasing in lactation (mamotropin)**
  - **stimulating the pigeon crop gland to secret "crop milk"**

Peptide and protein hormones

- **Neurohypophyseal hormones**

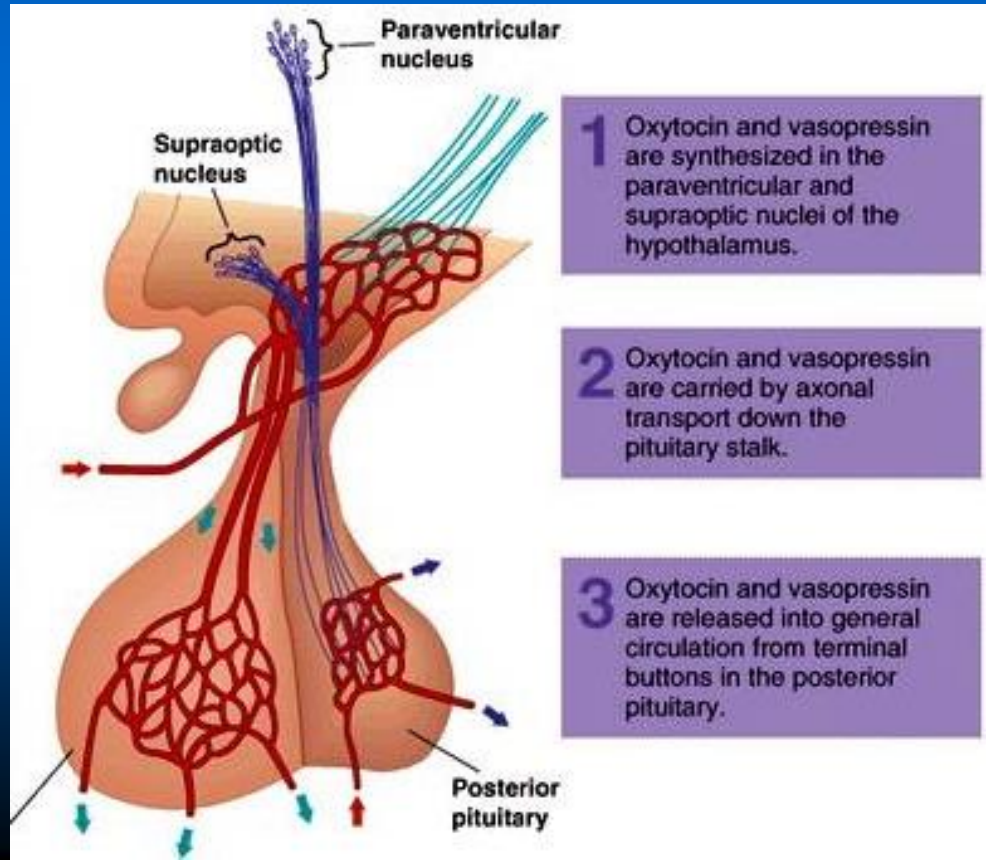
Oxytocin, Vasopressin (synthesized in hypothalamus)

↓ Axoplasmic fluid

Posterior pituitary

↓ Stimulation of the N cell

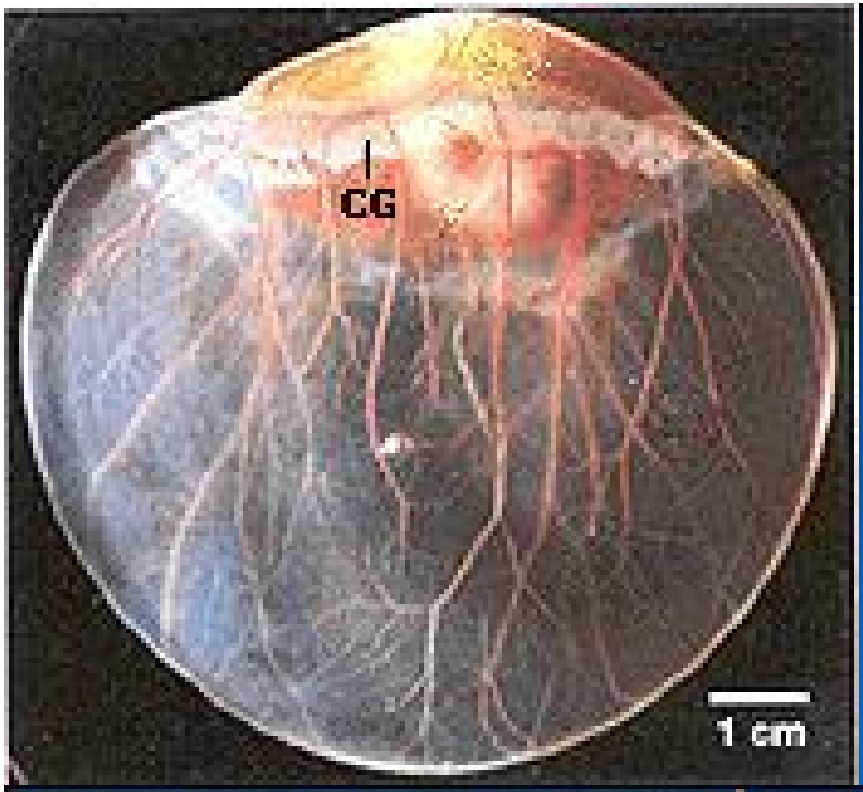
release



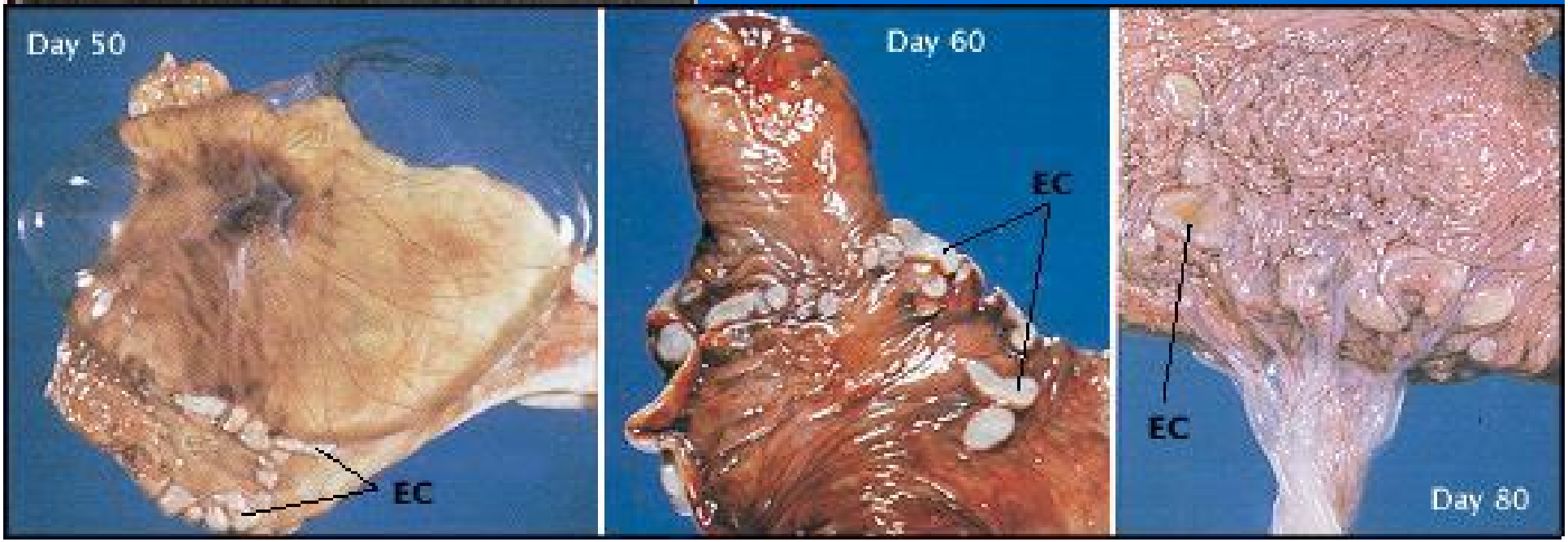
Peptide and protein hormones

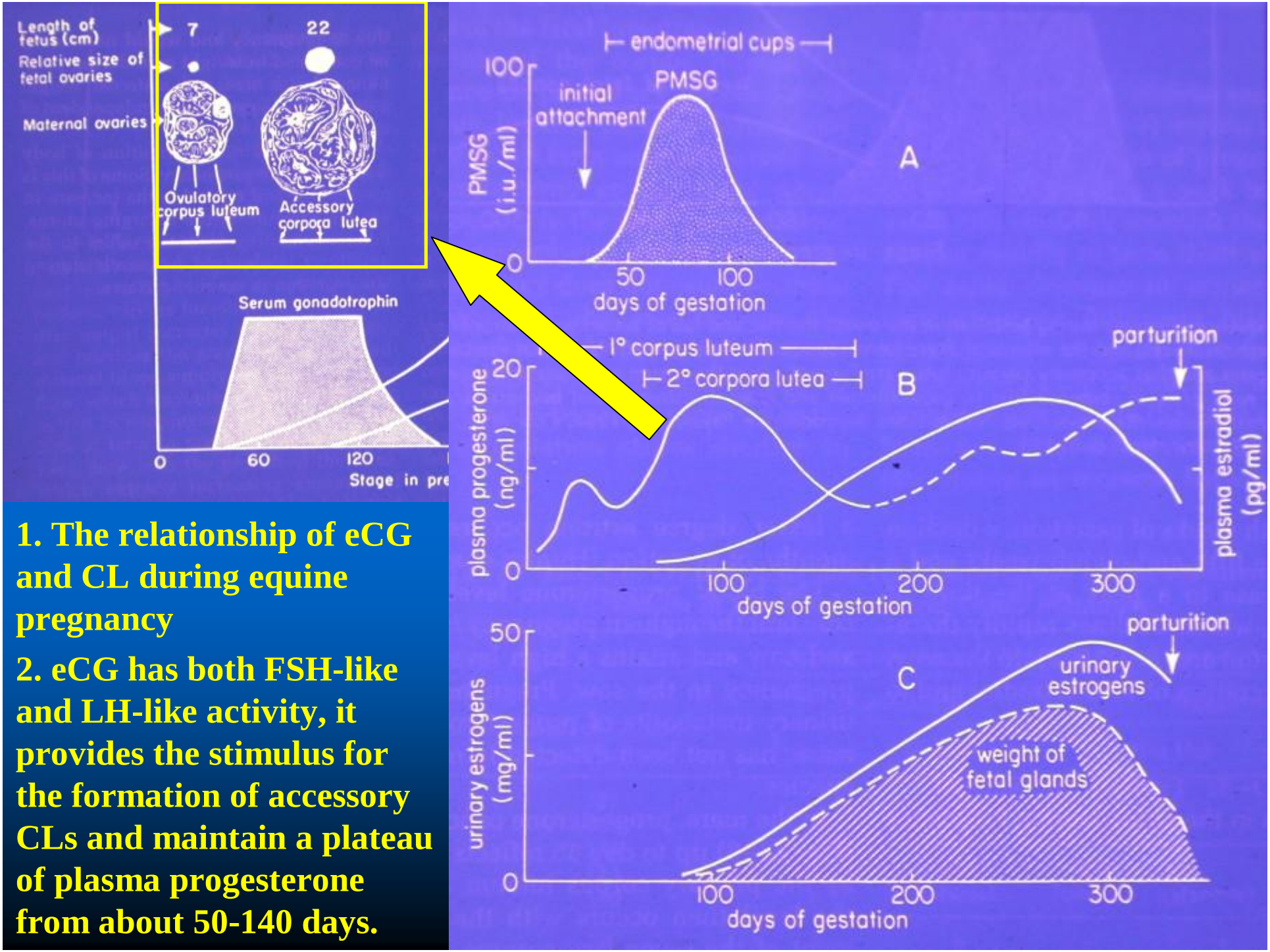
- **Placental gonadotropins**
  - **PMSG (eCG, MW @ 70,000), a protein hormone in serum, produced by fetal trophoblastic cells of pregnant mares**
    - **The function of endometrial cups begins on day 36 until 120 days of pregnancy.**
  - **hCG(MW @ 36,000), a protein hormone, extracted from the urine of pregnant women from 30-40 days of pregnancy**
  - **glucocortocoid, estrogen, progestin, relaxin…**

Endometrial cups



Endometrial cups develop from cells of the chorionic girdle, circumferentially around the conceptus at a point where the membranes of the allantois and yolk sac meet.



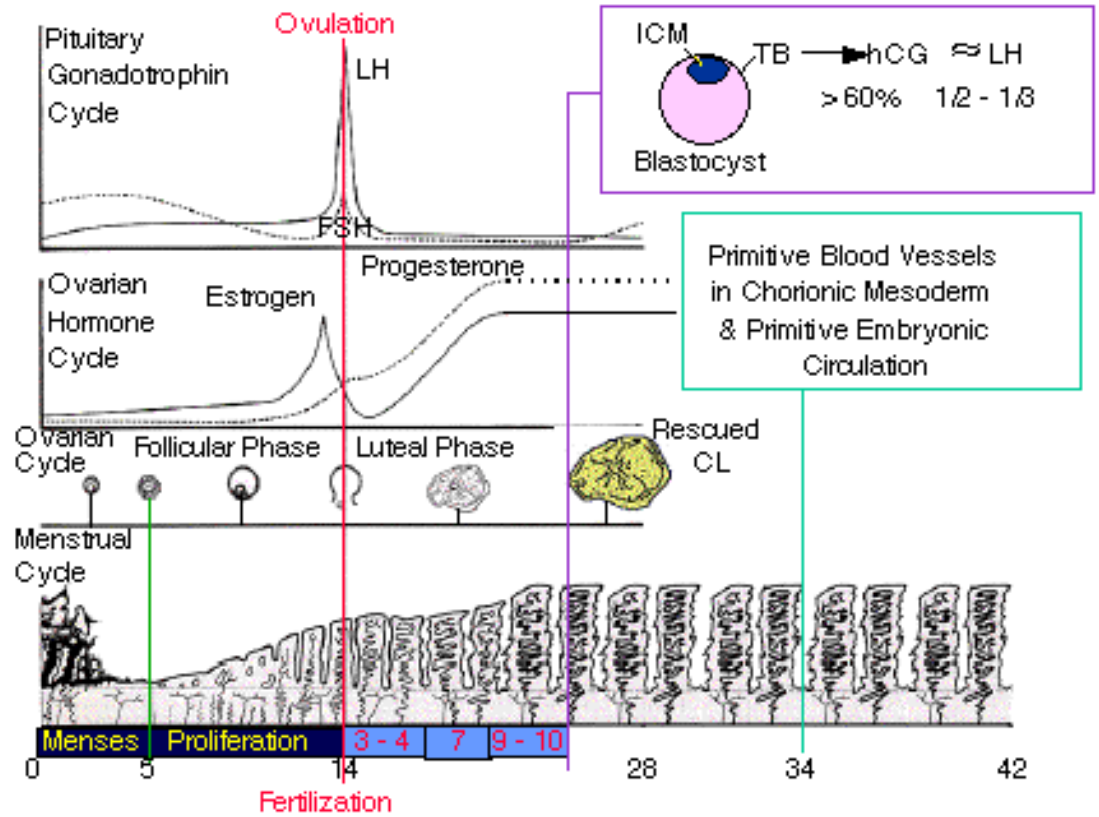


1. The relationship of eCG and CL during equine pregnancy

2. eCG has both FSH-like and LH-like activity, it provides the stimulus for the formation of accessory CLs and maintain a plateau of plasma progesterone from about 50-140 days.

Clinical use of hCG

1. One Step hCG Urine Pregnancy Test
2. **hCG is also used to treat women with certain ovarian disorders.**

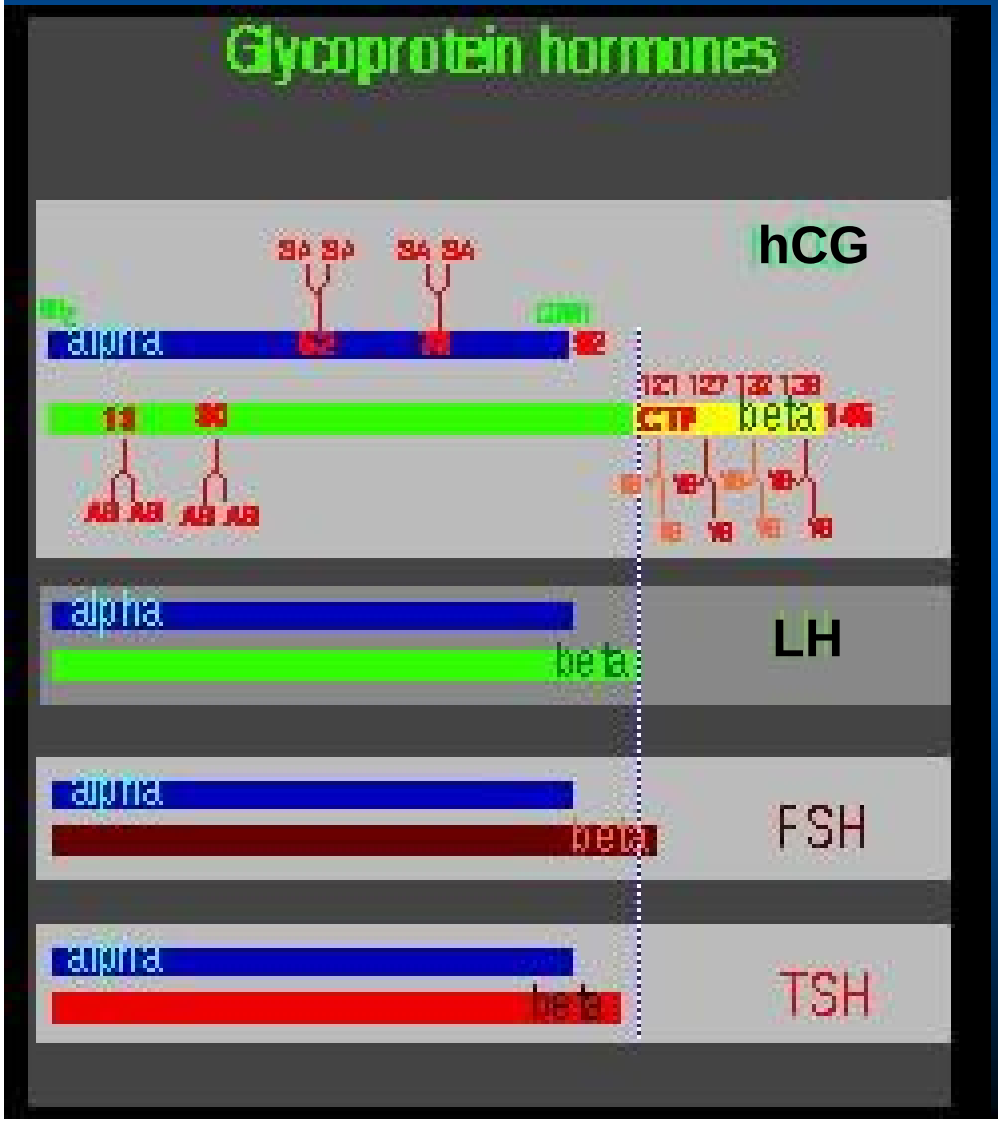


Clinical use of hCG

- **Athletes use hCG to increase the body natural production of testosterone which is often depressed by long term steroid use.**

- **HCG therapy has been found to be very effective in the prevention of testicular atrophy and to use the body own biochemical stimulating mechanisms to increase plasma testosterone level during training.**

Peptide and protein hormones



hCG - subunit α : 96 a.a
subunit β : N-terminal specific for hCG

LH - subunit α : 96 a.a
subunit β : 119 a.a specific for LH

FSH - subunit α : 96 a.a
subunit β : specific for FSH

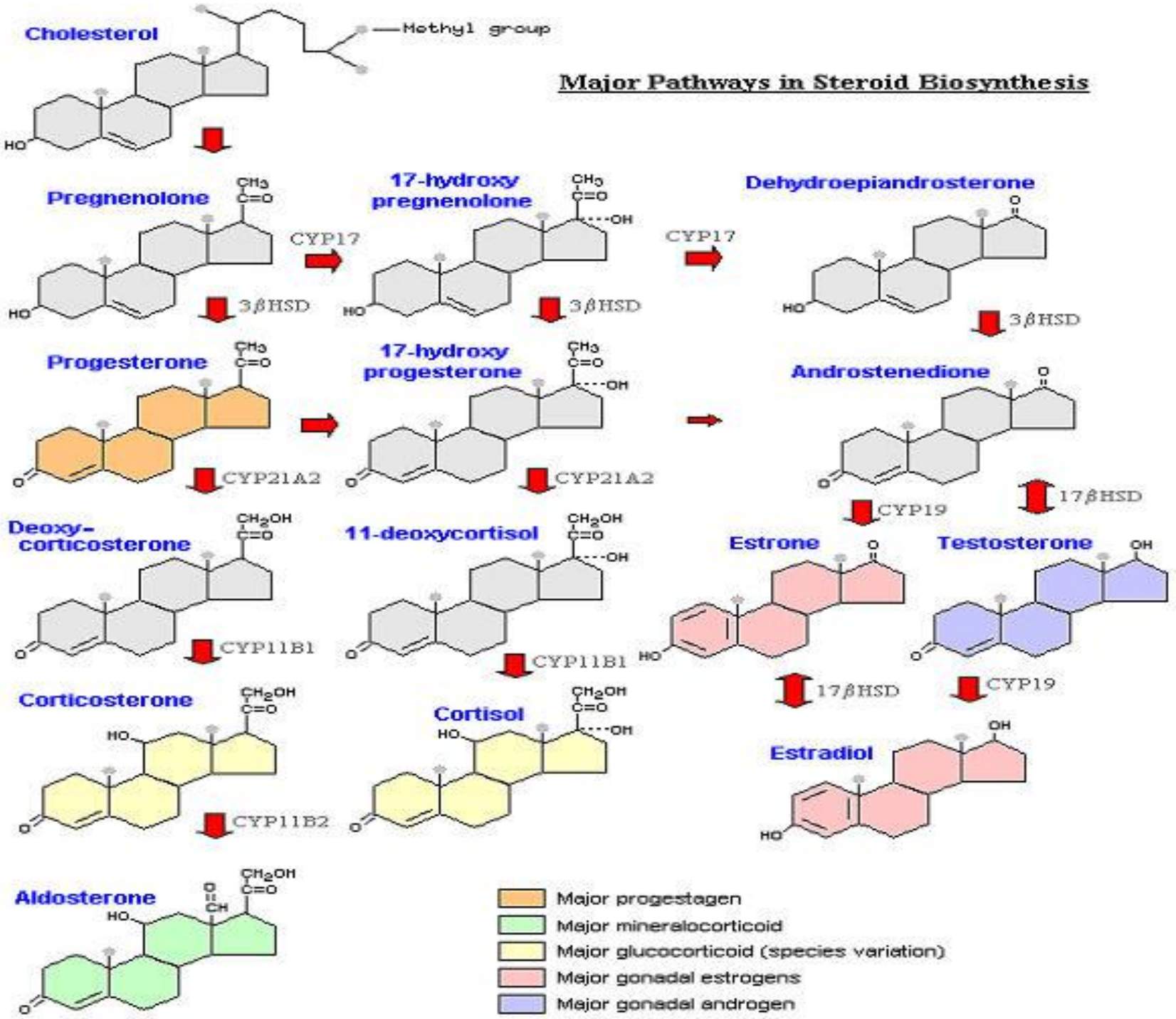
TSH - subunit α : 89 a.a in human
subunit β : 112 a.a in human

Peptide and protein hormones

- **Subunits,** a, b
  - b**-subunit, unique for each hormone within a species, determines the biological activity.**

Chemical classes of reproductive hormones

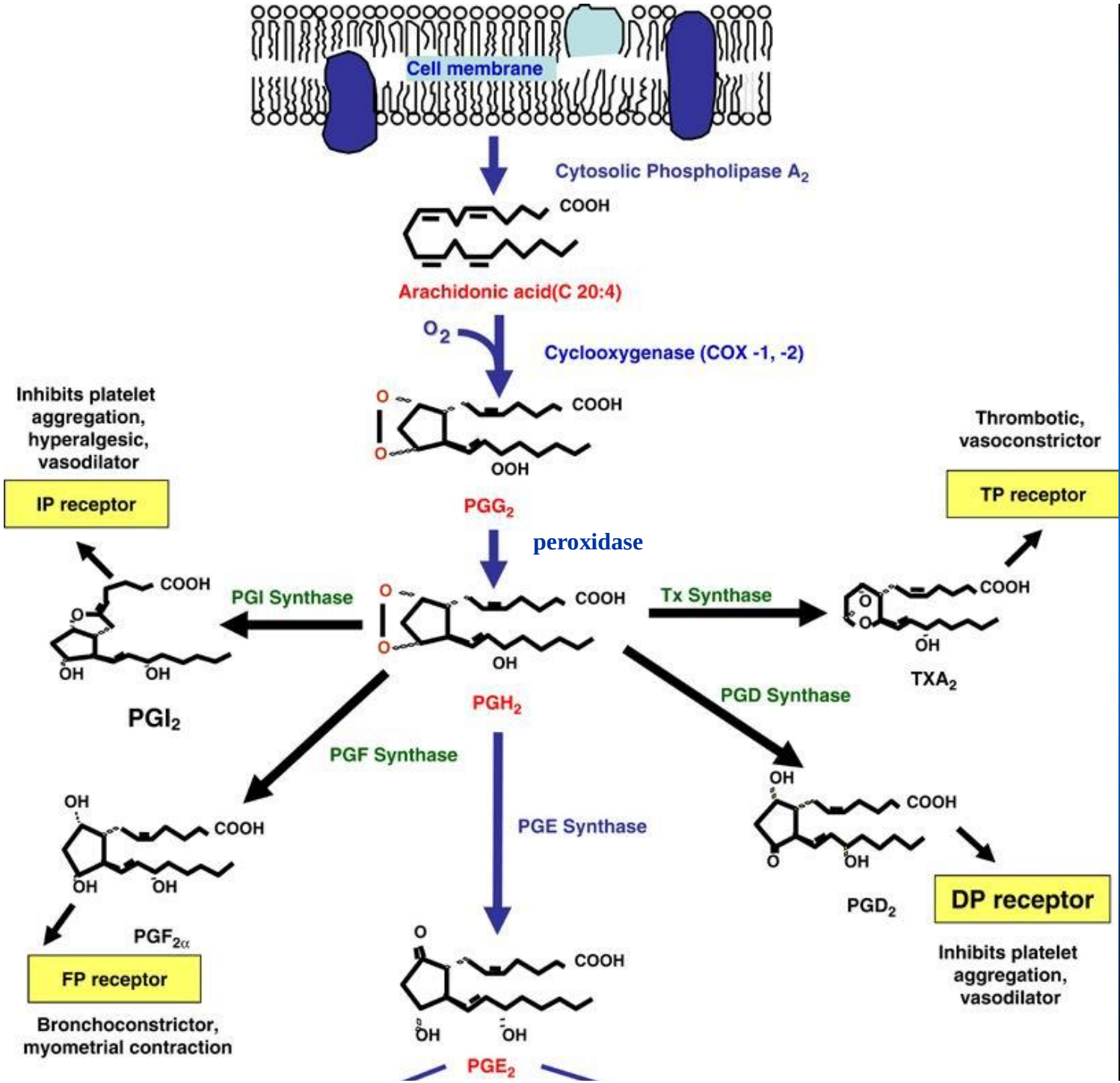
- Steroid hormones
- derived from the precursor, cholesterol
- MW < 500
  - Estrogens (estradiol)
  - Androgens (testosterone)
  - Progesterones (progesterone)


Major Pathways in Steroid Biosynthesis
Cholesterol
Methyl group
Pregnenolone
17-hydroxy pregnenolone
Dehydroepiandrosterone
CYP17
CYP17
3βHSD
3βHSD
3βHSD
Progesterone
17-hydroxy progesterone
Androstenedione
CYP21A2
CYP21A2
CYP19
17βHSD
Deoxy-corticosterone
11-deoxycortisol
Estrone
Testosterone
CYP11B1
CYP11B1
17βHSD
CYP19
Corticosterone
Cortisol
Estradiol
CYP11B2
Aldosterone
Major progestagen
Major mineralocorticoid
Major glucocorticoid (species variation)
Major gonadal estrogens
Major gonadal androgen

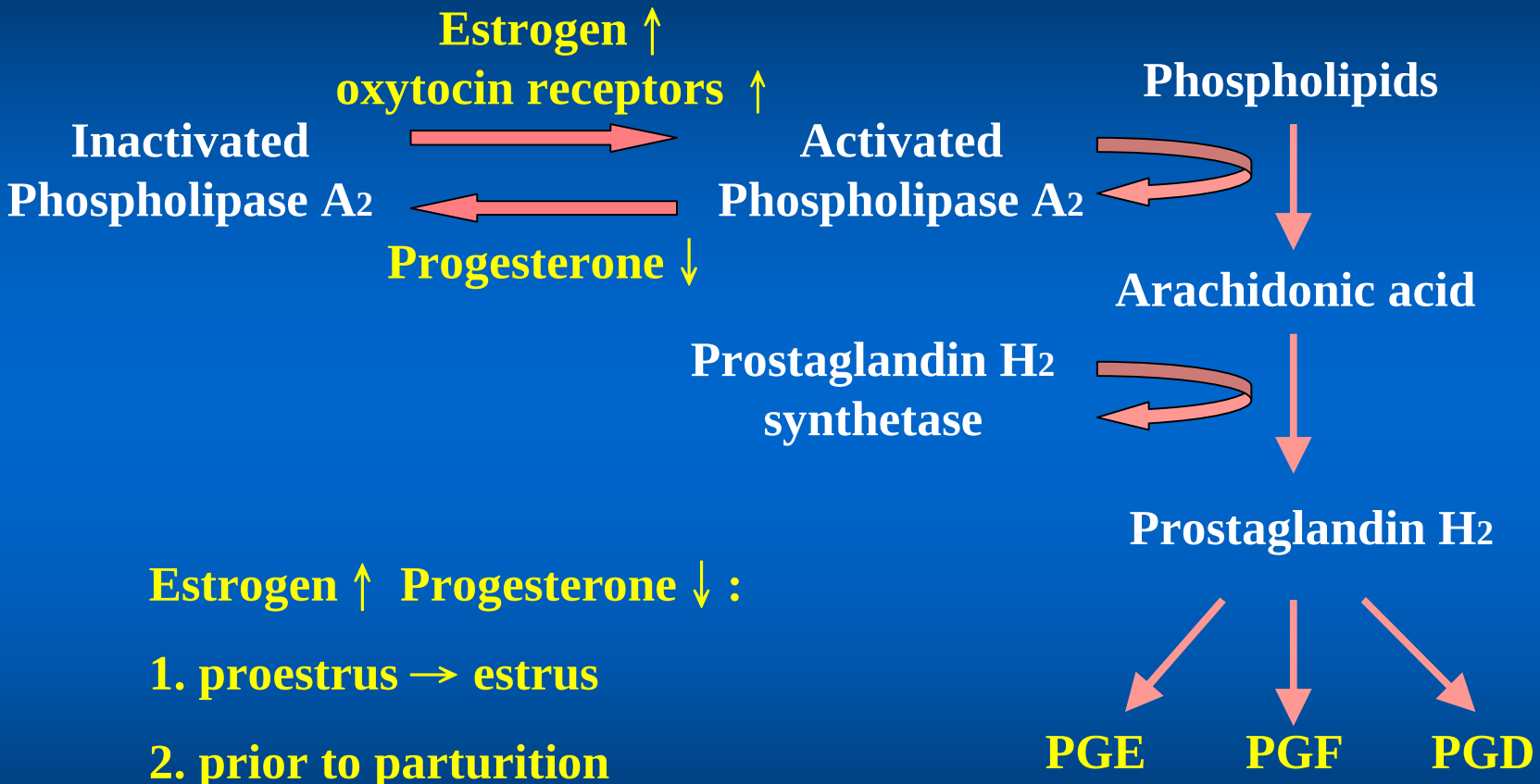
Chemical classes of reproductive hormones

- **Prostaglandins**
  - **a group of 20-C unsaturated fatty acids**
  - **MW: 300-400**
  - **local hormones, short biological half-life with a local action**
  - **found in mammalian tissues, uterus and prostata**

Biosynthesis of PG from arachidonic acid



Synthesis of prostaglandin affected by estrogen



Estrogen ↑ Progesterone ↓ :

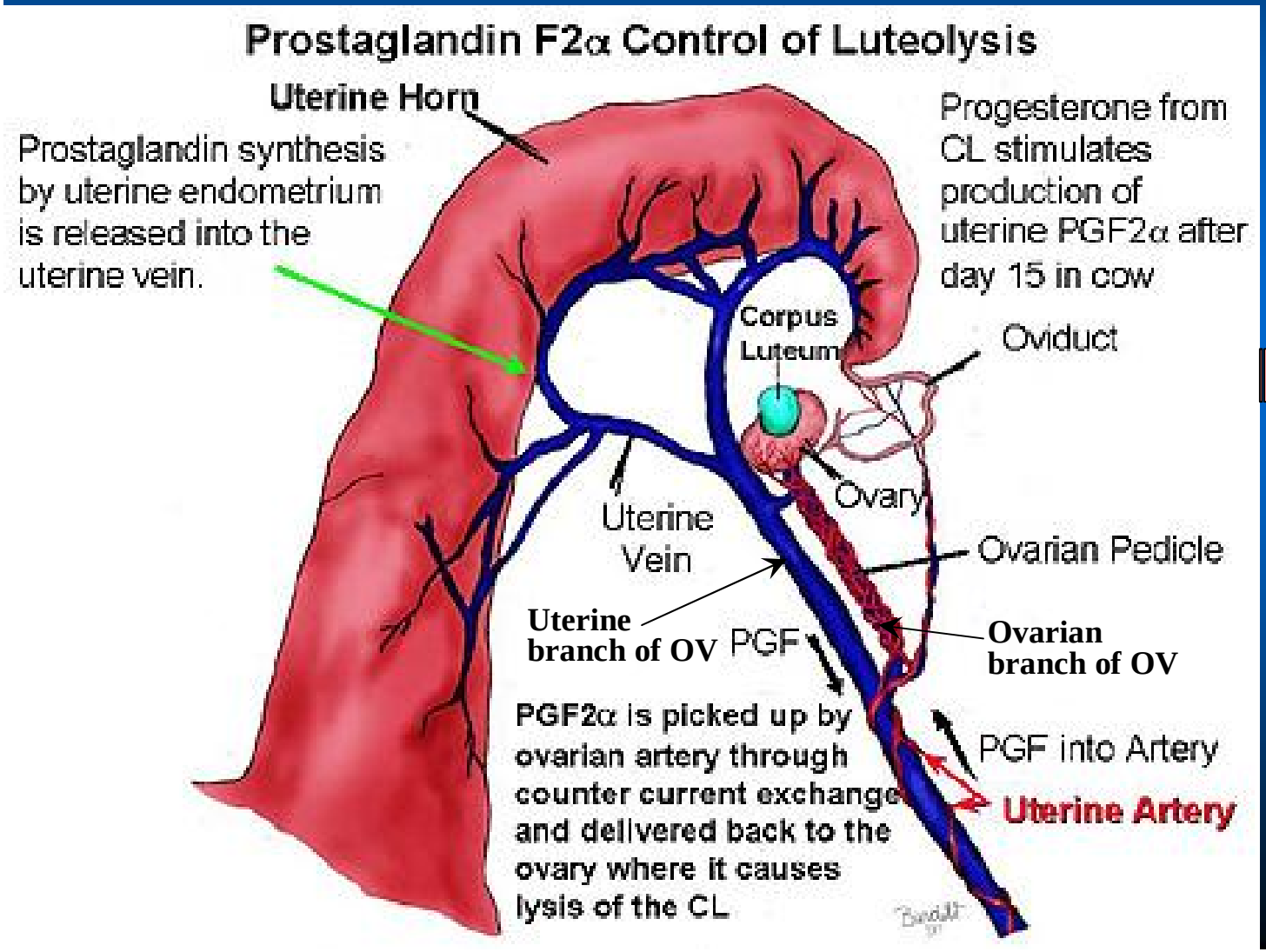
1. proestrus → estrus

2. prior to parturition

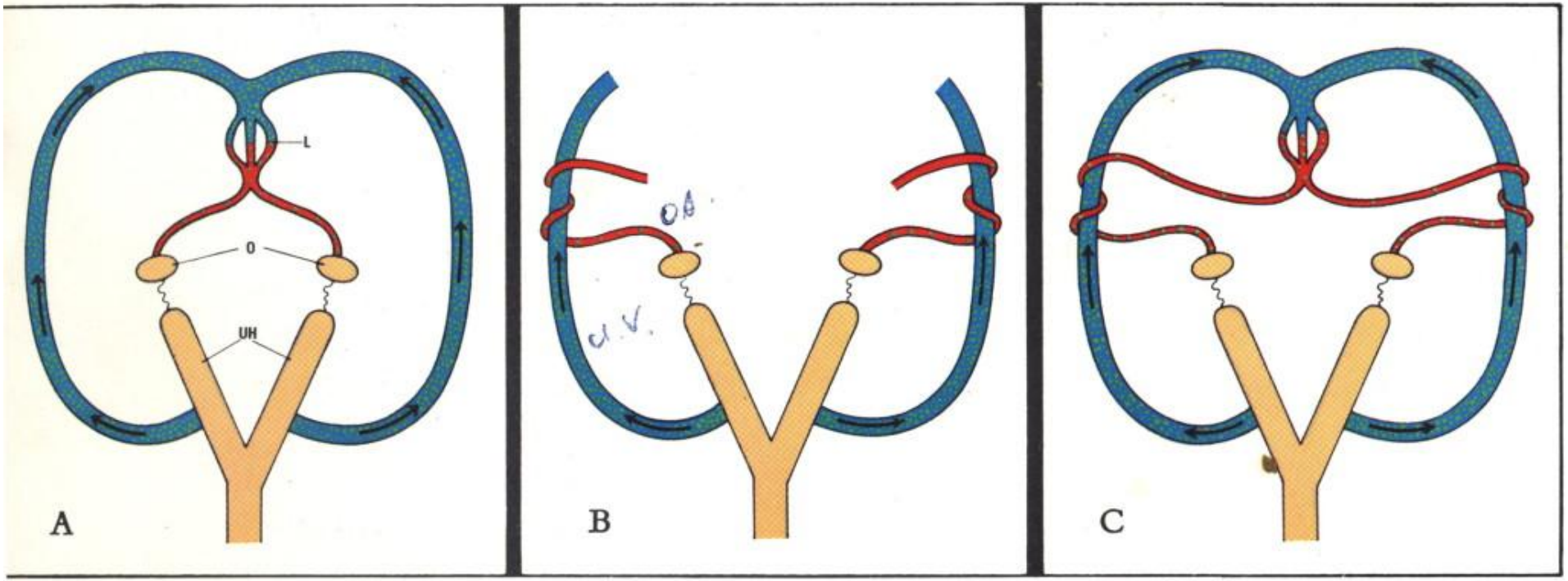
Function of prostaglandins

PGE_2	stimulates	vascular dilation bronchiodilation gastrointestinal and uterine contraction inflammatory response
	mimics	hormones that act through cyclic AMP
	inhibits	platelet aggregation
PGF_2	stimulates	vascular constriction bronchioconstriction smooth muscle contraction, e.g. intestinal tract, uterus
PGI_2	inhibits	platelet aggregation (increases cAMP) vascular constriction bronchioconstriction gastrointestinal and uterine contraction

vascular uteroovarian pathways for PGF2α



vascular uteroovarian pathways for $PGF_{2\alpha}$



Systemic uteroovarian pathway:

horse, rabbit

Local uteroovarian pathway:

sheep, cattle, (guinea pig, hamsters, rats)?

Combined systemic and local uteroovarian pathway:

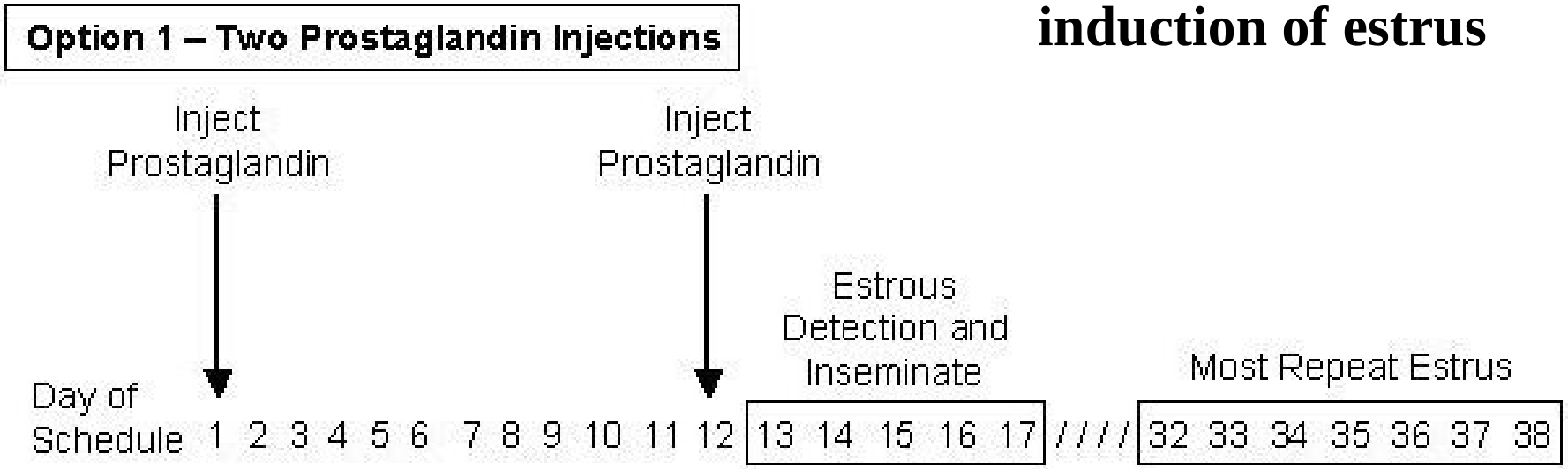
swine

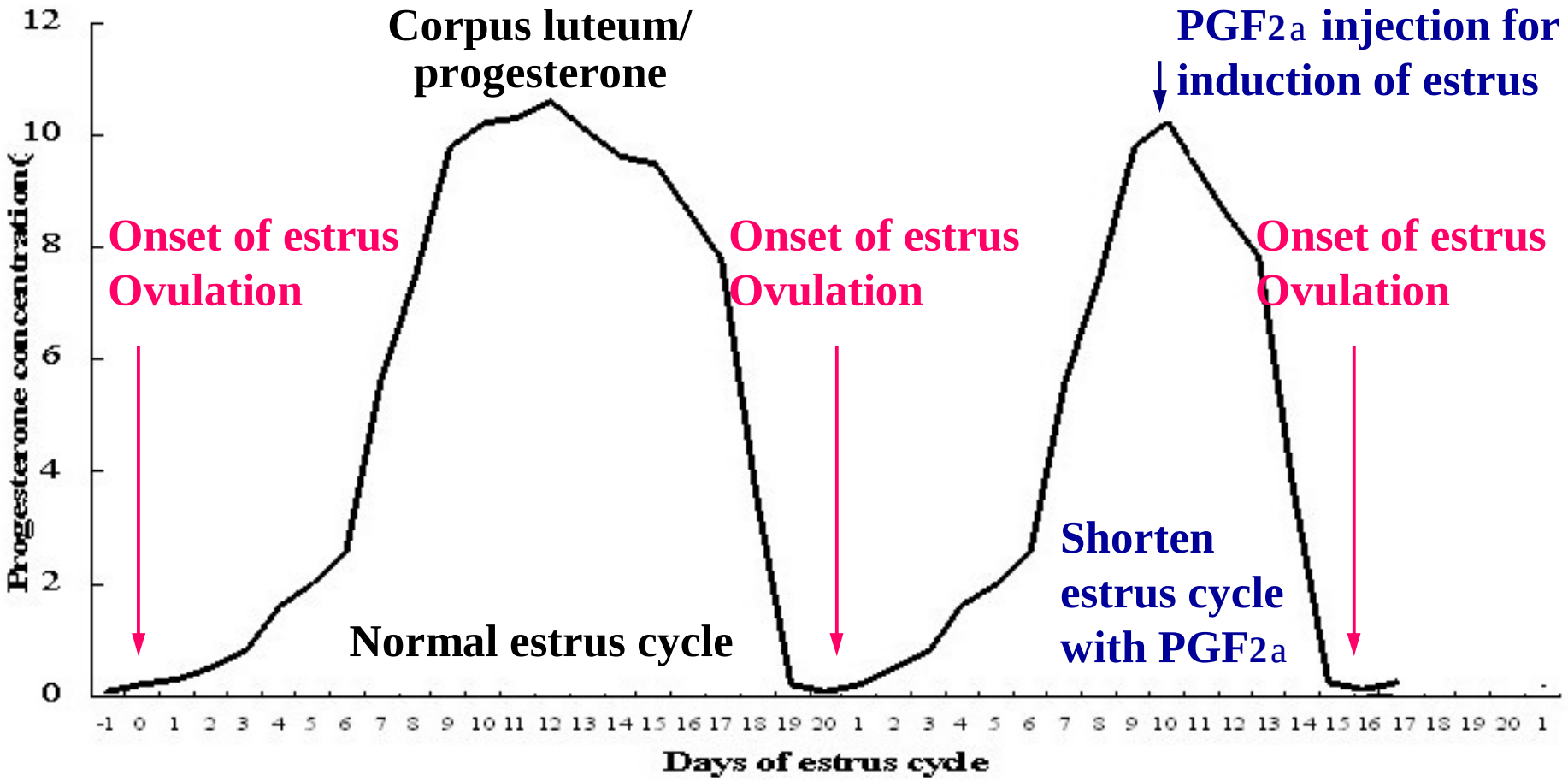
Clinical use of PGF_{2a}

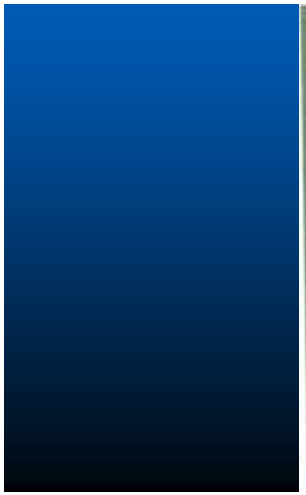
endometritis/pyometra

Clinical use of PGF_{2a}

induction of estrus

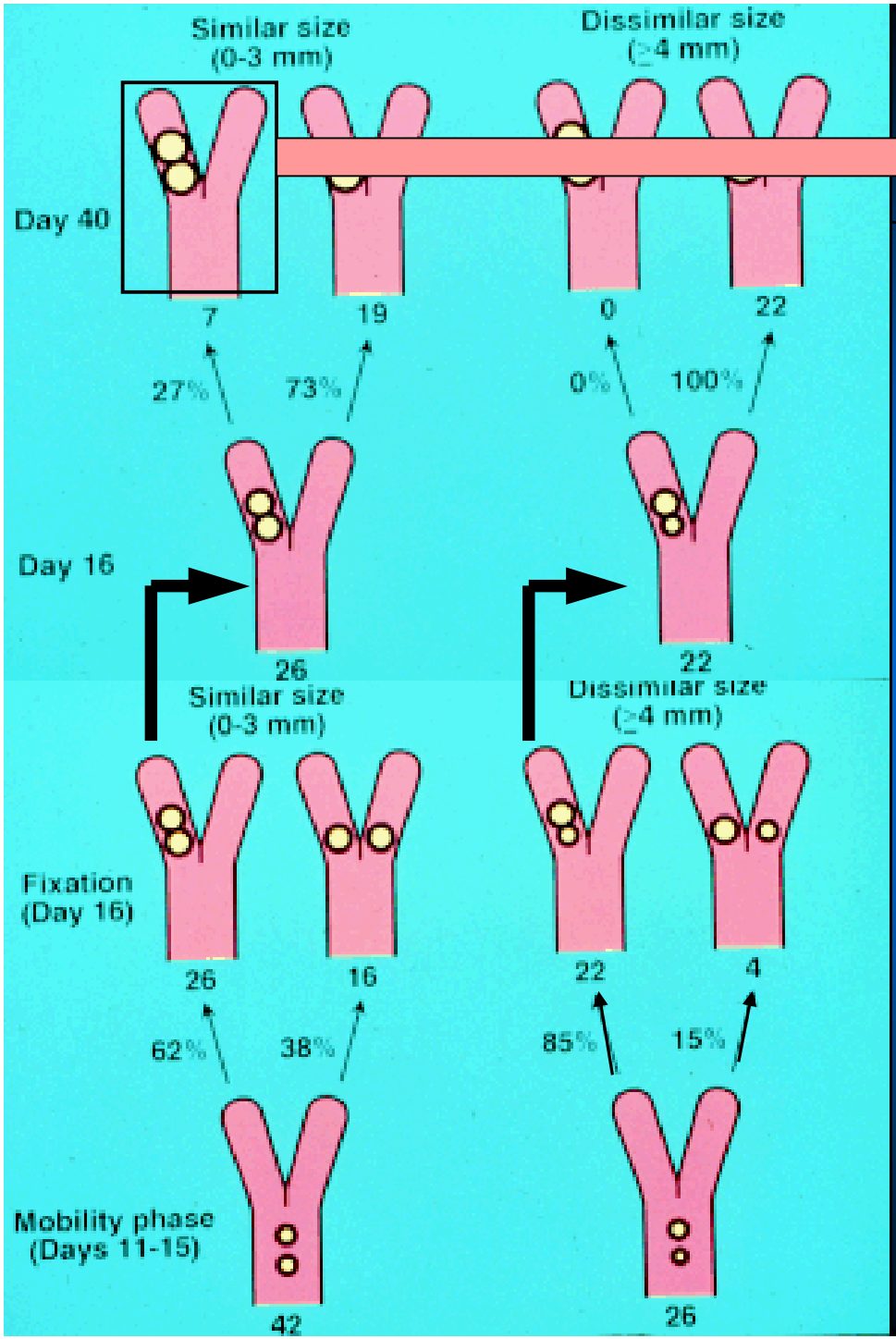



Corpus luteum/
progesterone
PGF2a injection for
induction of estrus
Onset of estrus
Ovulation
Onset of estrus
Ovulation
Onset of estrus
Ovulation
Normal estrus cycle
Shorten
estrus cycle
with PGF2a
Progesterone concentration(
12
10
8
6
4
2
0
-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 1
Days of estrus cycle

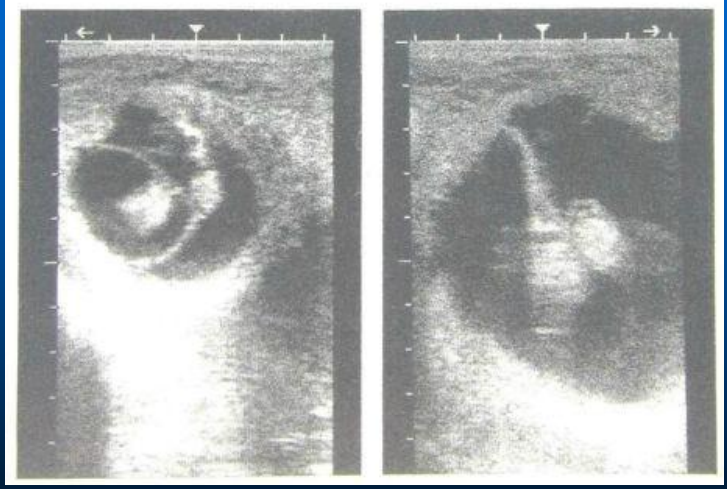


Clinical use of PGF_{2a}

induction of abortion/parturition



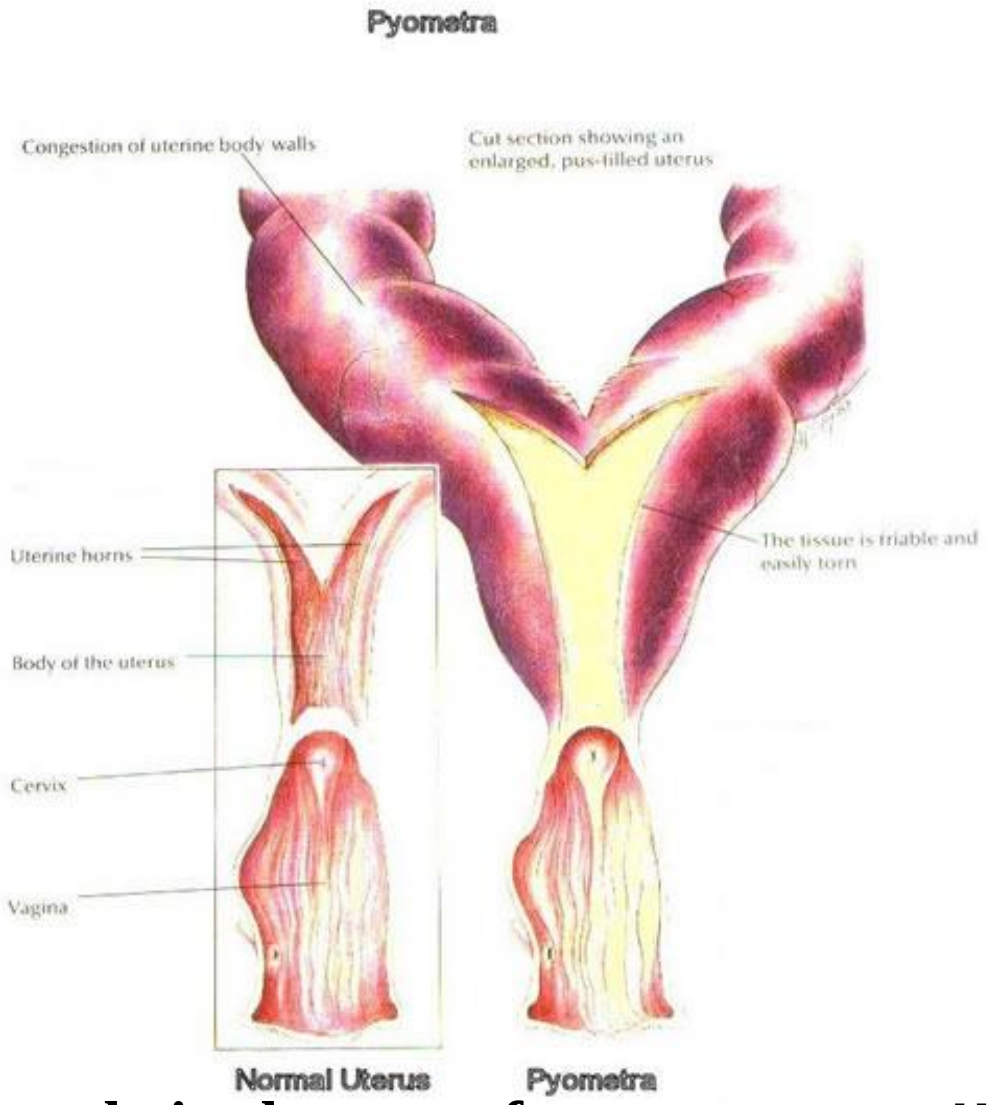
induction of abortion/parturition



Clinical use of PGF_{2a}

induction of abortion/parturition

Clinical use of PGF_{2a}



only in the case of open pyometra!!

induction of abortion
endometritis/pyometra

misalliance

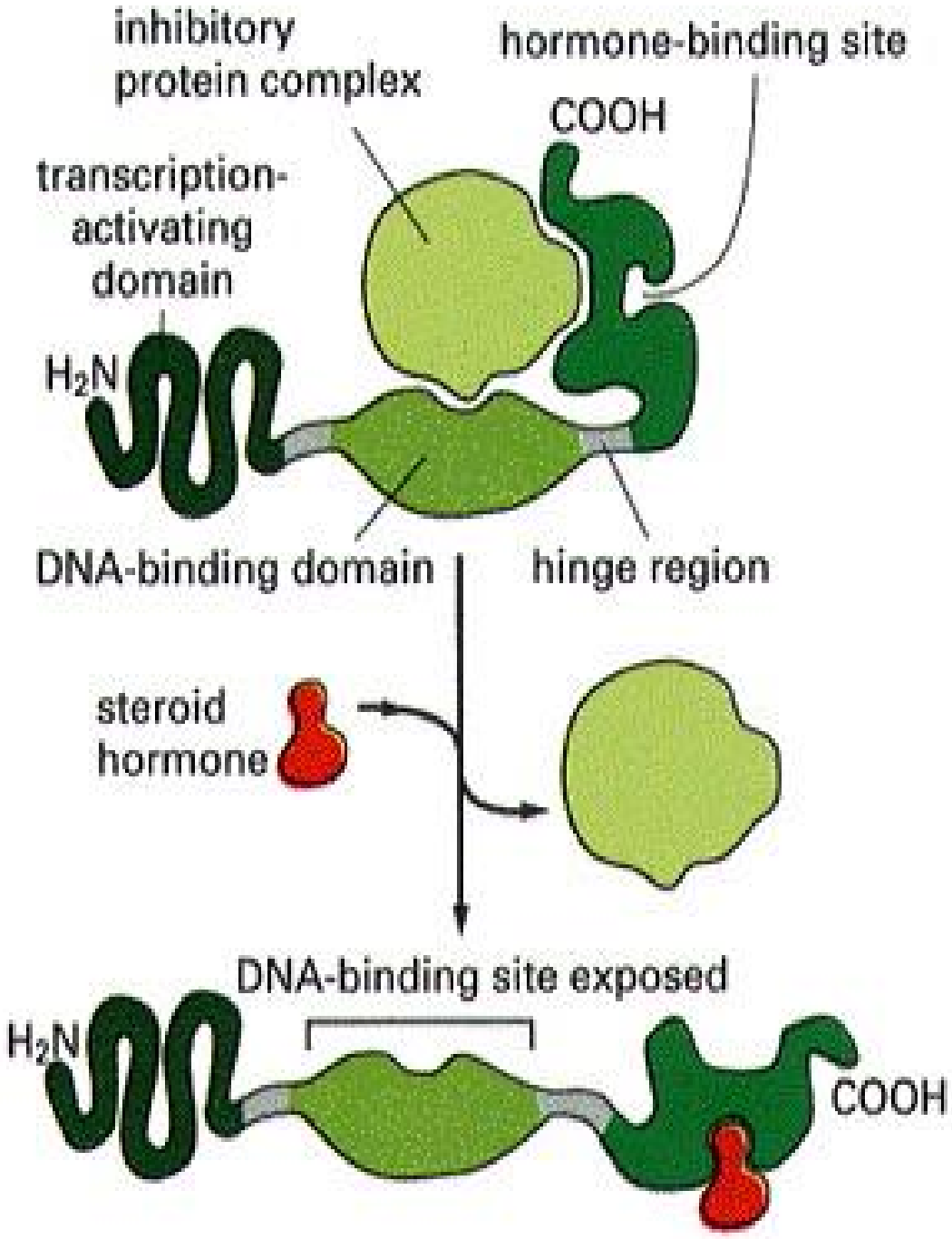
Hormone receptors

- **Steroid hormones**
  - **fat soluble, able to enter all cells**
  - **low concentration (estrogen: 10-150 pmol/L in non-pregnant female)**
    - **Receptors enable the target cells**

      -to concentrate the hormone within the cell

      -to elicit particular cellular responses

      -to create a biological response on specific binding



The steroid hormone receptor is made up of three domains:

1. **a modulating N-terminal domain (transcription-activation domain),**
2. **a DNA-binding domain**
3. **a C-terminal steroid-binding domain.**

Hormone receptors

protein-bound steroid hormone ⟷ non-protein-bound hormone

passive diffusion

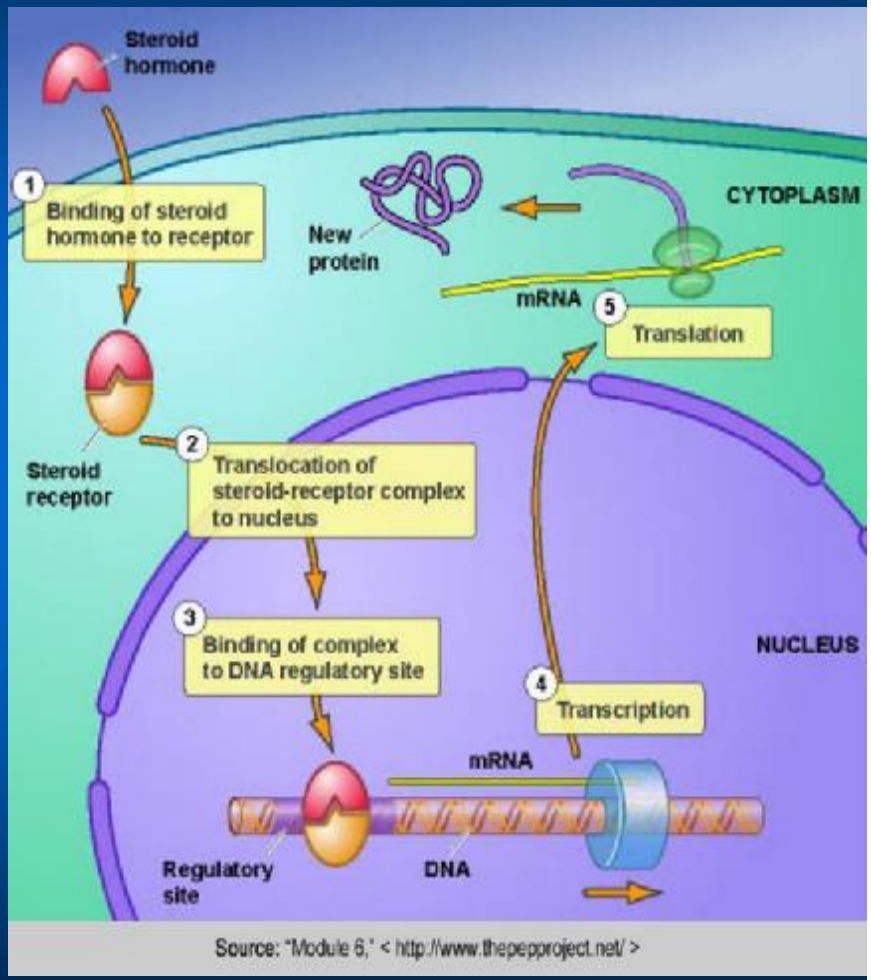
receptors

steroid-receptor complex

Translocation of complex into nucleus

RNA synthesis ↑ → protein synthesis ↑

Target cells

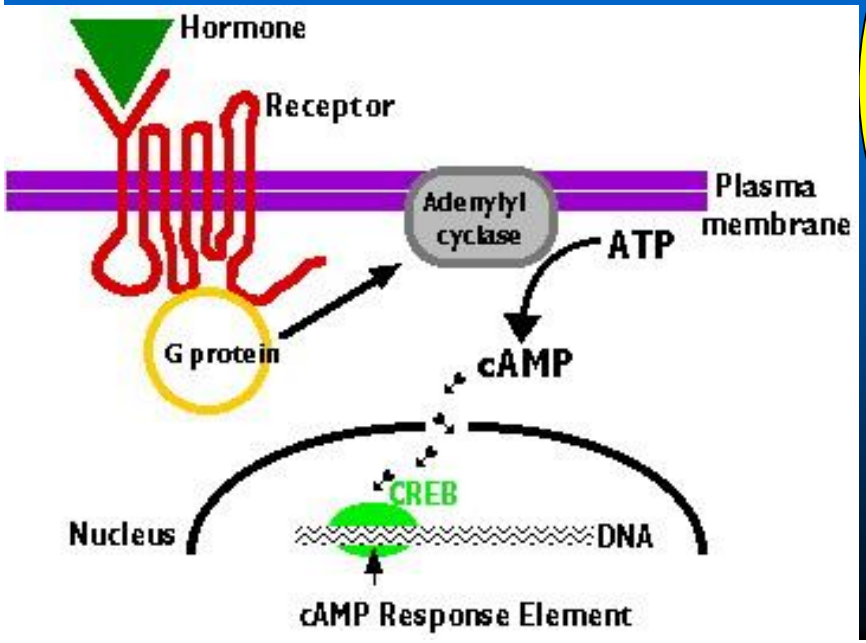


E2: mammary gland

P4: endometrium

T: sertoli cell

Hormone receptors



Protein hormone

protein-receptor complex

receptors

G protein

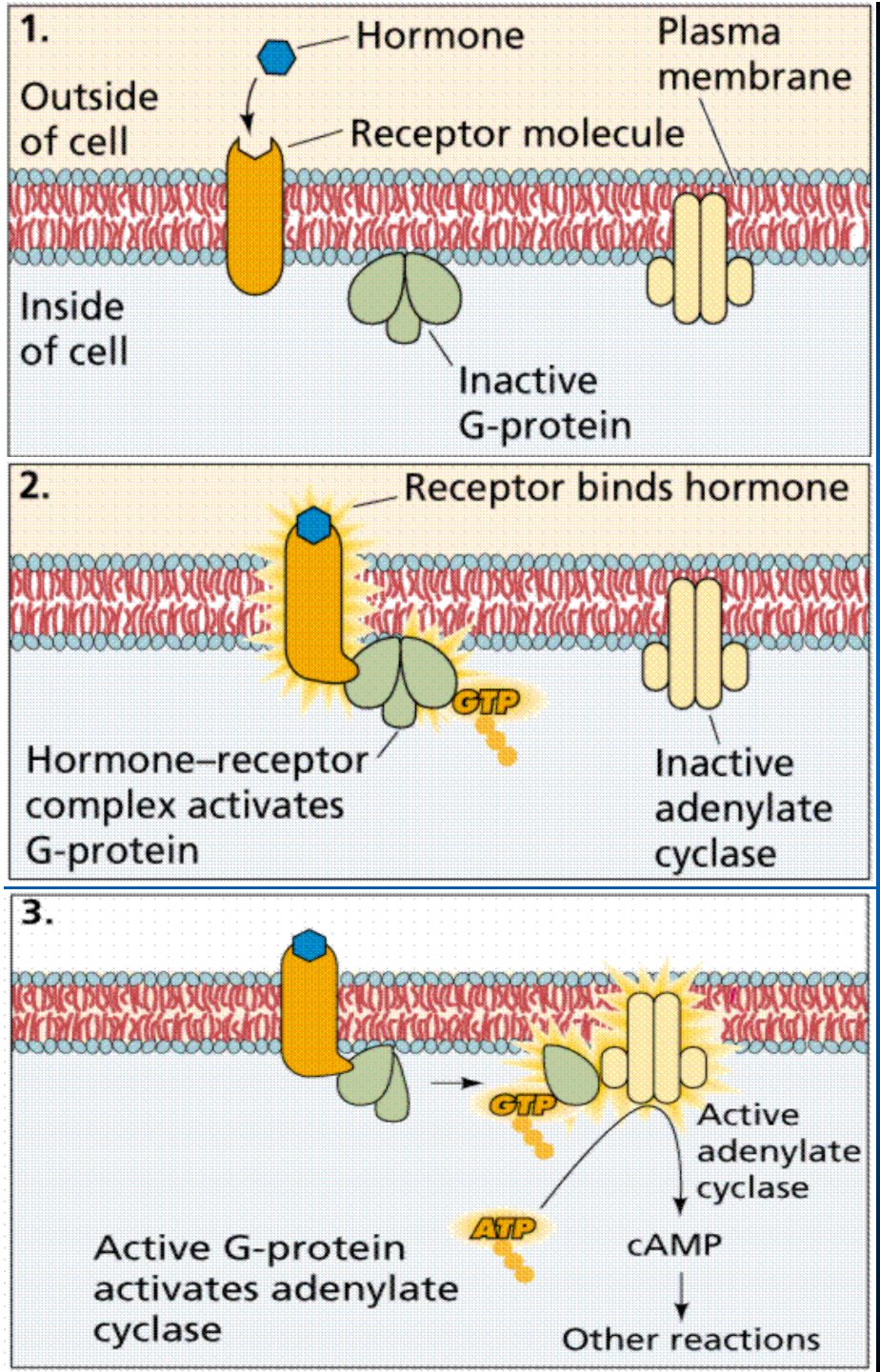
cyclic AMP

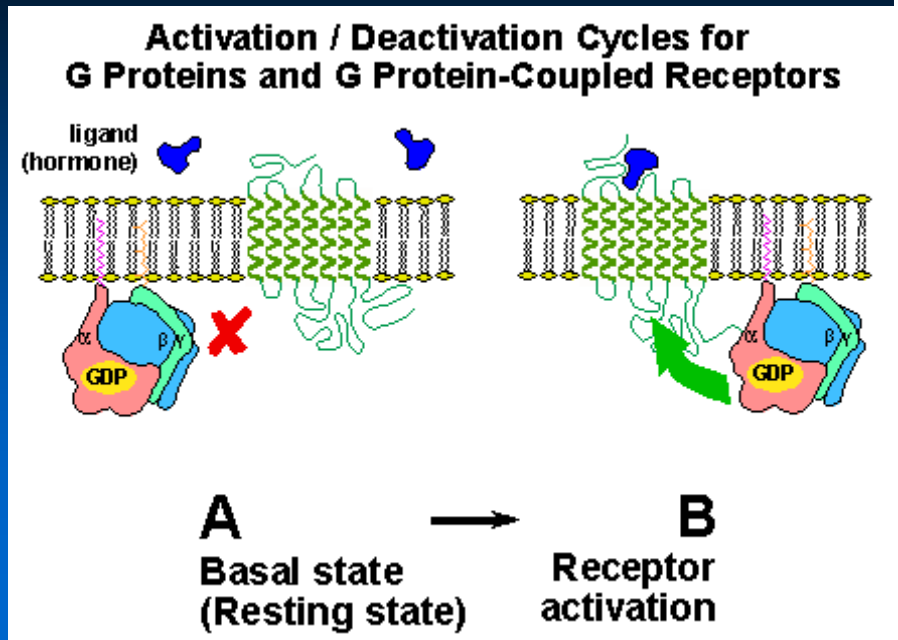
actions of FSH and LH in gonads

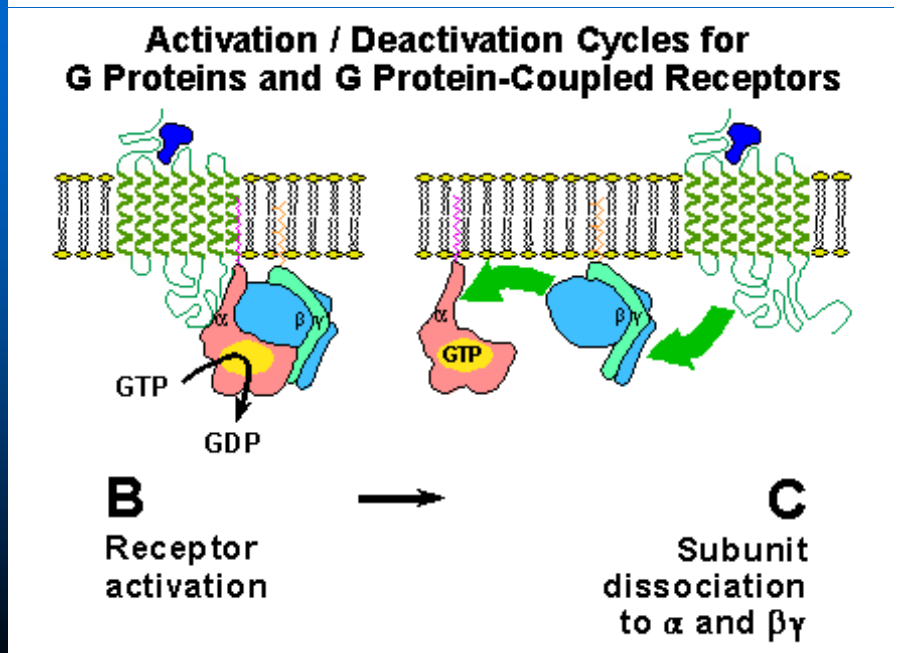
Target cells

protein kinase

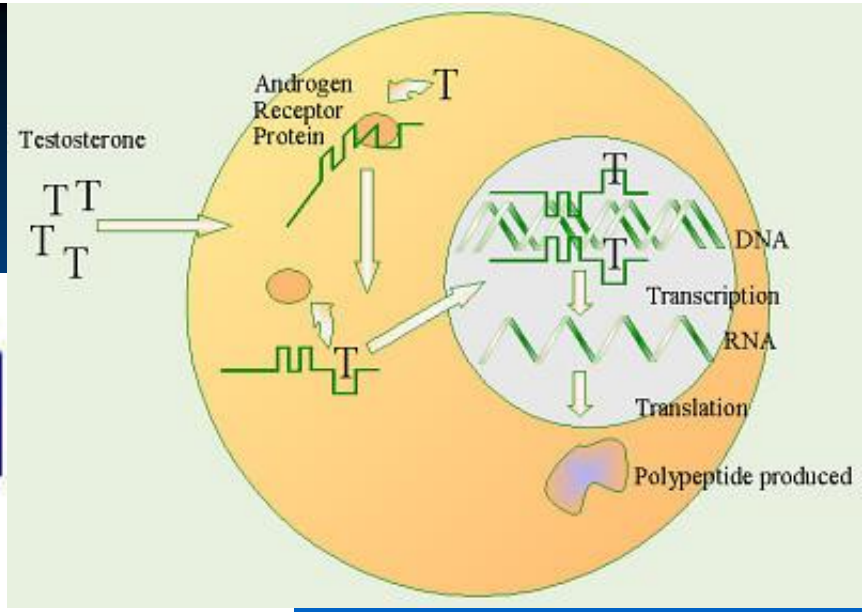
actions of cholesterol

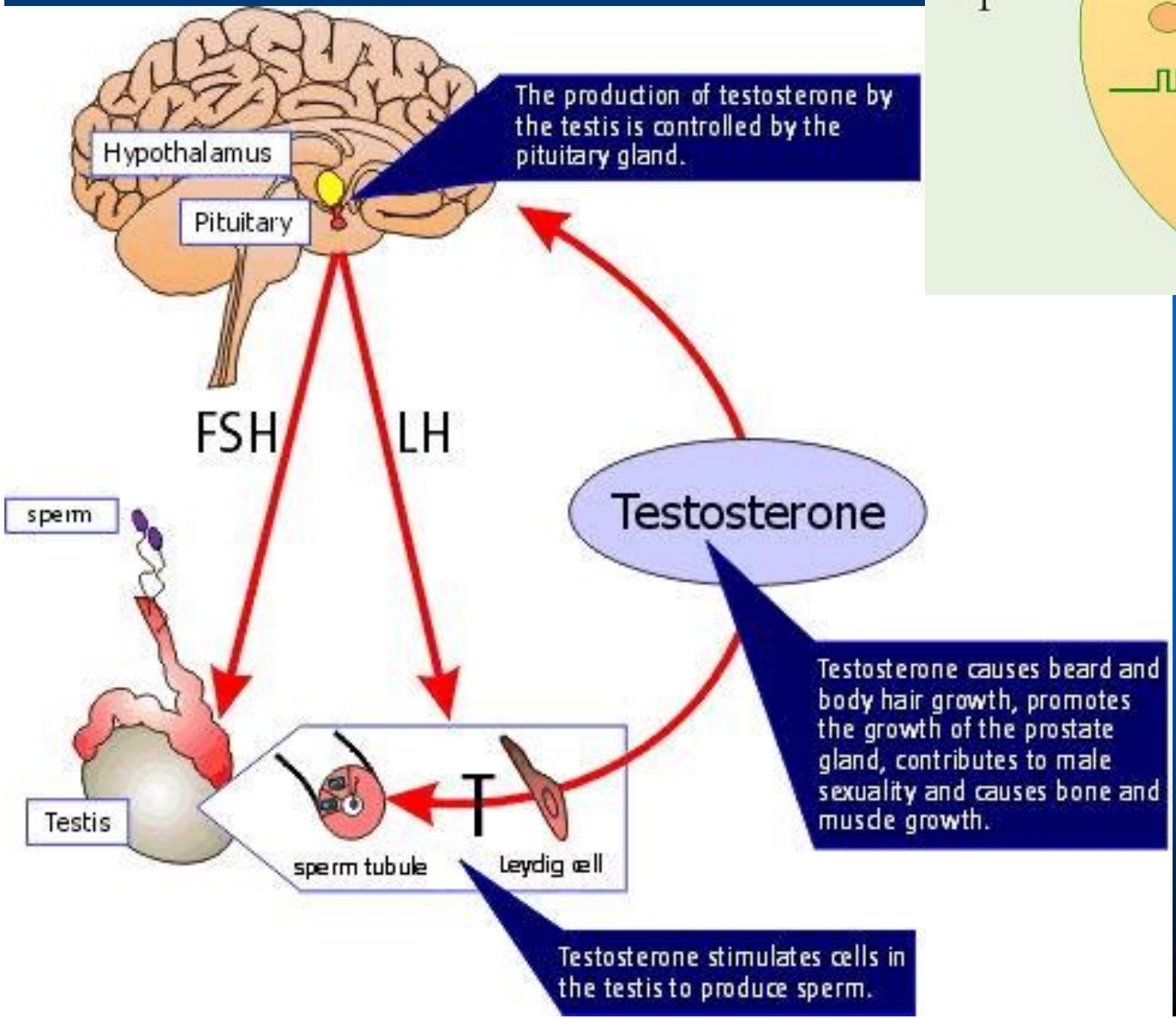

1.
Hormone
Plasma membrane
Outside of cell
Receptor molecule
Inside of cell
Inactive G-protein
2.
Receptor binds hormone
GTP
Hormone–receptor complex activates G-protein
Inactive adenylate cyclase
3.
GTP
Active adenylate cyclase
ATP
cAMP
Active G-protein activates adenylate cyclase
Other reactions


Activation / Deactivation Cycles for
G Proteins and G Protein-Coupled Receptors
ligand (hormone)
α
βγ
GDP
A
Basal state (Resting state)
B
Receptor activation


Activation / Deactivation Cycles for
G Proteins and G Protein-Coupled Receptors
α
βγ
GTP
GDP
B
Receptor activation
C
Subunit dissociation to α and βγ

Tissues responsive to androgens

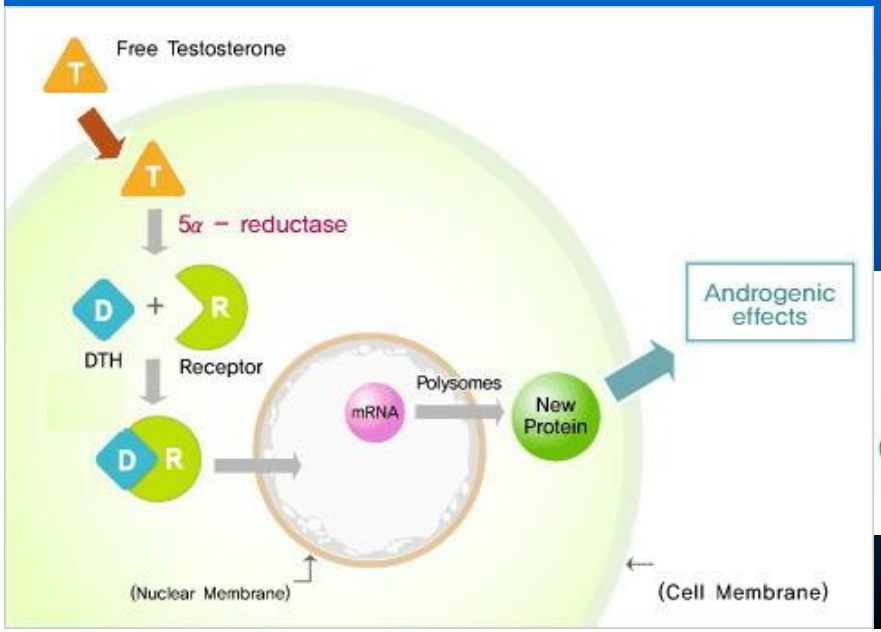


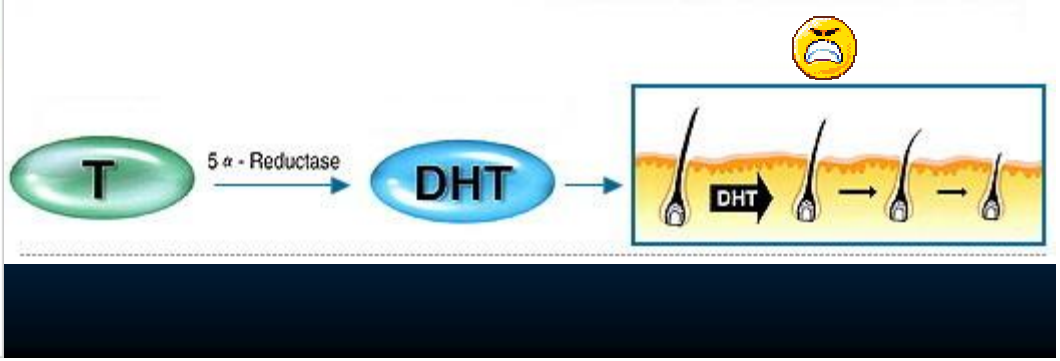


Interconversion of steroids in target tissues

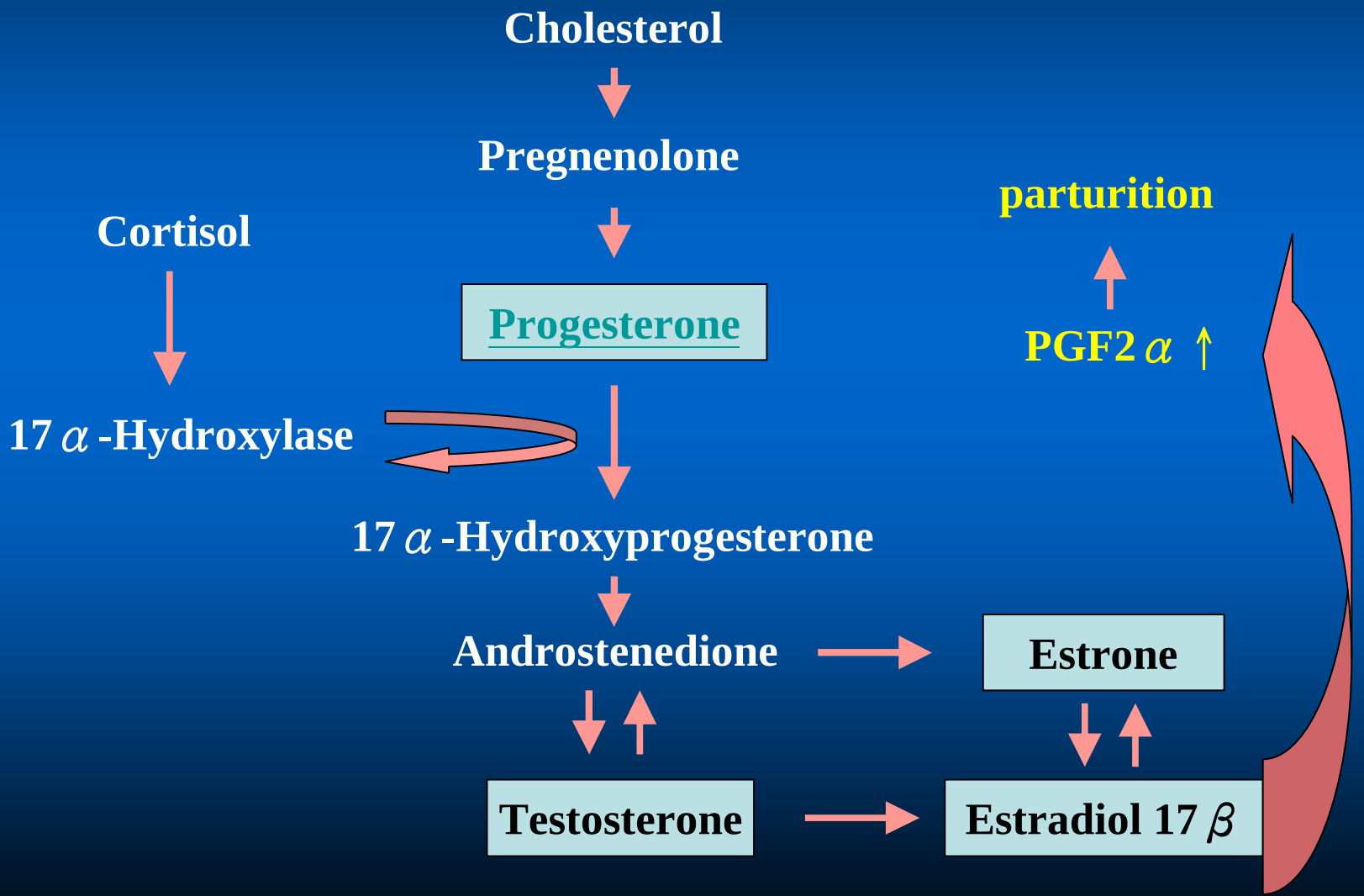
testosterone → 5 α -reductase → 5 α -dihydrotesterone (5 α -DHT)

Target cells





Progesterone conversion in the placenta



Synthesis and clearance of hormones

- **Synthesis and clearance are main the factors, which influence the hormone concentrations.**
  - **The concentration of a hormone reflects its rate of synthesis or secretion.**
  - **metabolized by liver**
  - **detection of the body fluids (plasma, urine, saliva, feces…)**

→ **reproductive condition, ex wild animals**

Synthesis and clearance of hormones

- **under the control of gonadotropins in pulsatile mode**

♂

  - **The secretion of testosterone changes in pulsatile rate.**
  - **varying from 3.5 – 20 nmol/L within hours**

♀

  - **The pulse rate of E_2 and P_4 remains stable.**

Synthesis and clearance of hormones

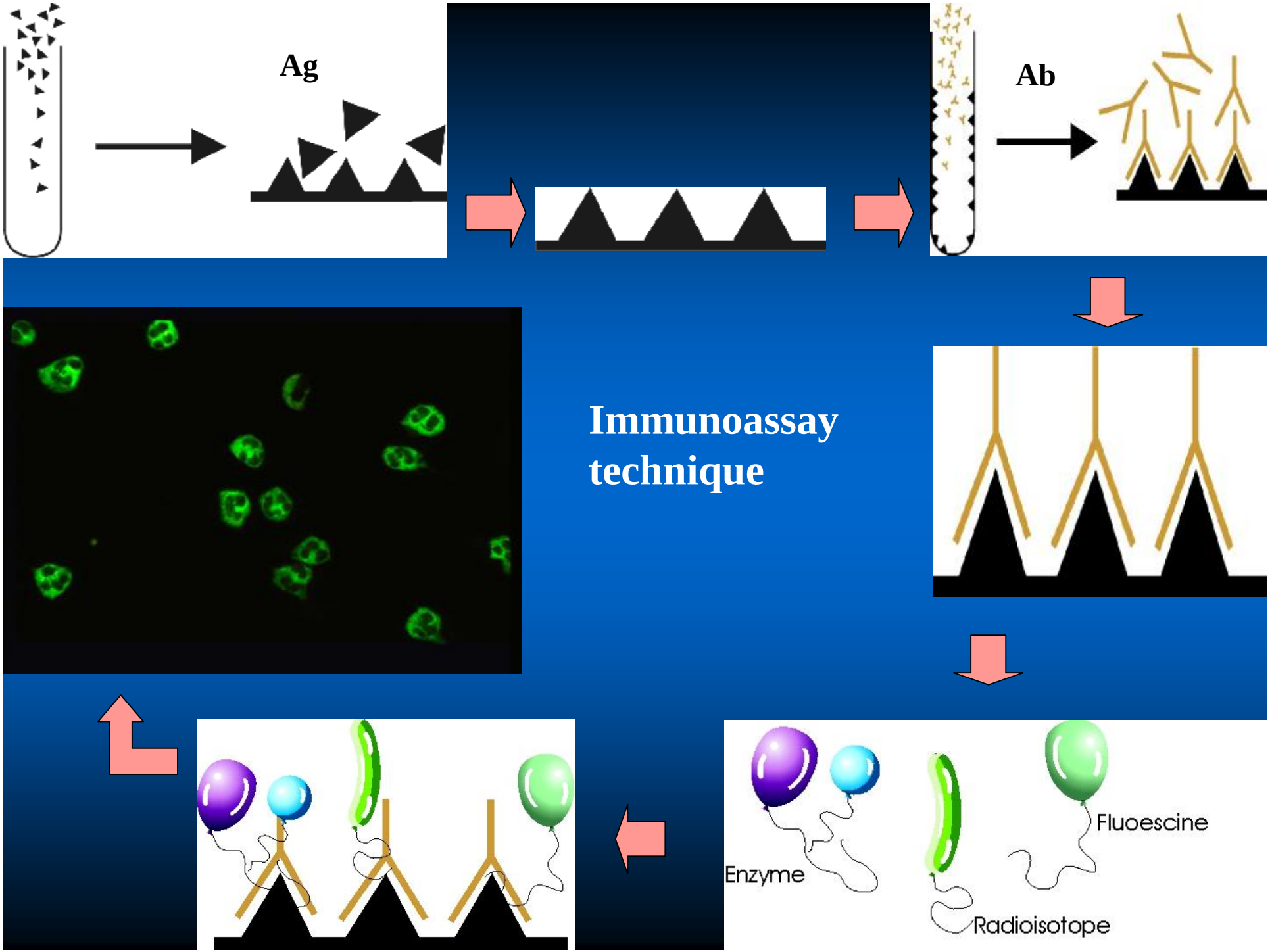
- **Placenta gonadotropins (hCG, eCG)**
  - **produced in high concentration**
  - **longer half-life than the pituitary gonadotropins**
    - **FSH, LH ≒ 10-30 minutes**
    - **hCG ≒ 1.5 days**
    - **eCG ≒ 6 days**
    - **equine LH > other species**

Synthesis and clearance of hormones

- **$PGF_{2\alpha}$ → 15-keto-13,14-dihydro- $PGF_{2\alpha}$**
  - **Half life**
    - **primary $PGF_{2\alpha}$ < 20 seconds**
    - **15-keto-13,14-dihydro-$PGF_{2\alpha}$ ≒ 8 minutes**
  - **25 mg $PGF_{2\alpha}$, IM in cows**
    - **> 90% excreted in the urine and feces (2:1) over 48 hours**

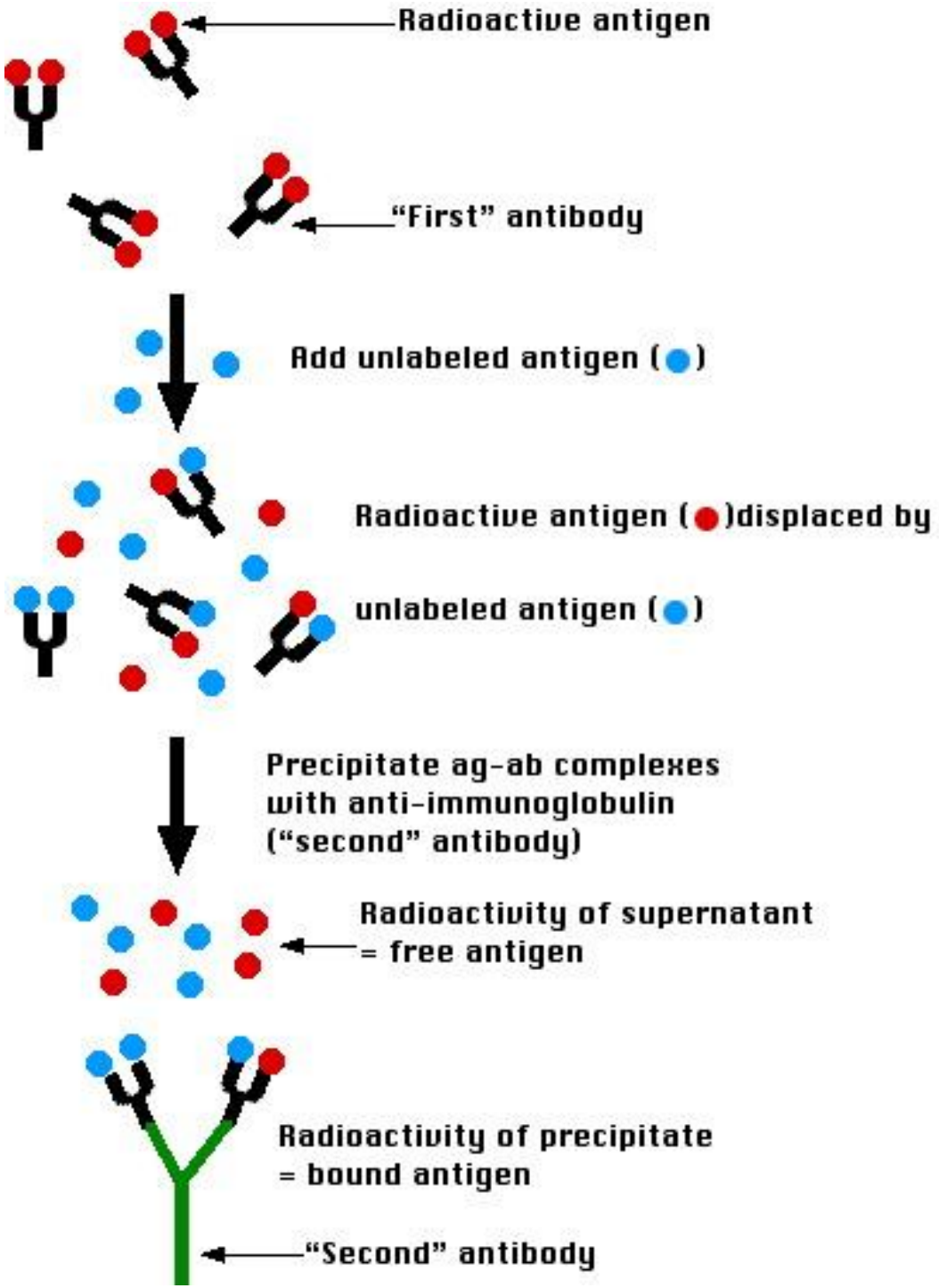
Assay Methods

- **Quantification of specific hormones is necessary for the endocrinological events.**
  - ~~**Weight-gain change**~~
  - ~~**chemical determinations for steroid hormones**~~
  - ~~**urine analysis**~~


Ag
Ab
Immunoassay technique
Enzyme
Fluoescine
Radioisotope

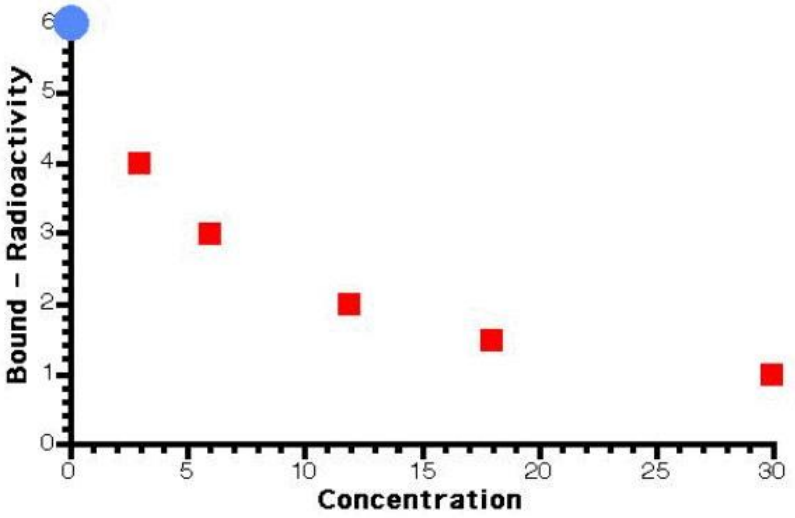
Assay Methods

- **Immunoassay**
  - **Radio-immunoassay (Berson *et al*., 1959), Sen: 10^{-9}**
  - **Enzyme multipled immunoassay technique, Sen:10^{-6}**
  - **Fluoescine immunoassay, Sen: 10^{-9}**
  - **EIA (Engvall *et al*., 1970s), Sen: 10^{-12}**
  - **Chemiluminescence immunoassay, Sen 10^{-15}**

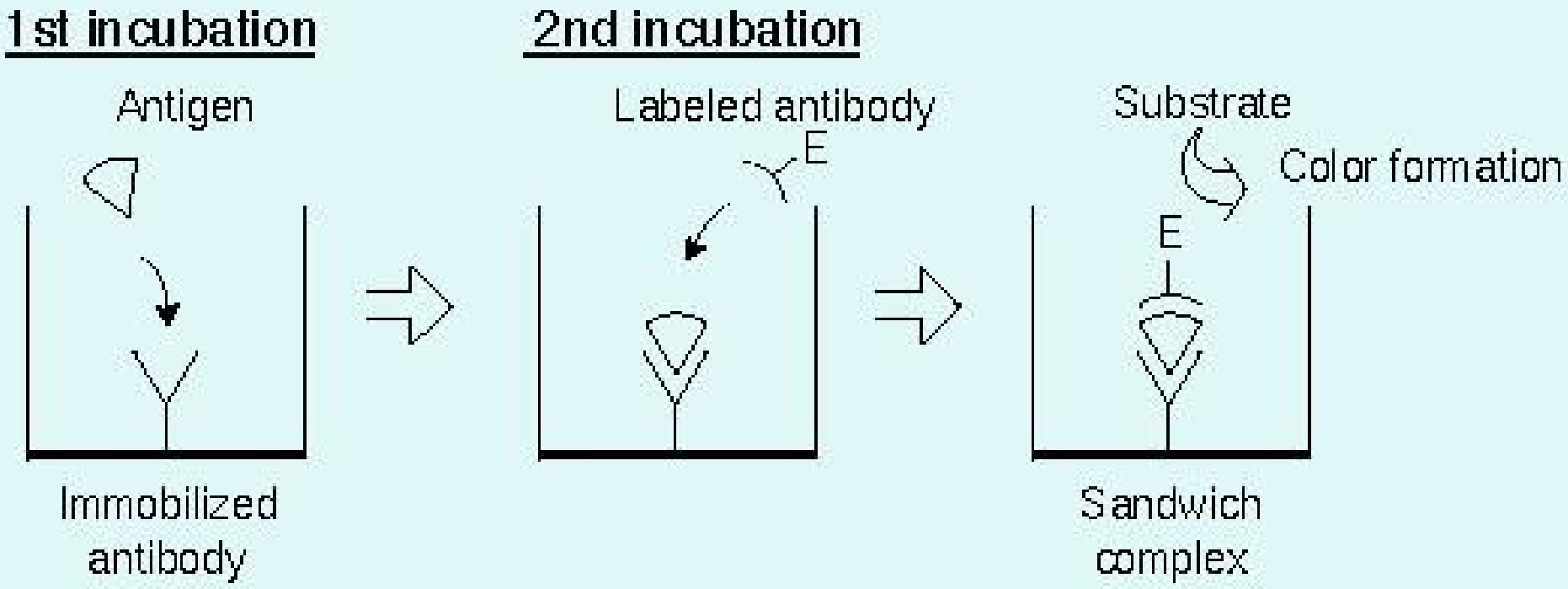


Radioimmunoassay

Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein, the radioactive isotopes ^{125}I or ^{131}I are often used as the radioactive antigen.

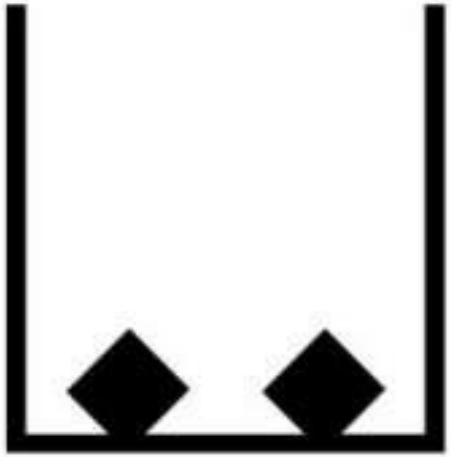


Sandwich EIA

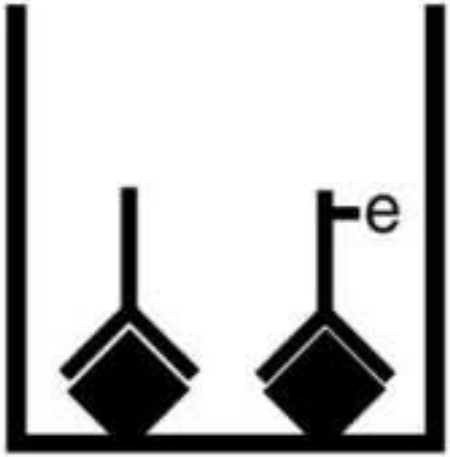


competitive EIA

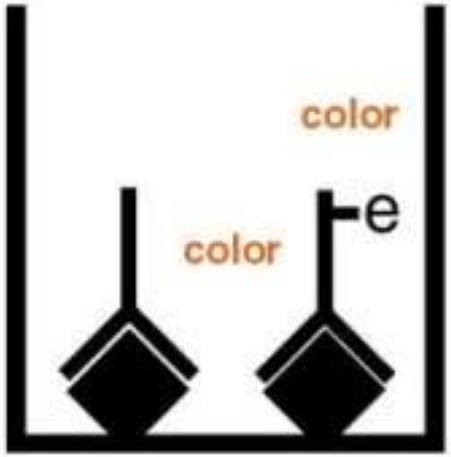
Step 1
Specific antigen is attached to a solid-phase surface



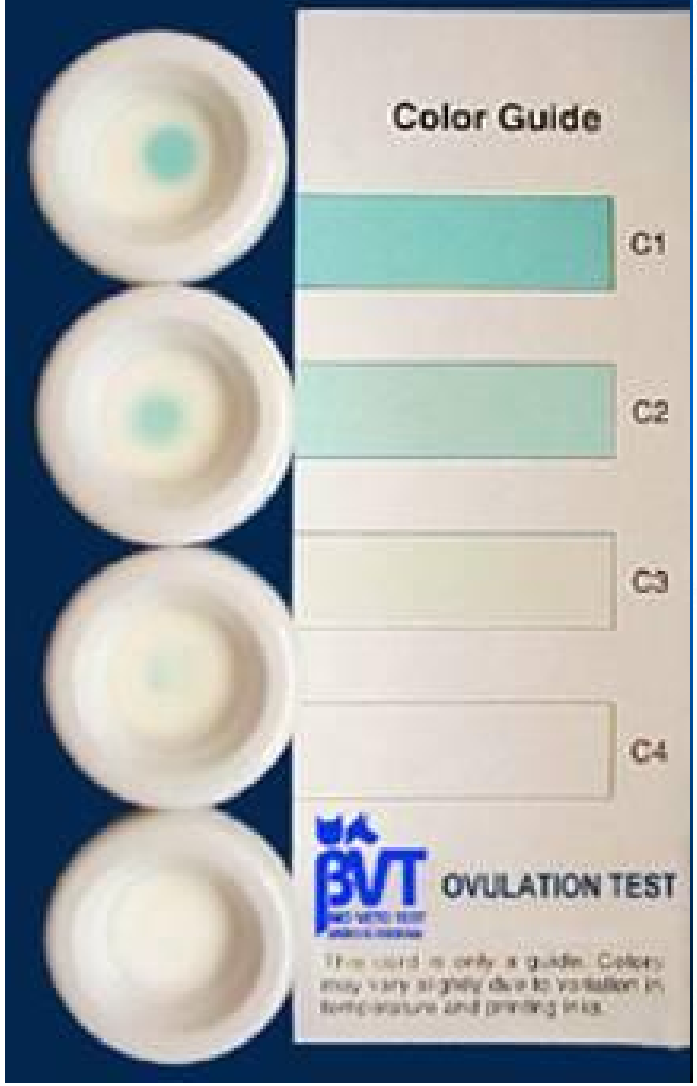
Step 2
Test specimen, which may or may not contain the antibody, and an enzyme-labeled antibody (conjugate) are added together

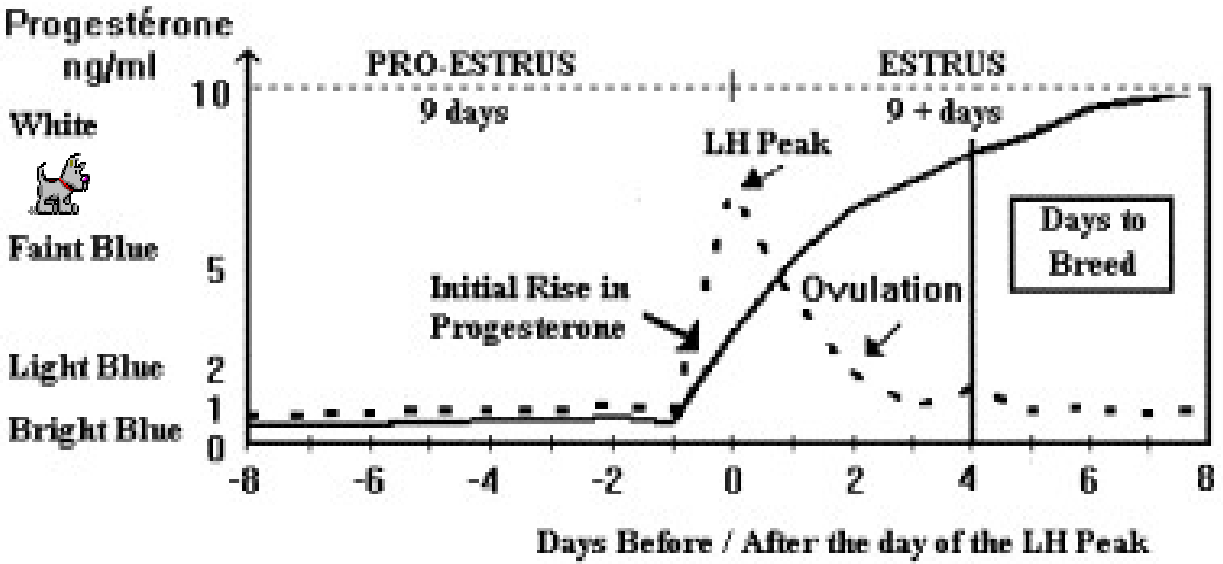


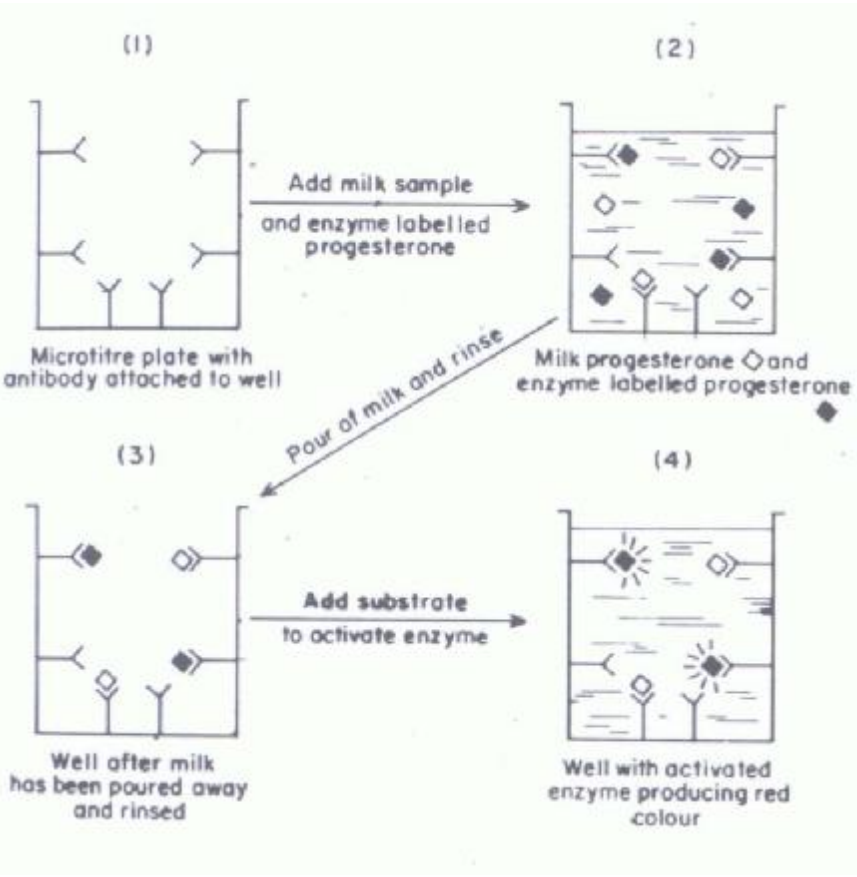
Step 3
Chromogenic substrate is added, which in the presence of the enzyme, changes color. The amount of color that develops is inversely proportional to the amount of antibody in the test specimen.

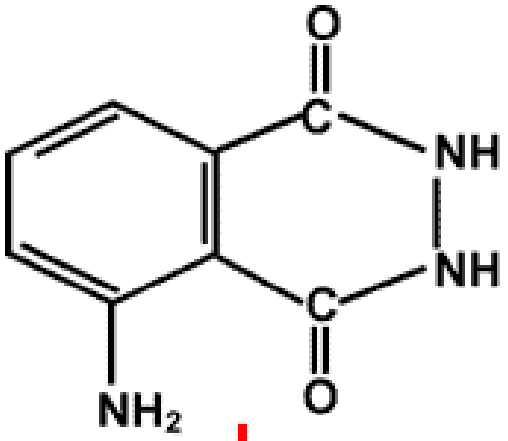


semi quantitative ELISA test



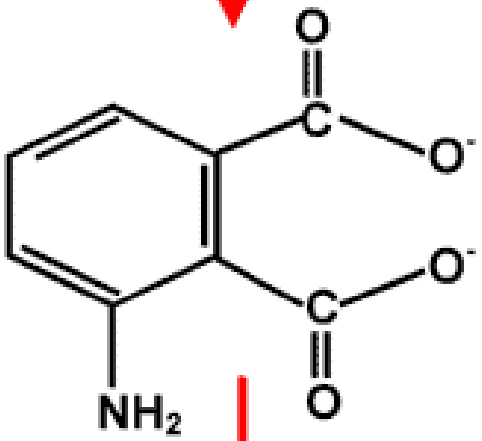




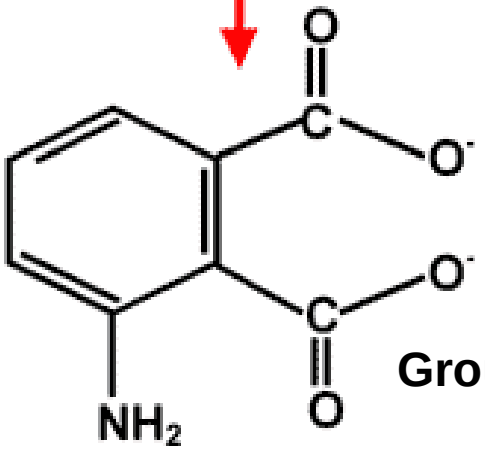


Luminol

+ H_2O_2



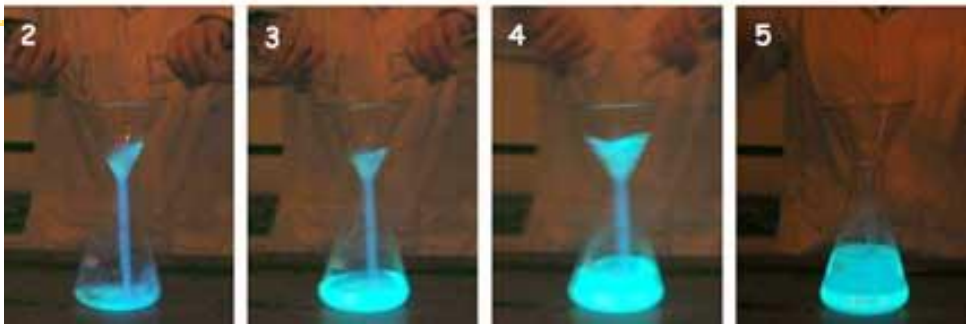
Excited State



Ground State

+

The chemical luminol is oxidised by H_2O_2, to produce a molecule in an excited state. When the excited electrons in the molecules fall back to their ground states, light is given out.



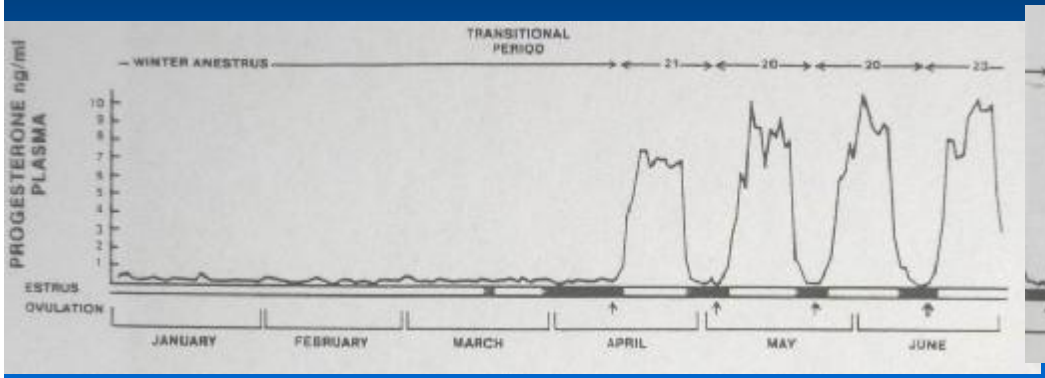
Assay Methods

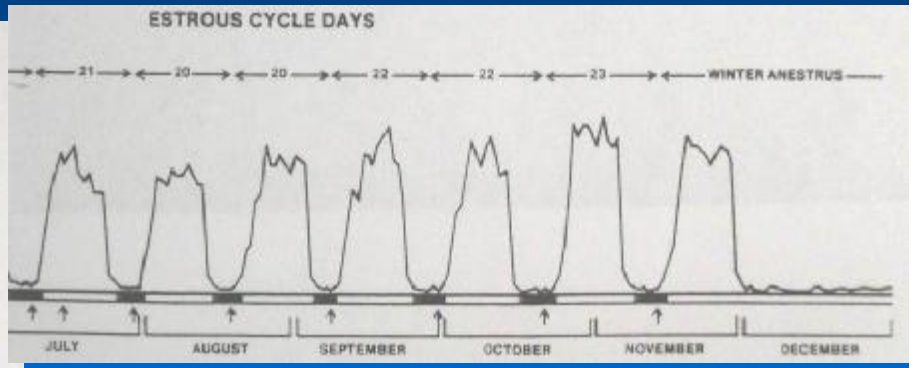
- **Immunoassay**
  - **Radio-immunoassay (Berson *et al*., 1959), Sen: 10^{-9}**
    - **Competitive protein binding**
  - **EIA (Engvall *et al*., 1970s), Sen: 10^{-12}**
  - **Chemiluminescence immunoassay, Sen 10^{-15}**

Diagnostic aids

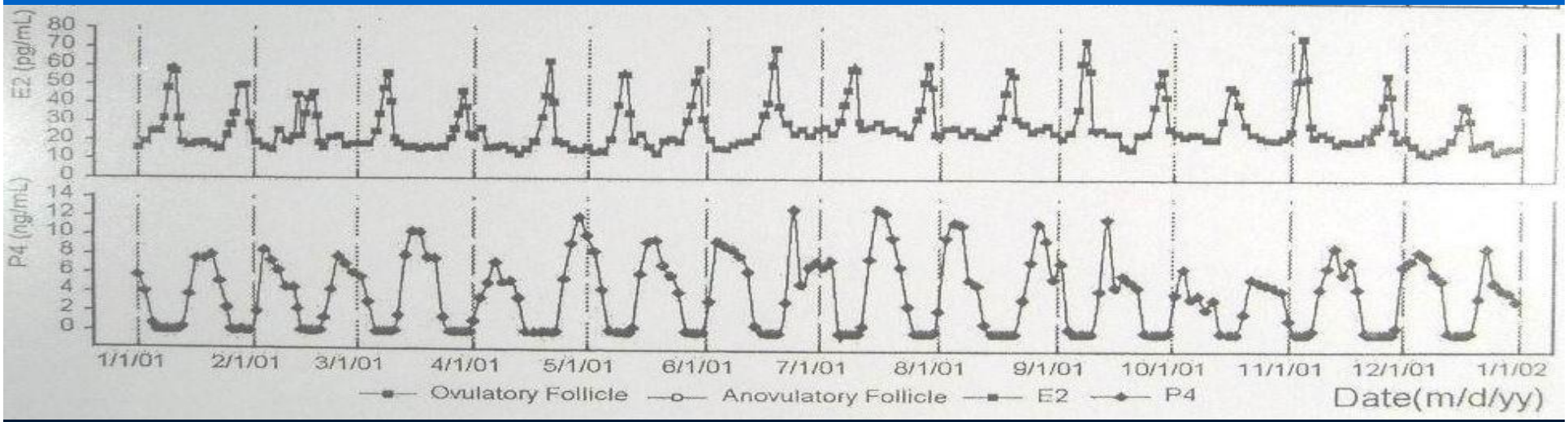
Therapy-monitoring

Monitoring the seasonal estrus activity of mare





Northern semi-sphere

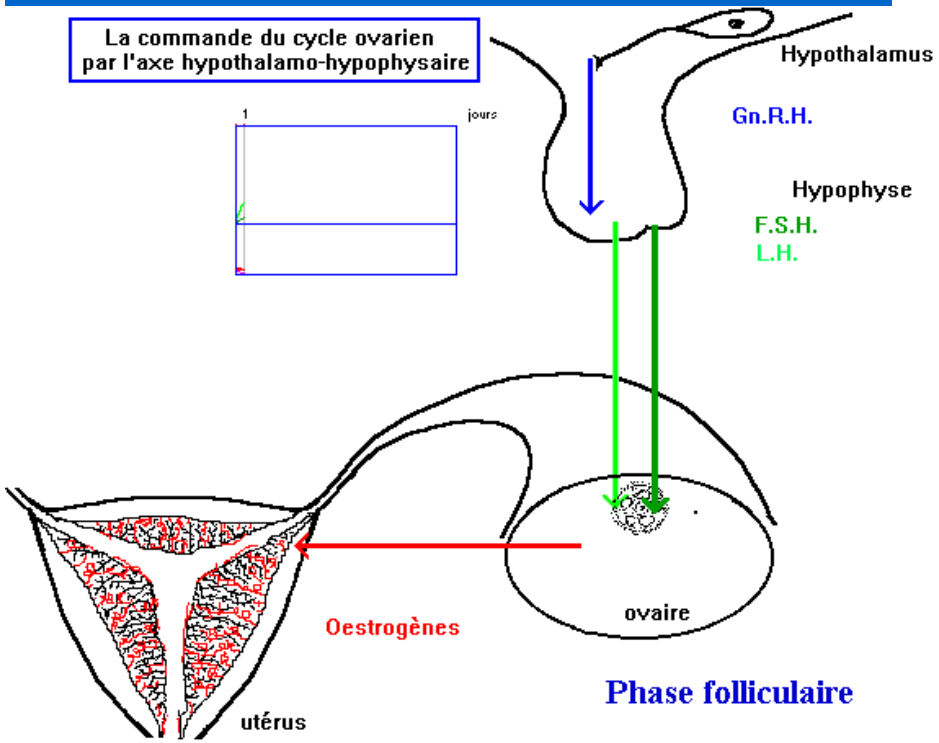


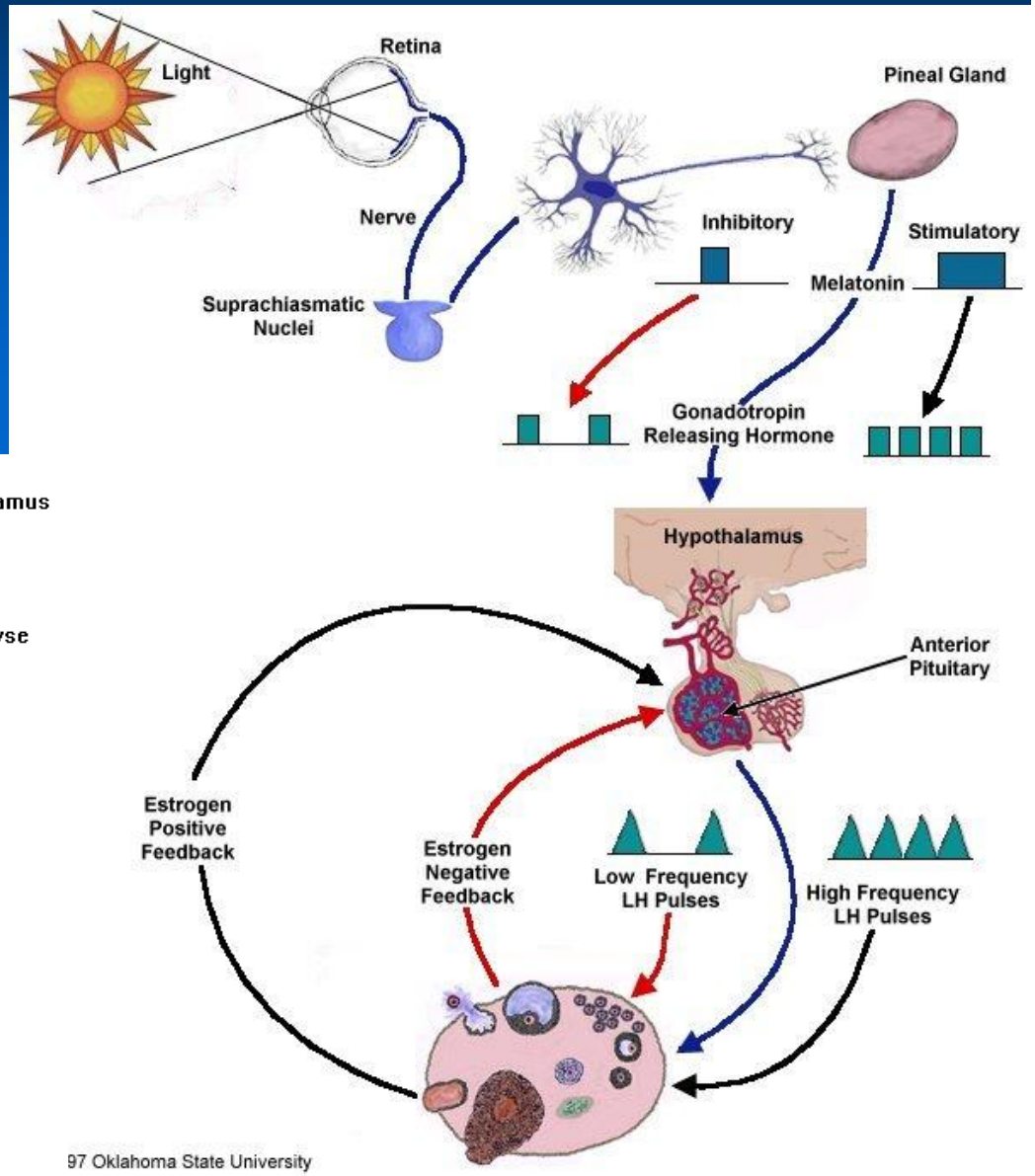
Subtropical Taiwan

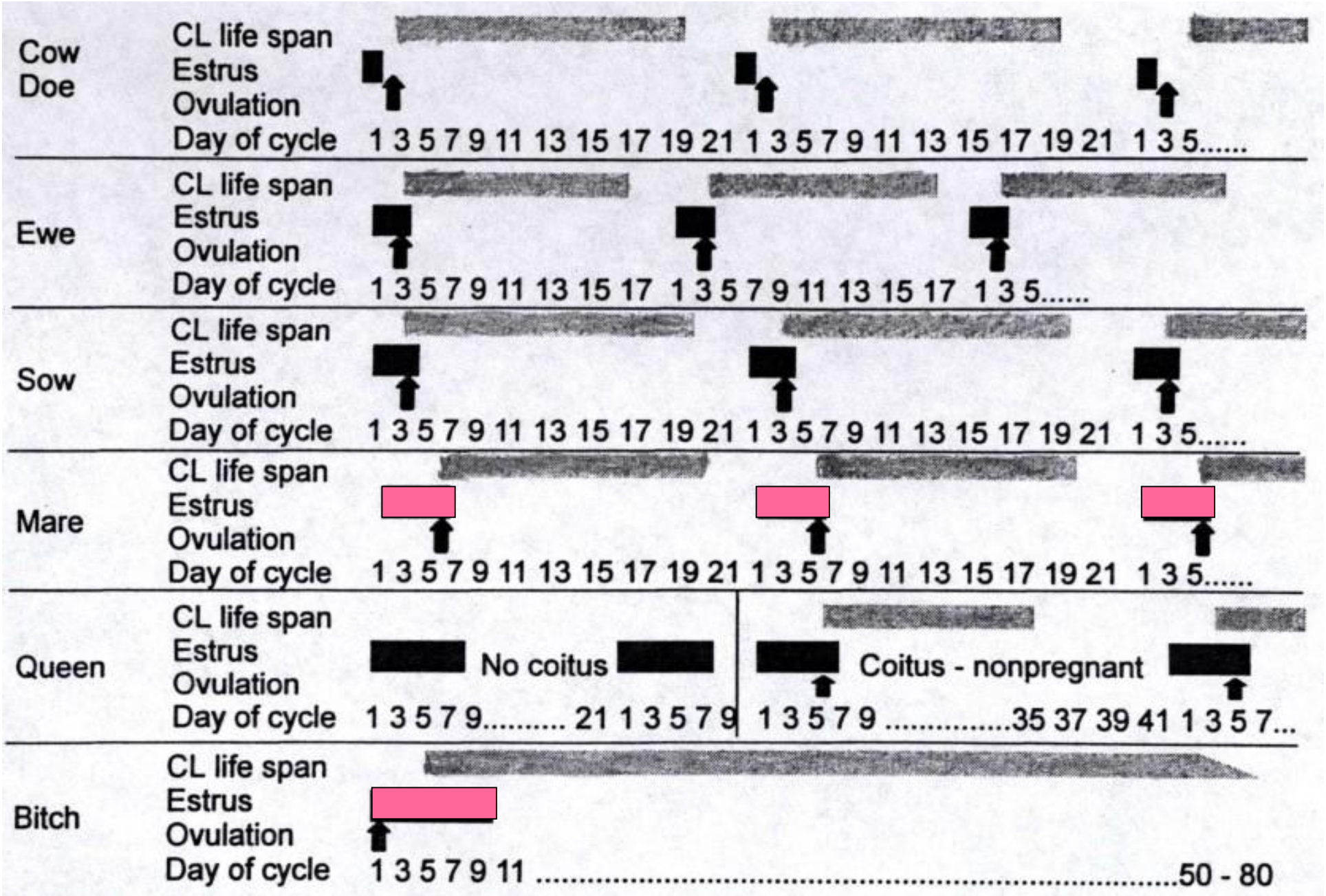
Physiology of reproductive hormones in the female

- Estrous cycle
- Control of the corpus luteum
- Early pregnancy
- Pregnancy and parturition

Endocrine control of cyclical reproductive activity







The duration of estrus, time of ovulation and duration of CL function in domestic animals

Influence of the length of day light on the ovary function in mares

increasing day light

decreasing day light

Photoreceptors in the eyes

neurotransmission

Pineal

Melatonin ↓ ↑ **Melatonin**

Hypothalamus

portal system

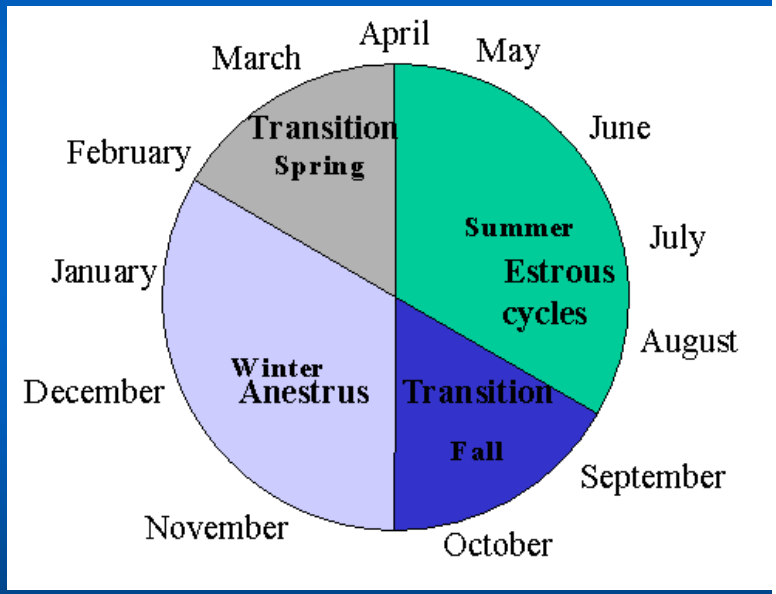
GnRH ↑ ↓ **GnRH**

Anterior pituitary

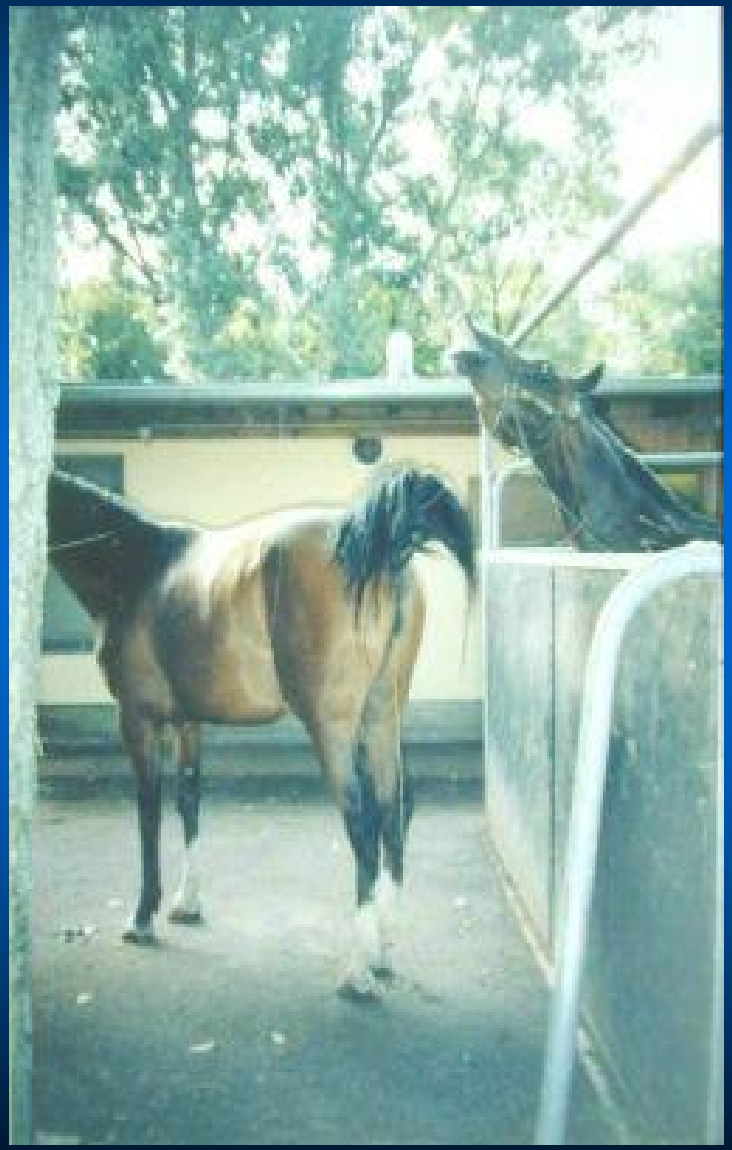
blood circulation

Gonadotropine ↑ ↓ **Gonadotropine**

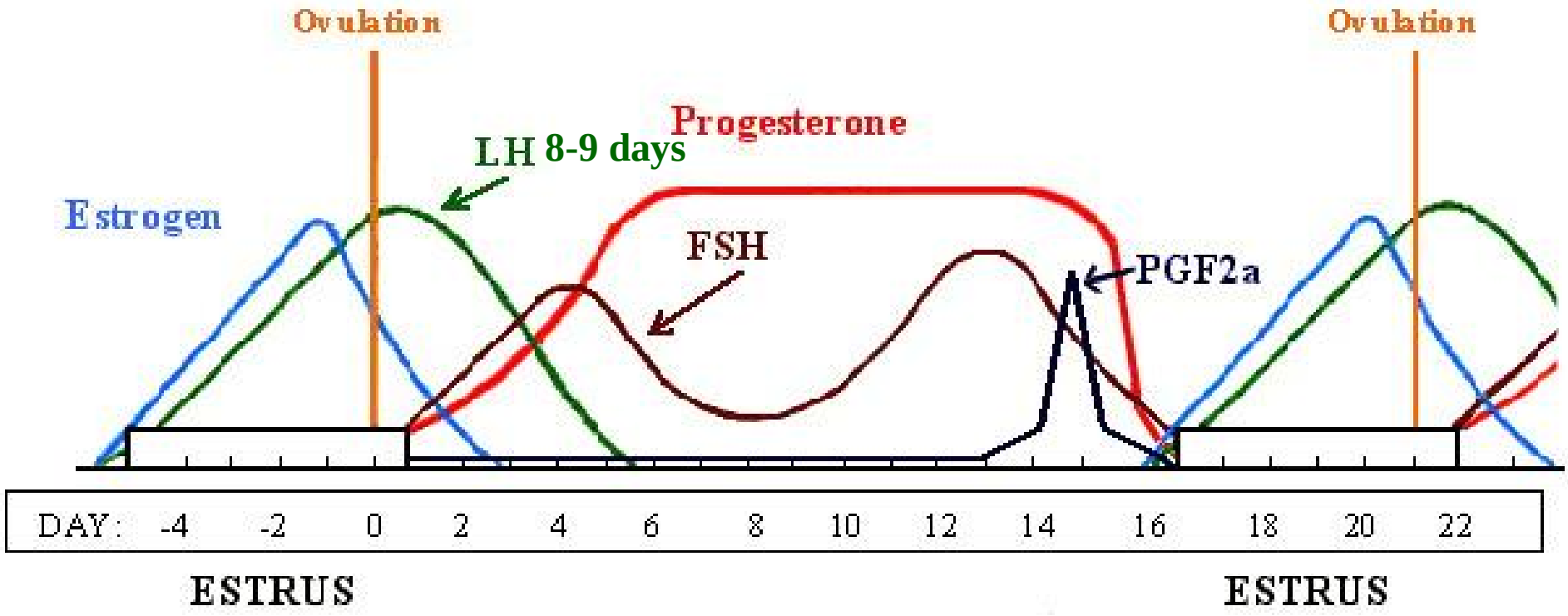
Ovaries



estrous behavior of mares



Large amounts of equine LH are released during an 8- to 9-day period with ovulation occurring on the 3rd to 5th day.



Control of the corpus luteum

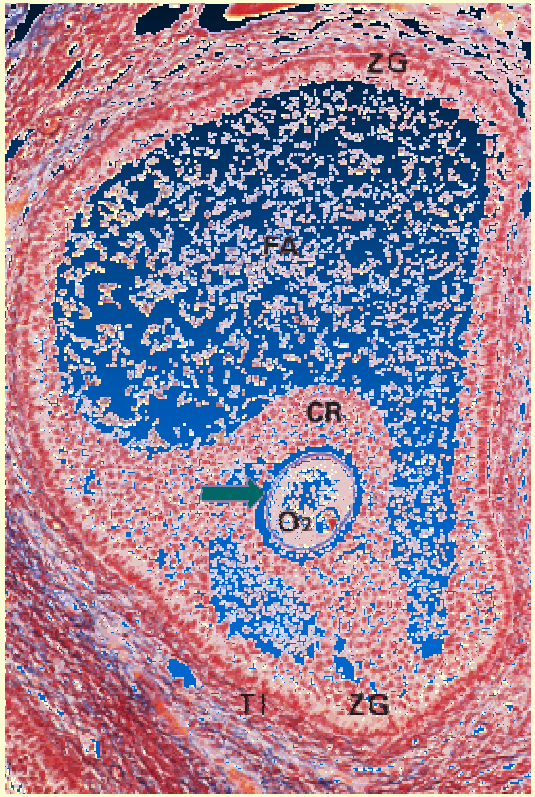
The Ovum

Graafian follicle

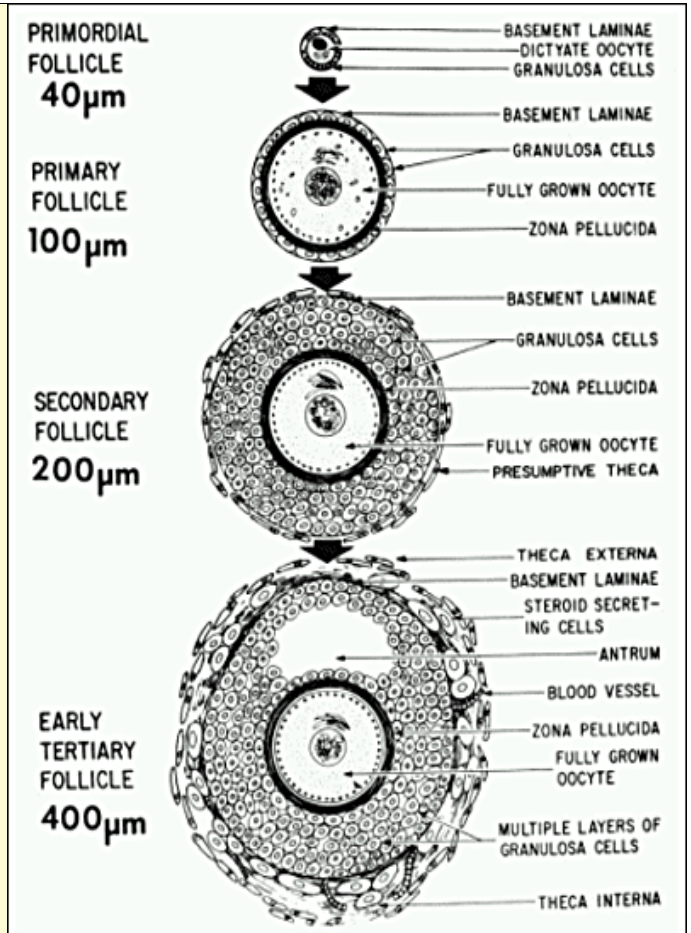
Azan _ 75

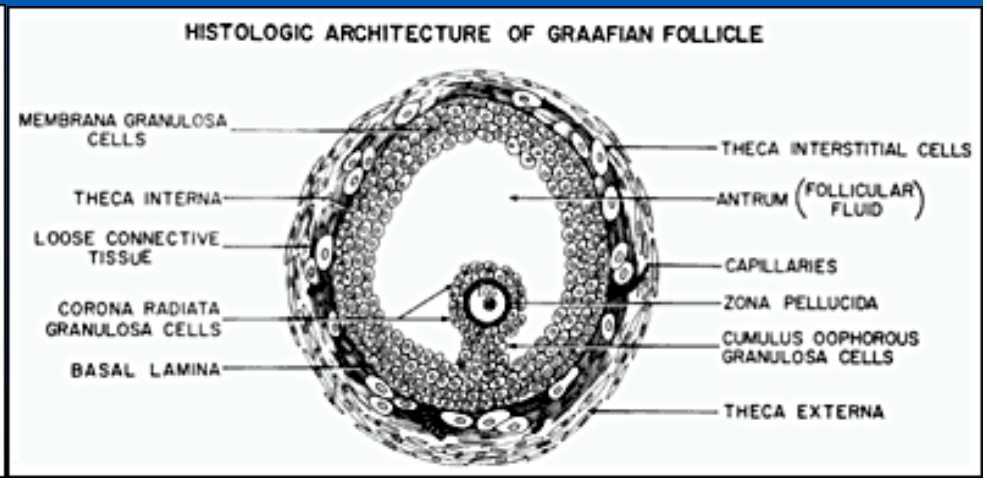
- FA = follicular antrum
- CR = corona radiata
- O2 = secondary oocyte
- ZG = zona granulosa
- TI = theca interna

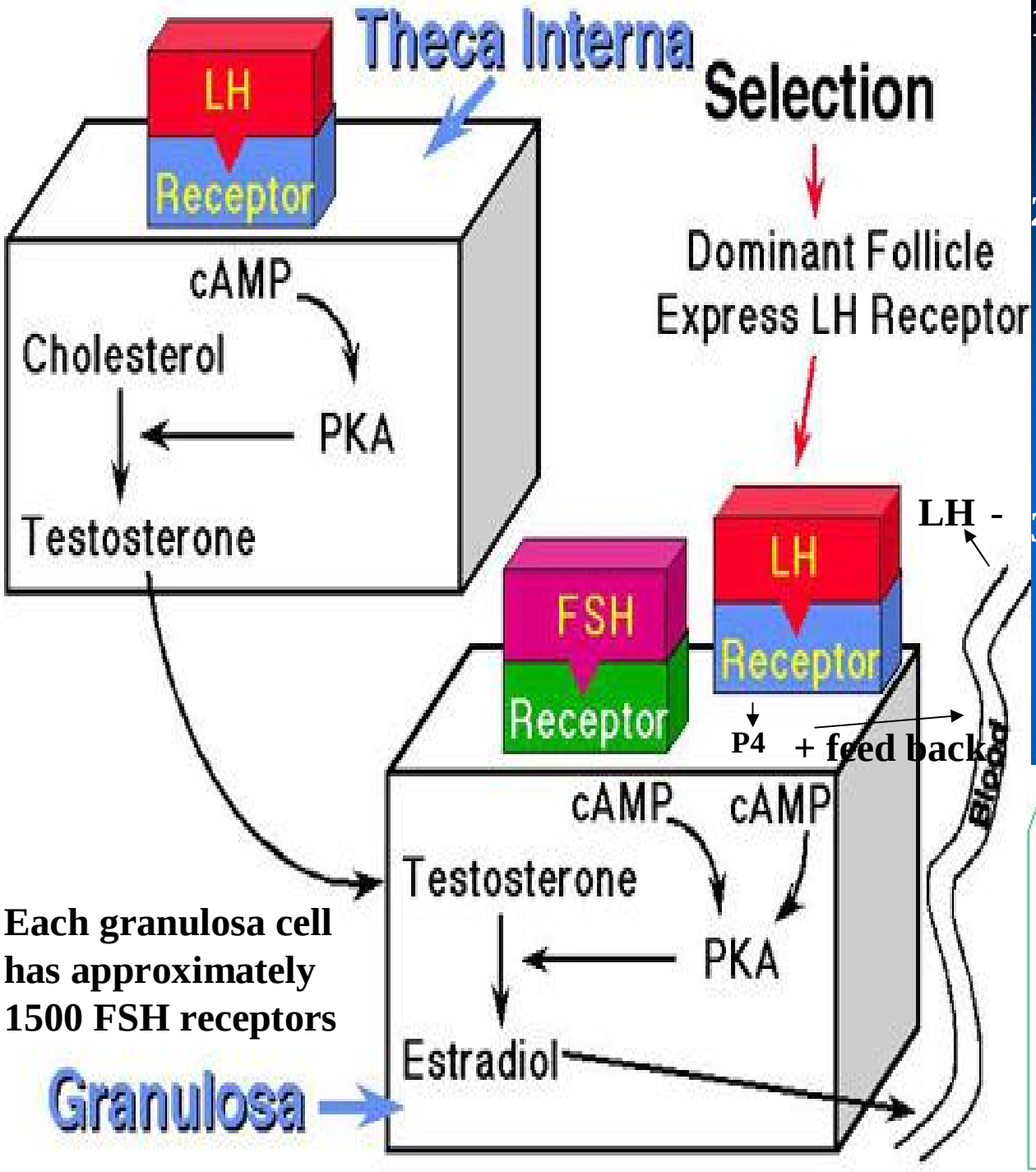
- The oocyte is released along with the corona radiata.
- Notice the clear ring just around the ovum (arrow tip): this is the zona pellucida



From Wheater's Functional Histology, 4th ed. 2000.

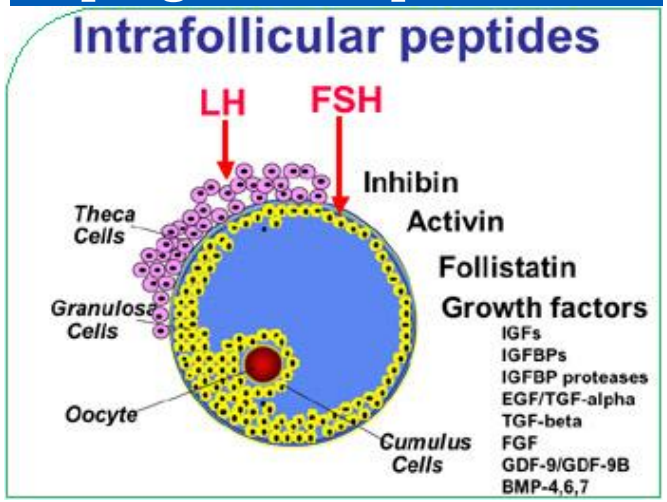


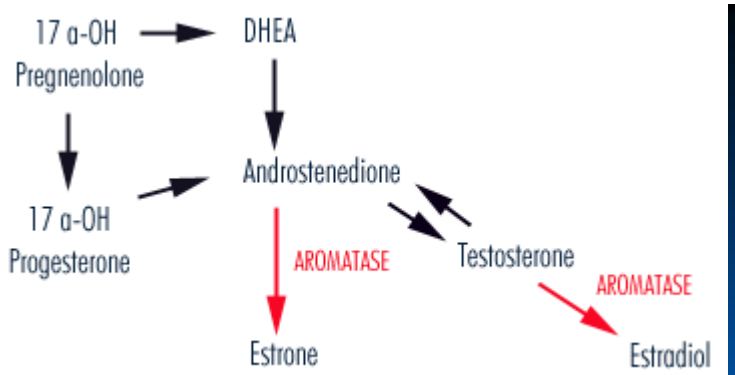


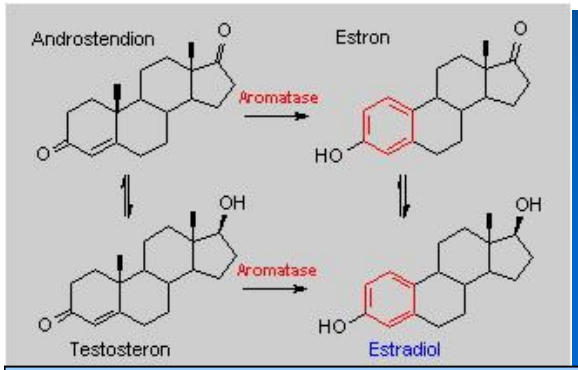


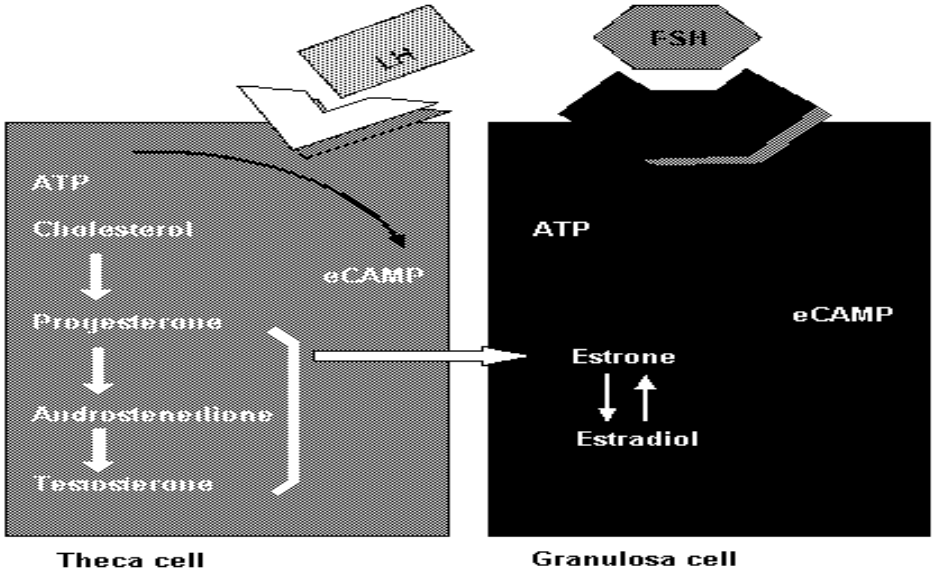
Each granulosa cell has approximately 1500 FSH receptors

1. theca interna has LH receptor and produces testosterone.
2. granulosa cell has FSH receptor which causes cell to take testosterone from theca and convert it to estradiol.
3. dominant follicle has LH receptors on granulosa which can respond to LH to drive progesterone production.









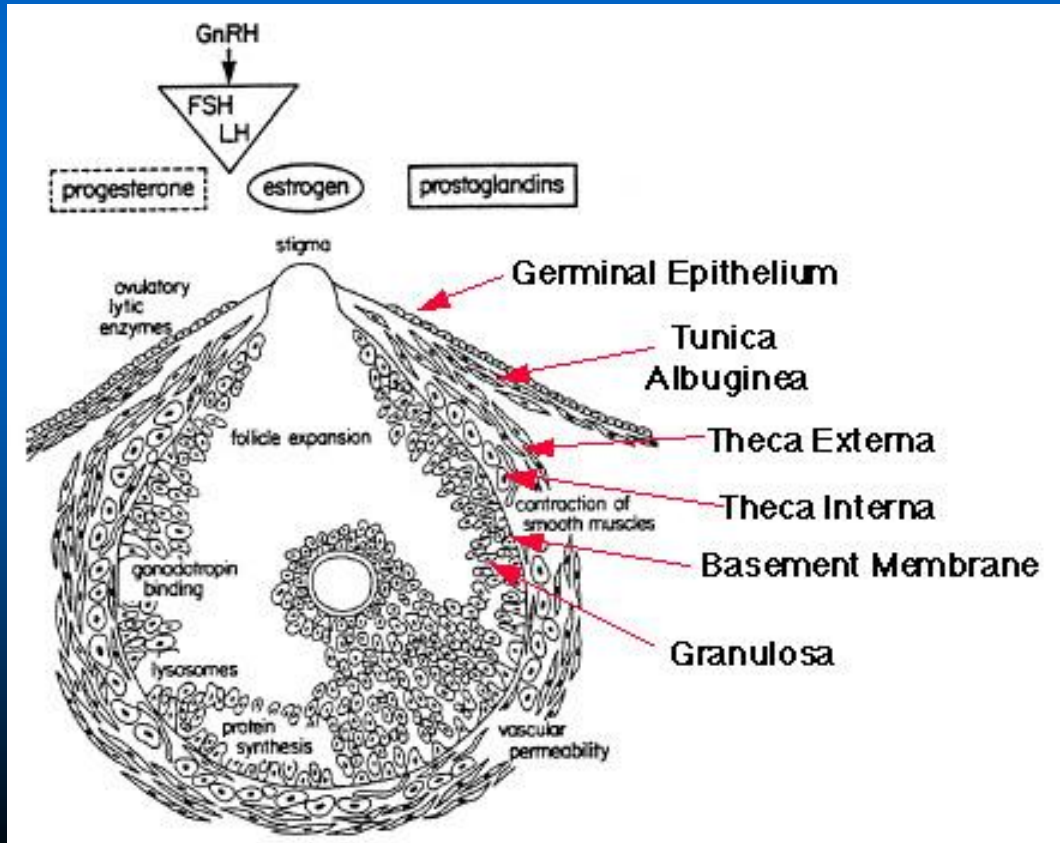
Ovarian steroidogenesis

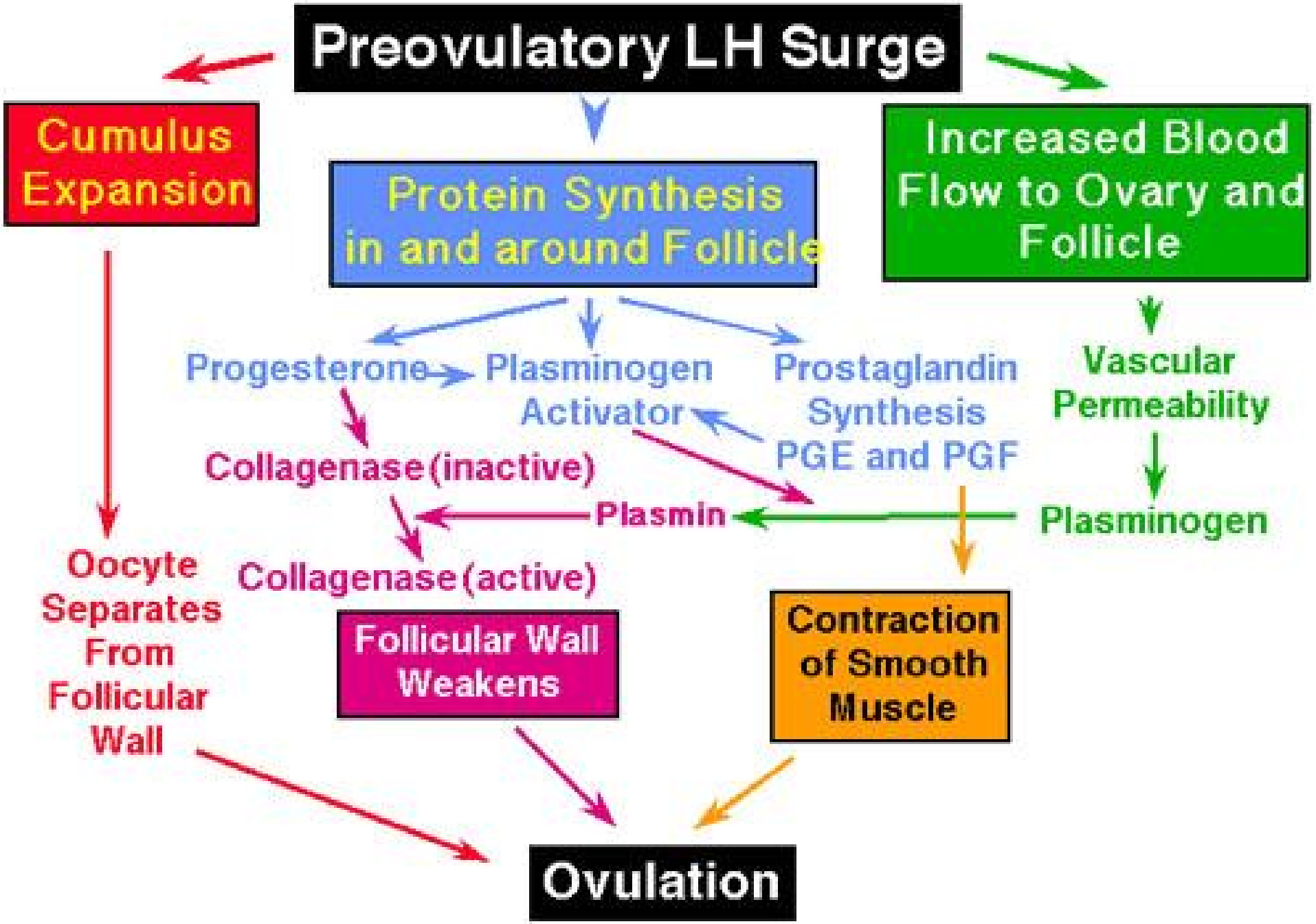
Production Rate of Sex Steroids in Women at Different Stages of the Menstrual Cycle			
	DAILY PRODUCTION RATE		
SEX STEROIDS*	Early Follicular	Preovulatory	Midluteal
Progesterone (mg)	1	4	25
17Hydroxyprogesterone (mg)	0.5	4	4
Dehydroepiandrosterone (mg)	7	7	7
Androstenedione (mg)	2.6	4.7	3.4
Testosterone (mg)	144	171	126
Estrone (mg)	50	350	250
Estradiol (mg)	36	380	250

FSH causes increased aromatase activity, which converts testosterone to estradiol

ovulation

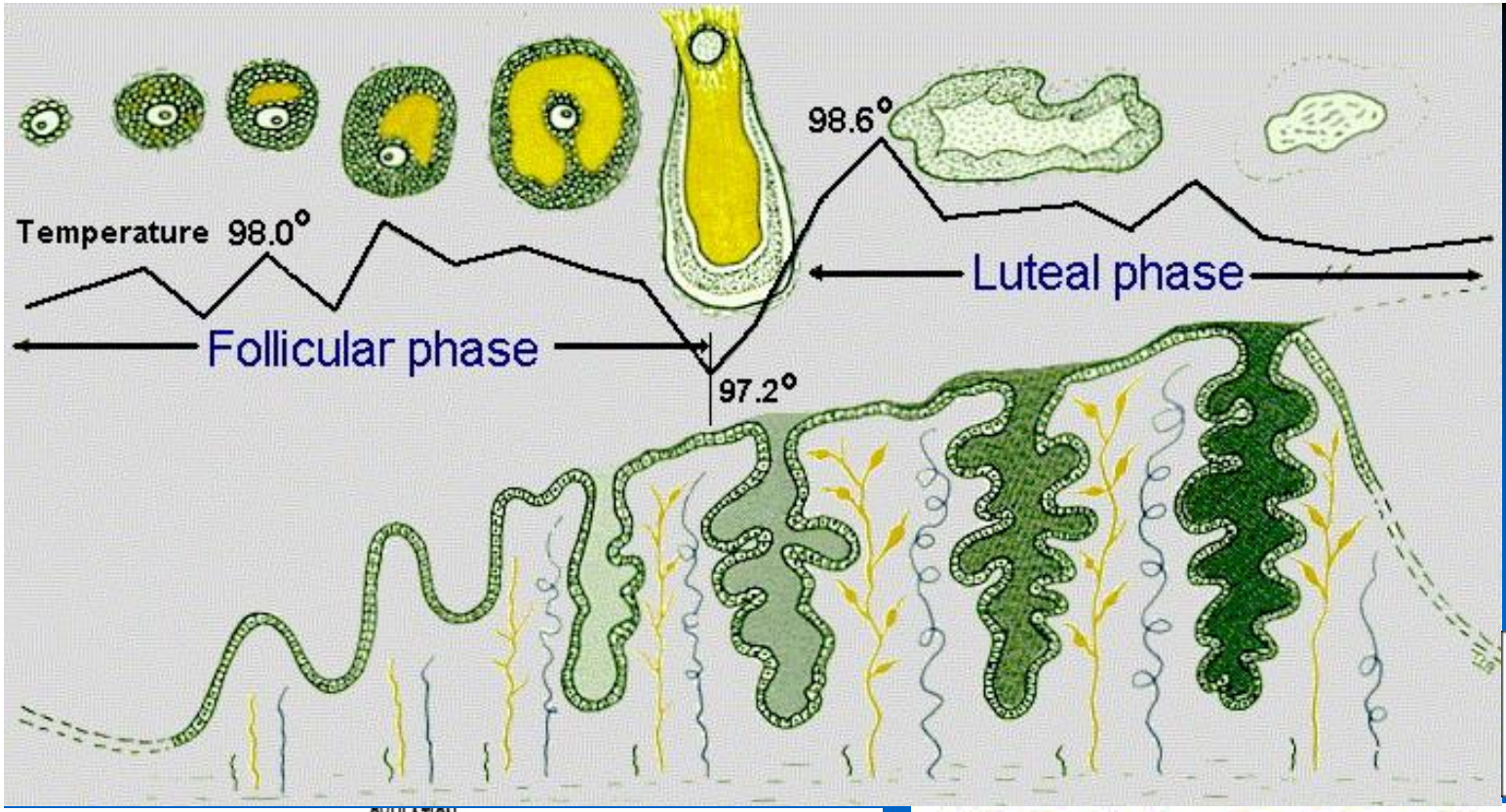
- cumulus cells produce hyaluronic acid and proteins to cause cumulus expansion
- increased blood flow to follicle (vascular permeability ↑)



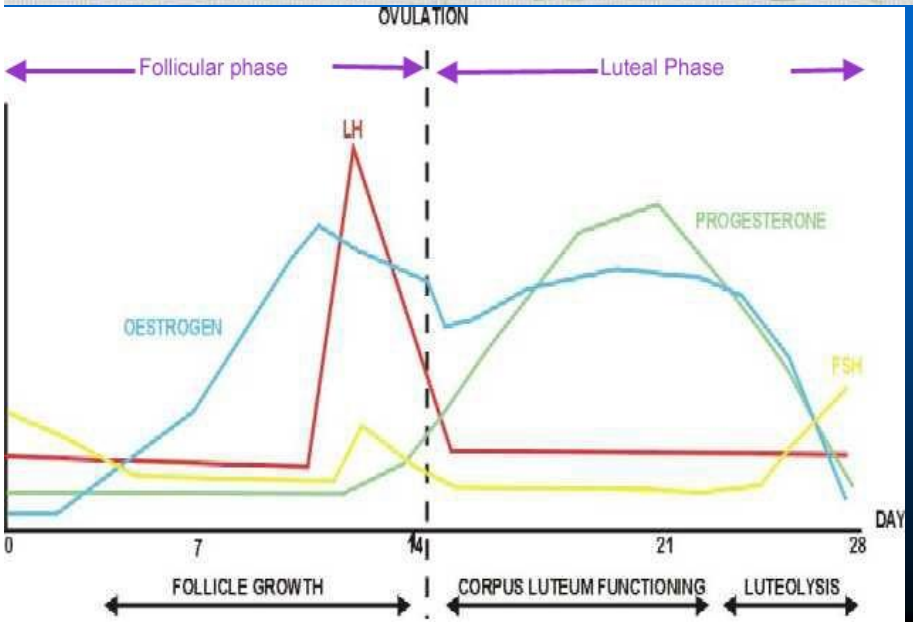

Preovulatory LH Surge
Cumulus Expansion
Protein Synthesis in and around Follicle
Increased Blood Flow to Ovary and Follicle
Progesterone
Plasminogen Activator
Prostaglandin Synthesis PGE and PGF
Vascular Permeability
Collagenase (inactive)
Plasmin
Plasminogen
Oocyte Separates From Follicular Wall
Collagenase (active)
Follicular Wall Weakens
Contraction of Smooth Muscle
Ovulation

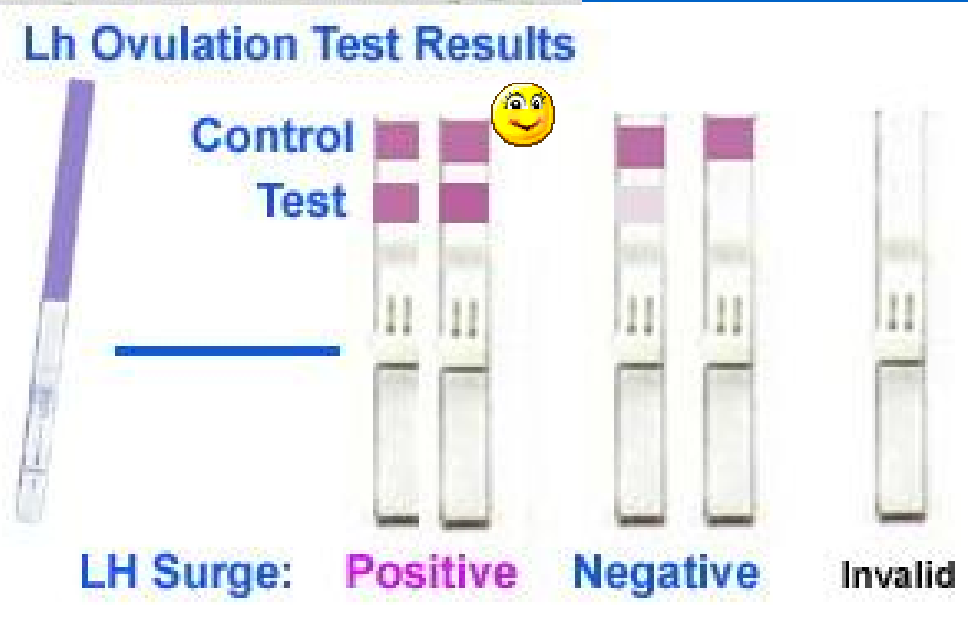
ovulation

- Triggers increase protein and steroid synthesis, and cellular differentiation within follicle
- Plasminogen is converted to plasmin by plasminogen activator (active collagenase ↑).
- Prostaglandin stimulates smooth muscle to contract and force the ruptured follicle to empty.


Temperature 98.0°
98.6°
97.2°
Follicular phase
Luteal phase

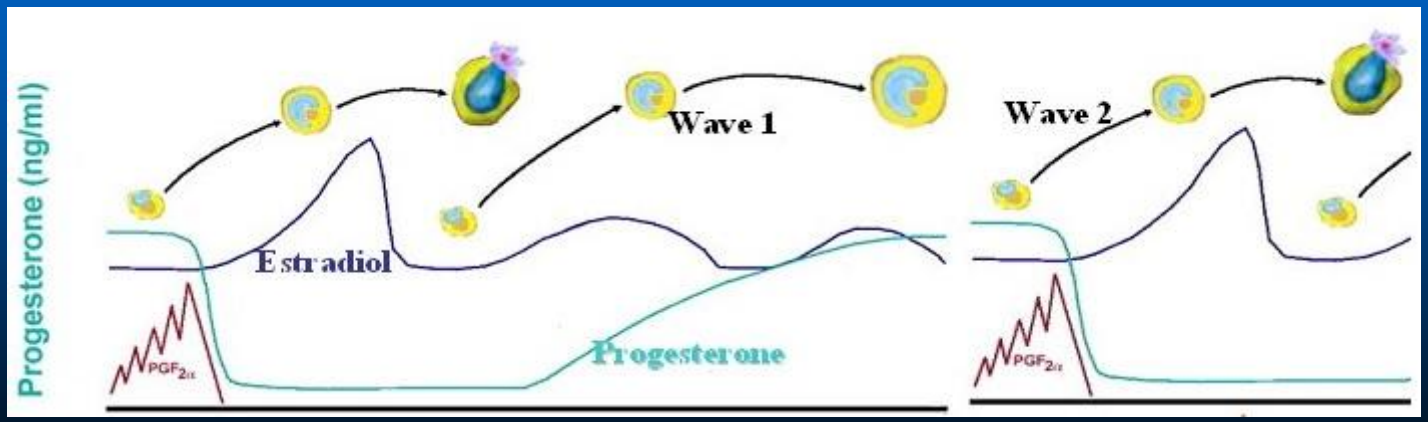

LH Surge
No LH Surge


OVULATION
Follicular phase
Luteal Phase
LH
PROGESTERONE
OESTROGEN
FSH
DAY
0
7
14
21
28
FOLLICLE GROWTH
CORPUS LUTEUM FUNCTIONING
LUTEOLYSIS


Lh Ovulation Test Results
Control
Test
LH Surge:
Positive
Negative
Invalid

Control of the corpus luteum

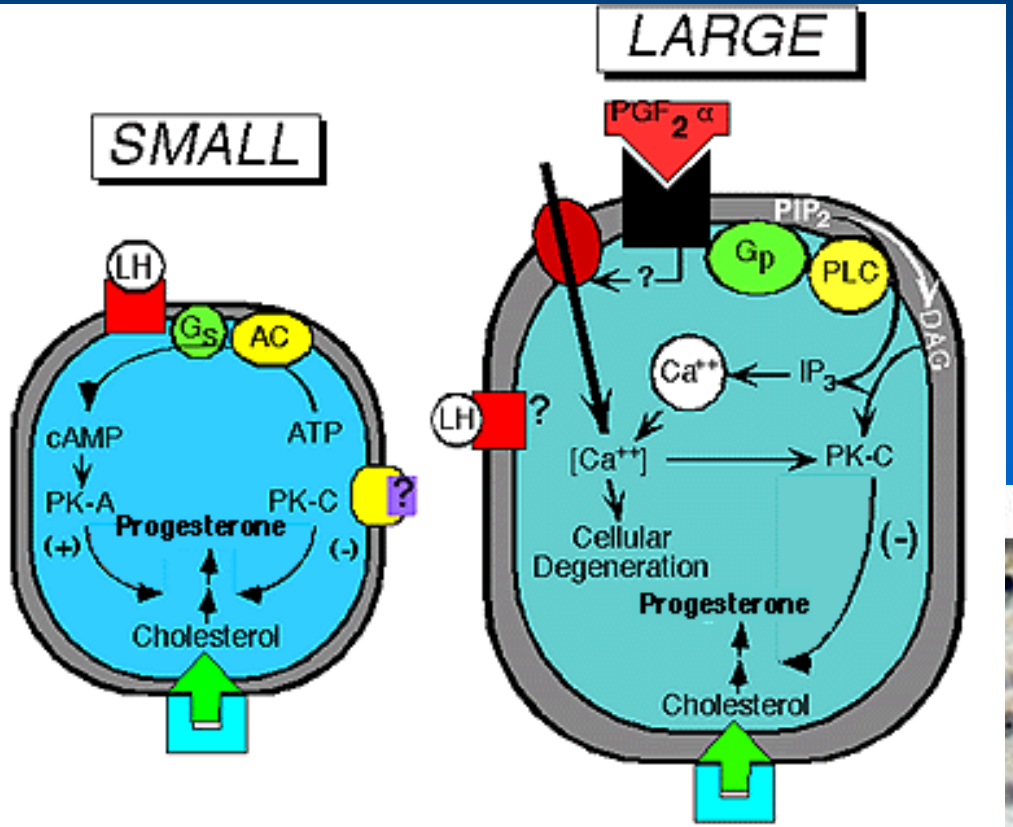
- CL is responsible for the estrous cyclicity.
- Uterus controls the lifespan of the CL in the cow, ewe, sow and mare.
  - Estrogens initiate uterus to synthesizes $PGF_{2\alpha}$.
  - Regression of CL is accomplished within 48 hrs.



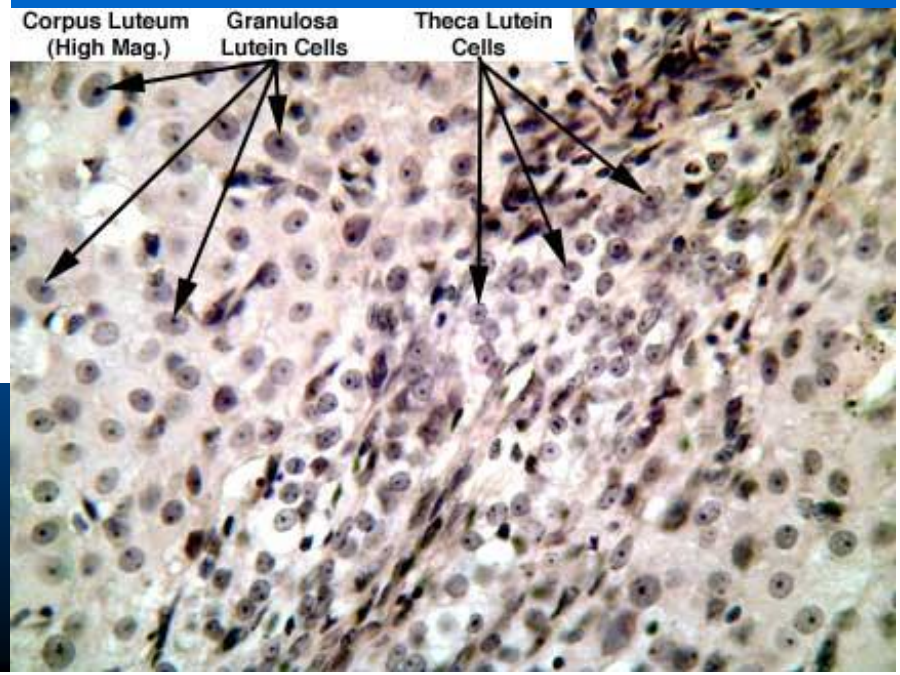
Control of the corpus luteum

- Estrogens → endometrial oxytocin receptors initiate the release of $PGF_{2\alpha}$, which causes the reaction of luteal regression.
- In ruminants, releasing of $PGF_{2\alpha}$ is induced by oxytocin secreted by the CL.
- In dog and cat, $PGF_{2\alpha}$ → CL?

Control of the corpus luteum



LH stimulates the secretion of progesterone from small luteal cells while $PGF_{2\alpha}$ inhibits secretion of progesterone from large luteal cells and causes degeneration of these cells.



Early pregnancy

- **Maternal recognition**
  - **modification of $PGF_{2\alpha}$ by the luteotropic products from the conceptus and uterus**
    - **Trophoblast proteins (cow, ewe, doe)**
    - **Estrogens (sow, mare)**
    - **intrauterine mobility of the embryo (mare)**
    - **? (bitch, queen)**

→ • **antiluteolytic action or luteotrophic effect**

→ • **to extend the luteal activity**

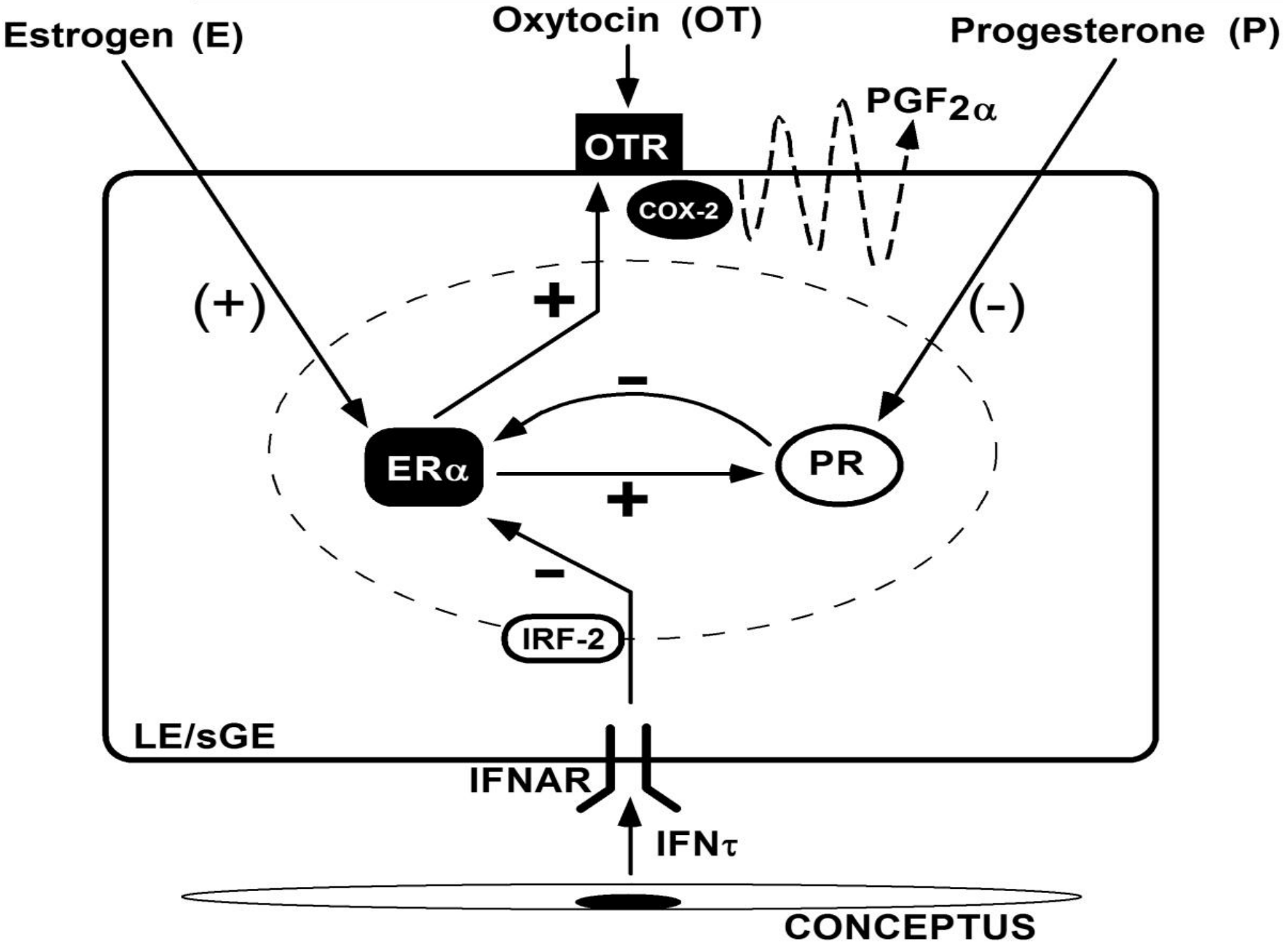
Early pregnancy

- **Trophoblast proteins (cow, ewe, doe)**
  - **oTP-1 secreted between 10 and 21-24 days**
  - **bTp-1 secreted between 16-19 days**
  - **cTP-1 secreted on Day 17**
    - **to bind uterine oxytocin receptors**
    - **to block the uterine $PGF_{2\alpha}$ secretion**

Trophoblast proteins

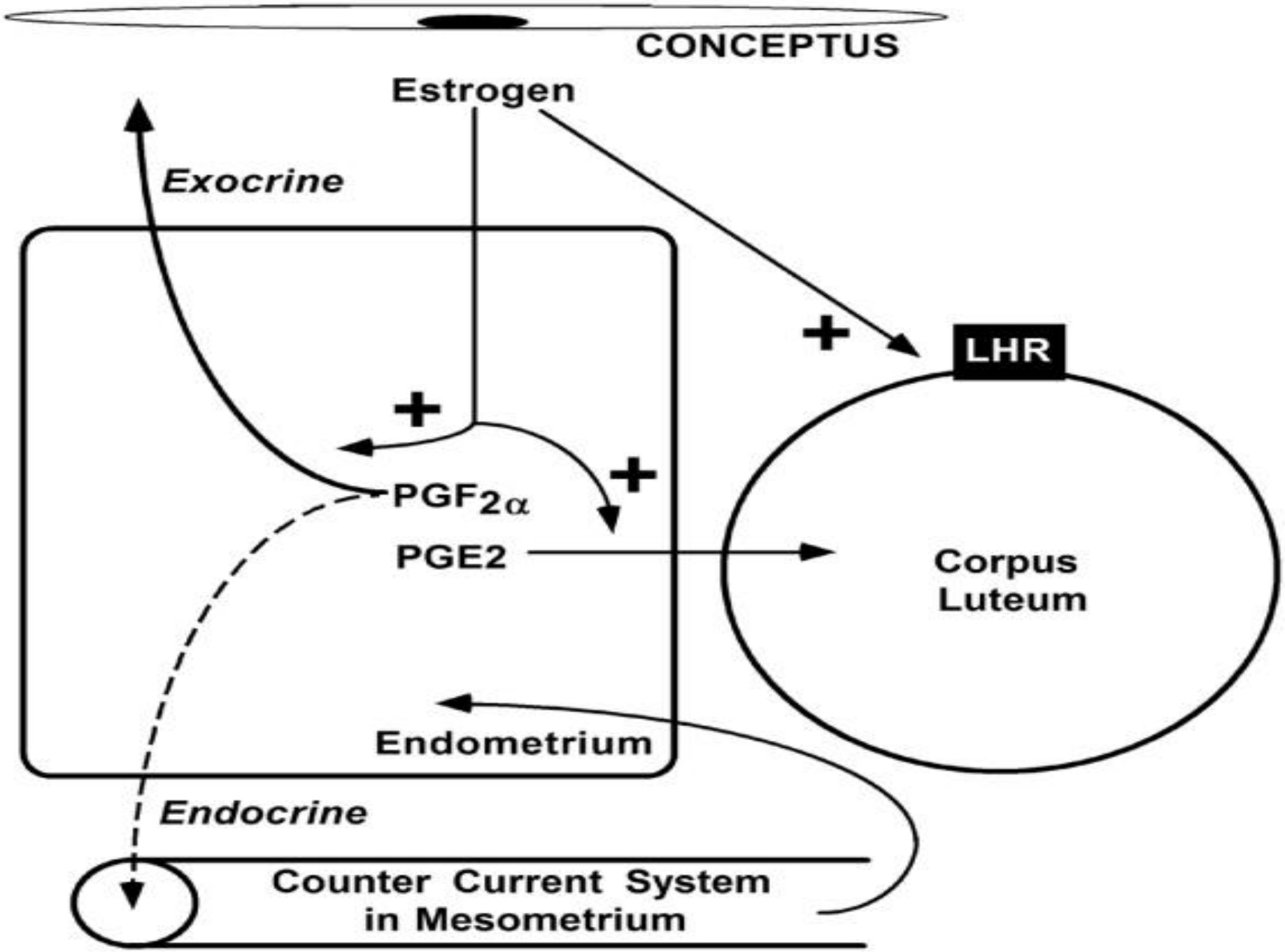
- **Ovine trophoblast protein (oTP-1, IFN-τ)**
  - **MW 18,000, produced on Day 10**
  - **inhibiting the uterine oxytocin receptors**
  - **inhibiting the synthesis of $PGF_{2\alpha}$**

→ **PGF2↓, PGE2↑**


Estrogen (E)
Oxytocin (OT)
Progesterone (P)
PGF2α
OTR
COX-2
(+)
+
(-)
−
ERα
PR
+
−
IRF-2
LE/sGE
IFNAR
IFNτ
CONCEPTUS

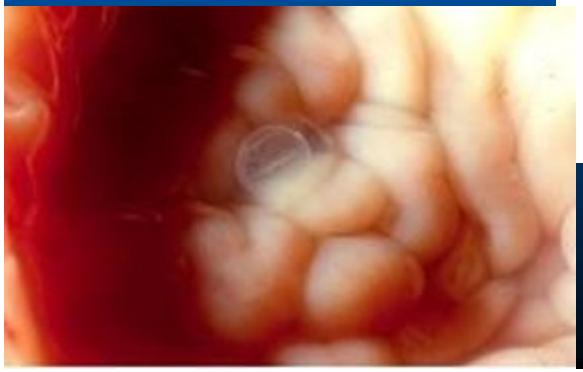
Early pregnancy

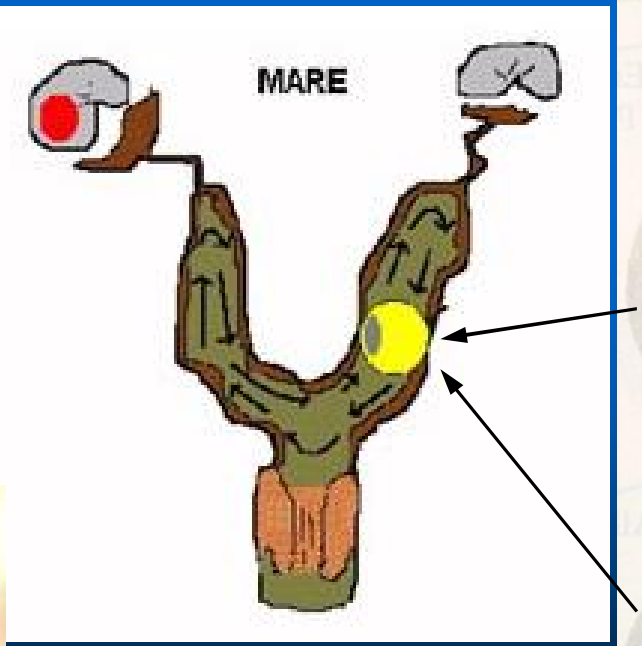
- **Conceptus-derived estrogens (sow, mare)**
  - **estrone and estradiol 17-β converted from progesterone on Day 11, Day14-18**
  - **rapid enlongation of the blastocyst**
  - **releasing of calcium, specific proteins**
    - **altering an endocrine of PG to an exocrine PG**
    - **metabolized to inactive PGFM**
    - **PGF2 → PGE2**

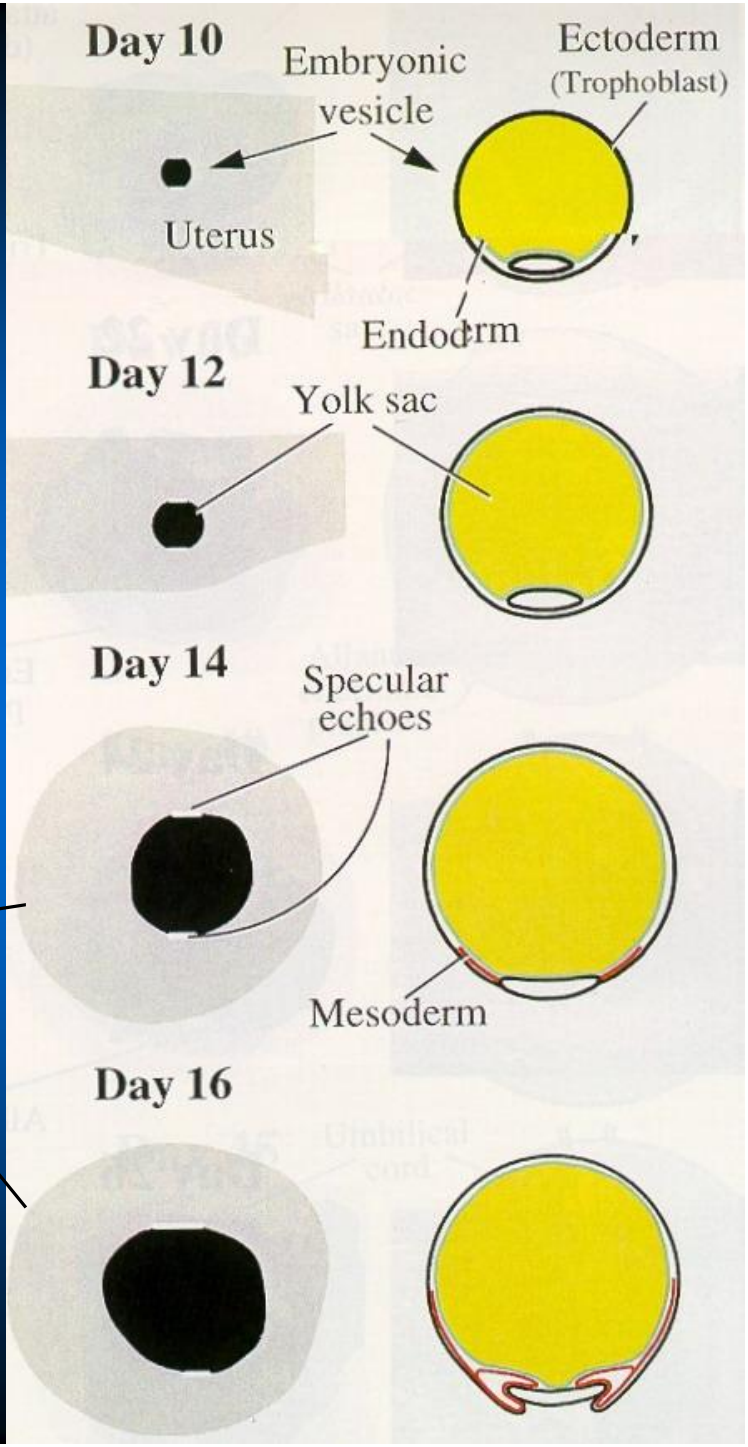

CONCEPTUS
Estrogen
Exocrine
+
LHR
+
+
PGF2α
+
PGE2
Corpus
Luteum
Endometrium
Endocrine
Counter Current System
in Mesometrium

- **There is extensive mobility of the equine conceptus within the horns and uterine body before fixation occurs between days 16 and 18.**

- **?? Endometrial Prostaglandin Synthesis Inhibitor, (EPSI) from endometrium**





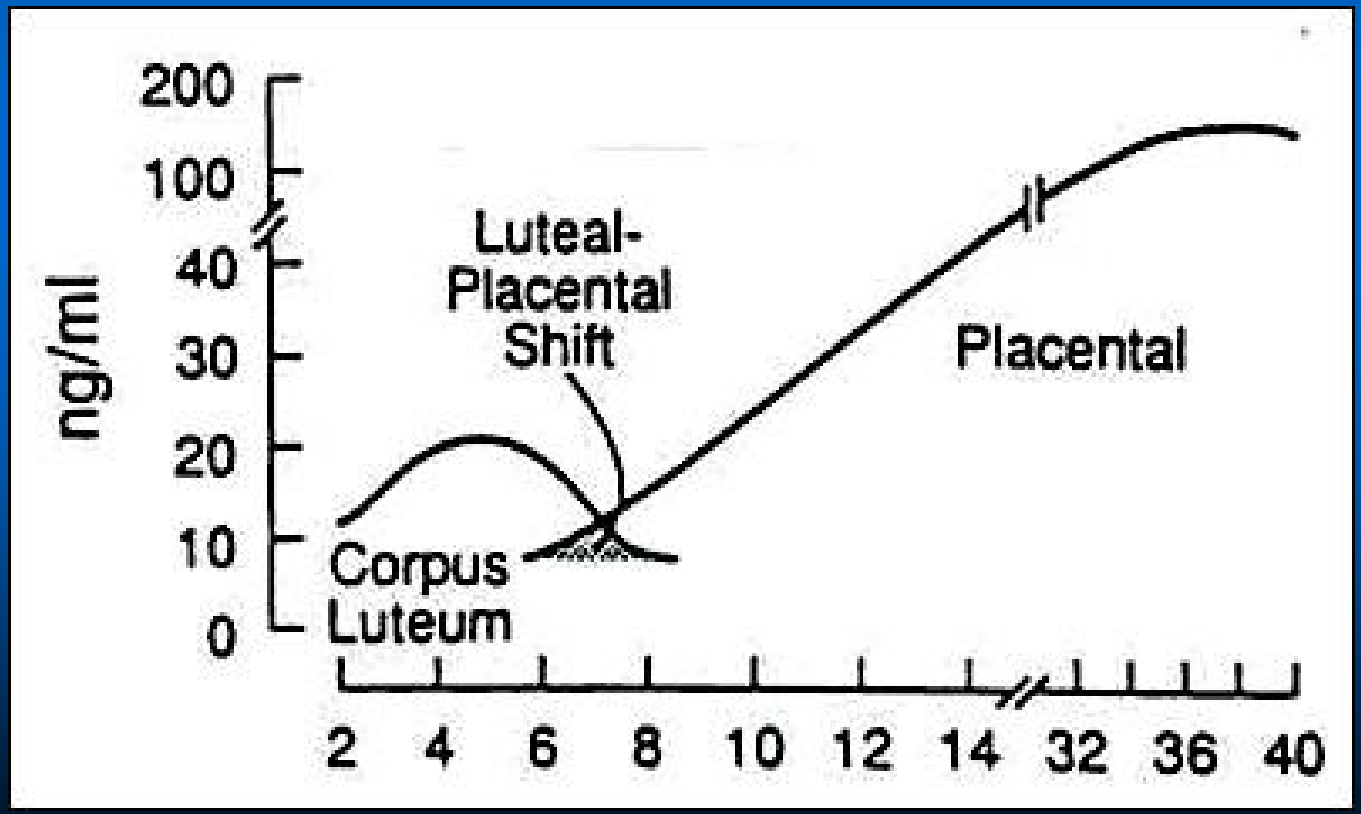


Time of maternal recognition of pregnancy

	Day of recognition	Day of definite attachment
Mare	14-16	36-38
Cow	16-17	18-22
Sow	12	18
Ewe	12-13	16
Goat	17	

Corpus luteum & Pregnancy

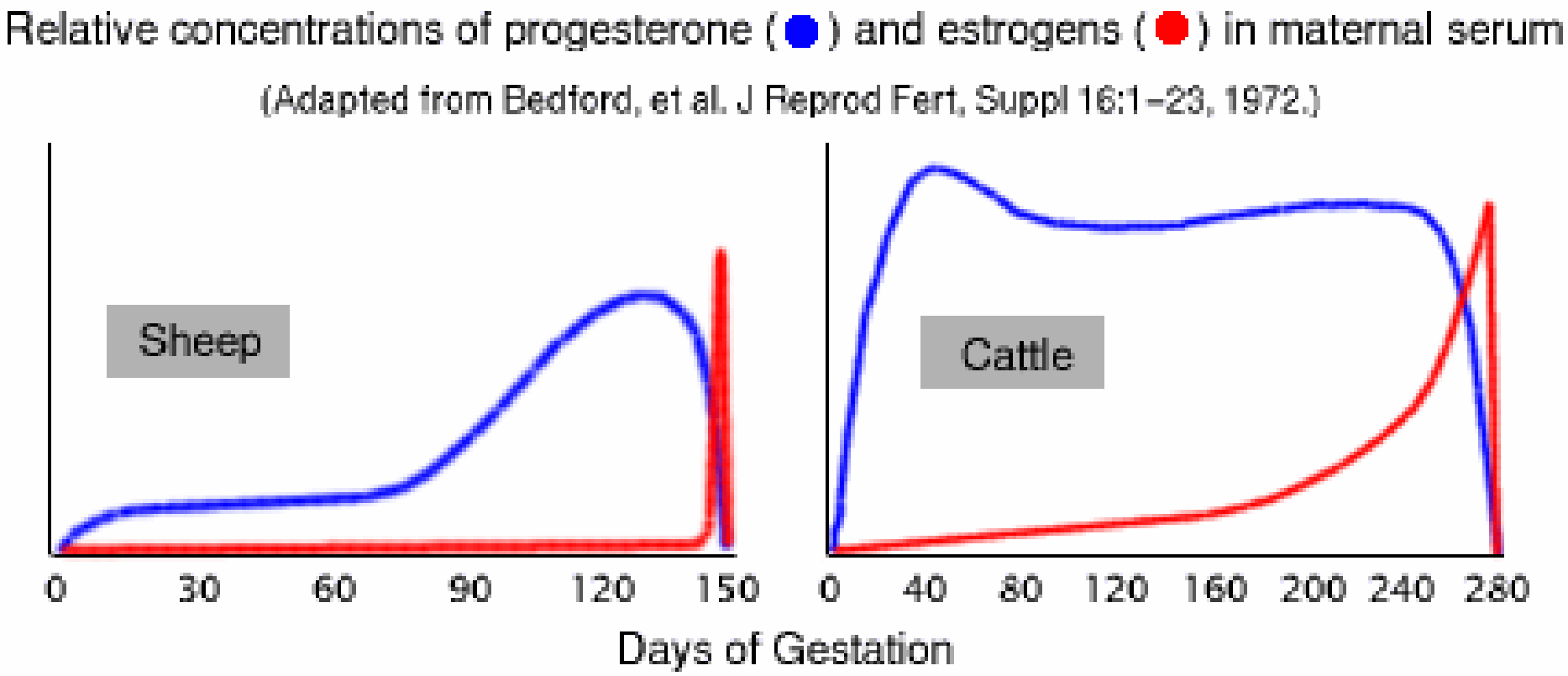
- **P_4 from CL: cow, sow, goat, dog and cat**
- **P_4 from CL + fetoplacental unit: mare, sheep**

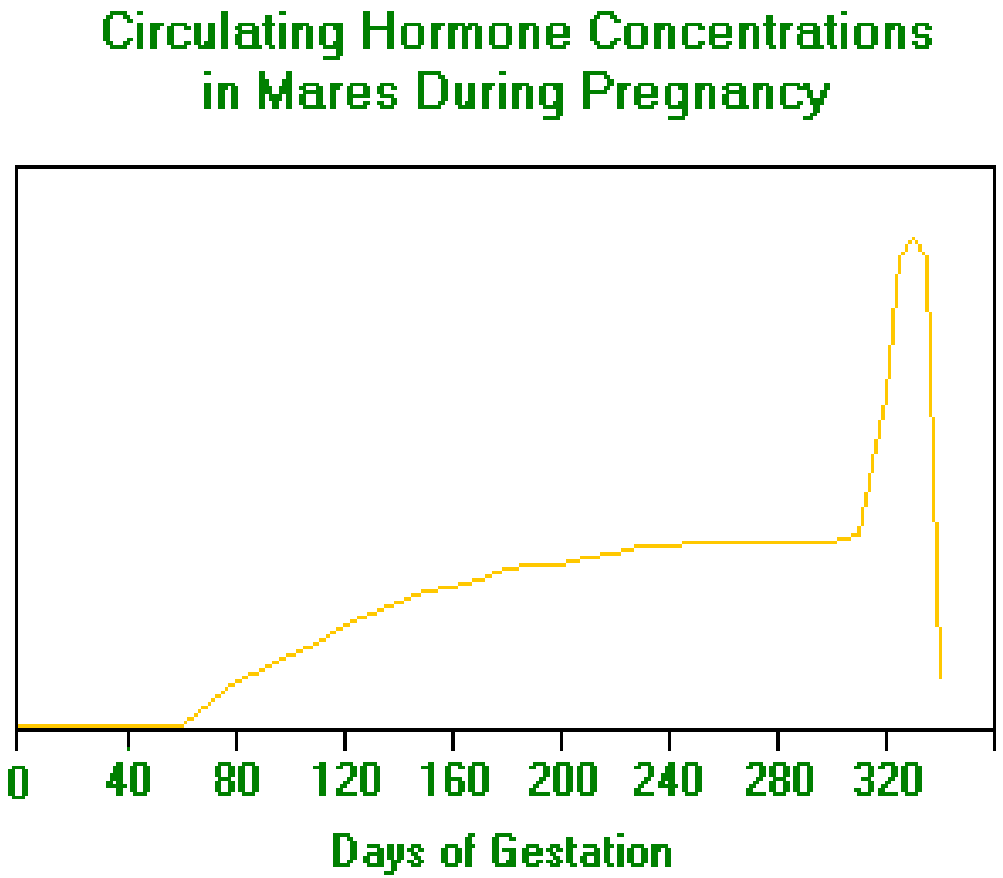


Pregnancy

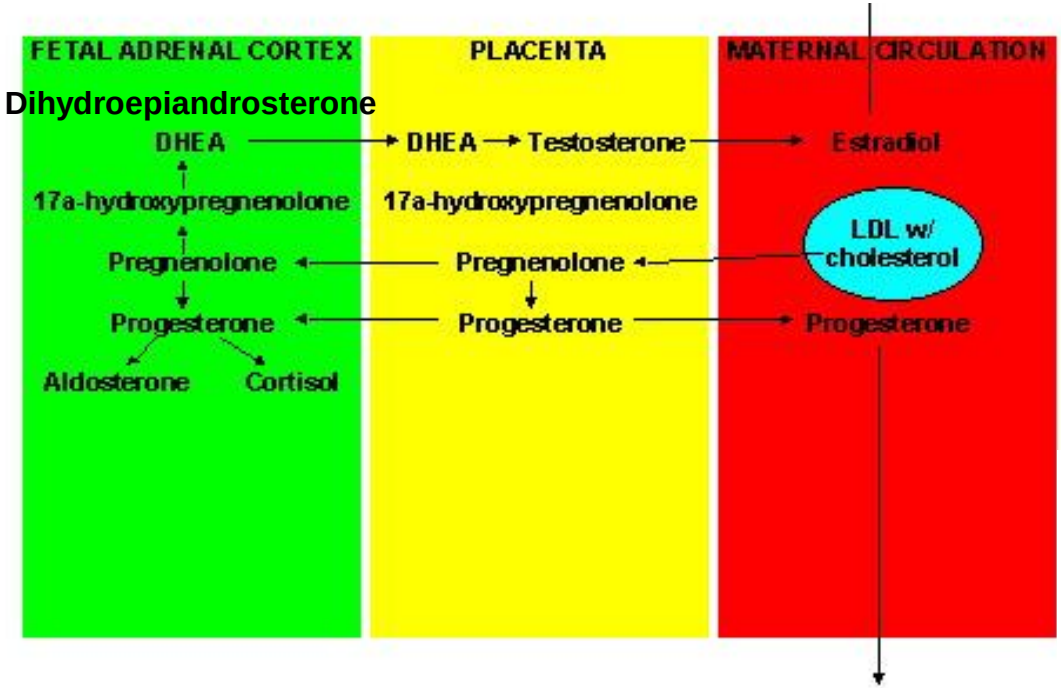
1. The sheep placenta produces enough progesterone by day 60-70.

2. Luteal progesterone is required throughout gestation in cattle and goats because their placenta secrete much smaller quantities of progesterone.

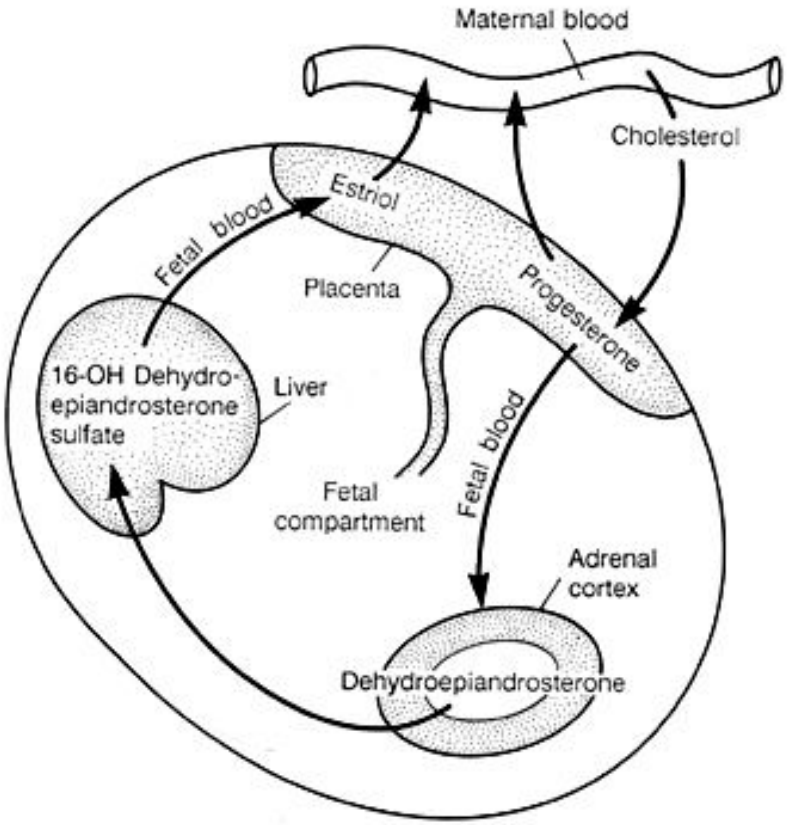




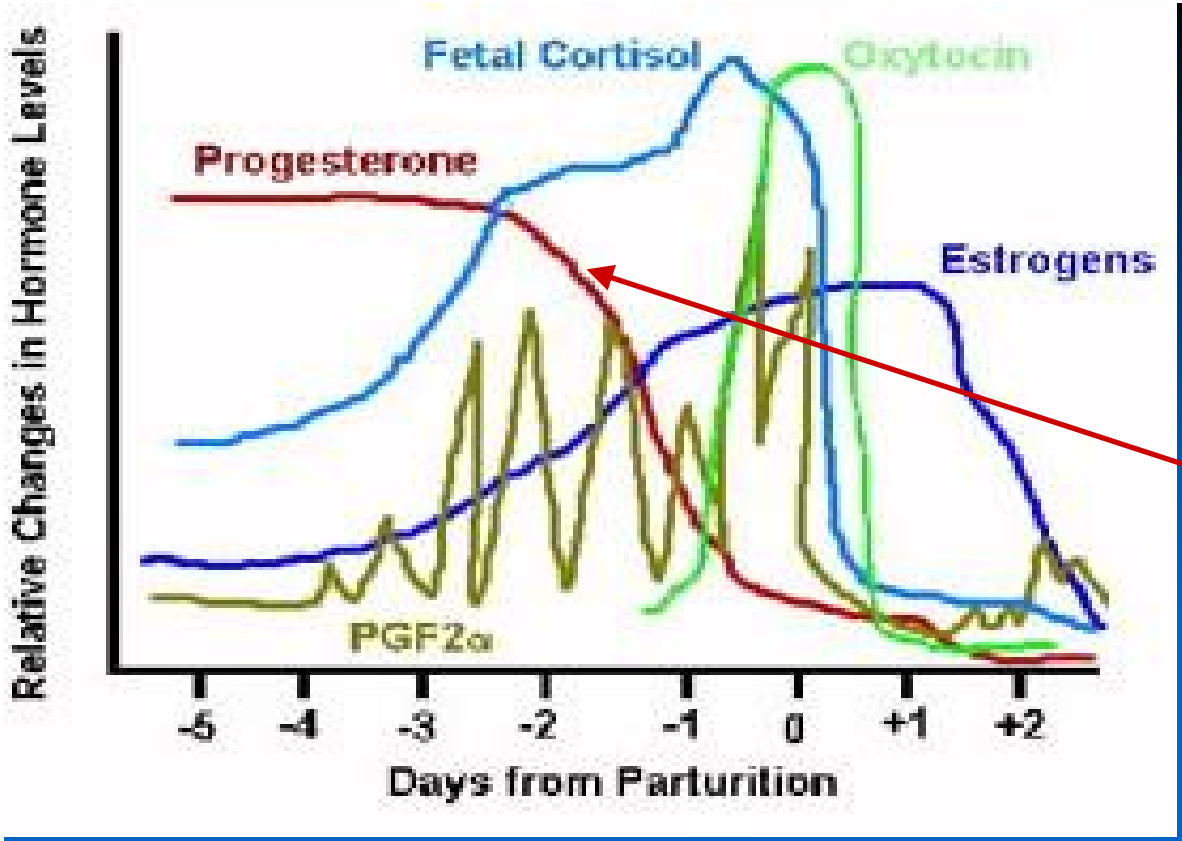
- [ ] Chorionic gonadotropin
- [ ] Estrogens - from ovaries
- [ ] Estrogens - from placenta
- [ ] Progesterone - from ovaries
- [x] Progestins - from placenta
- [ ] Relaxin



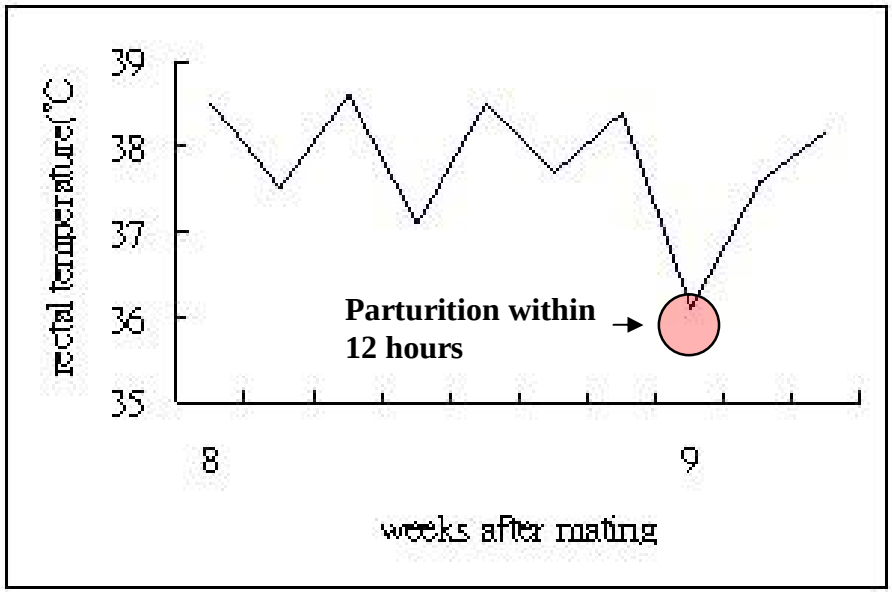
Fetal-Placental Unit



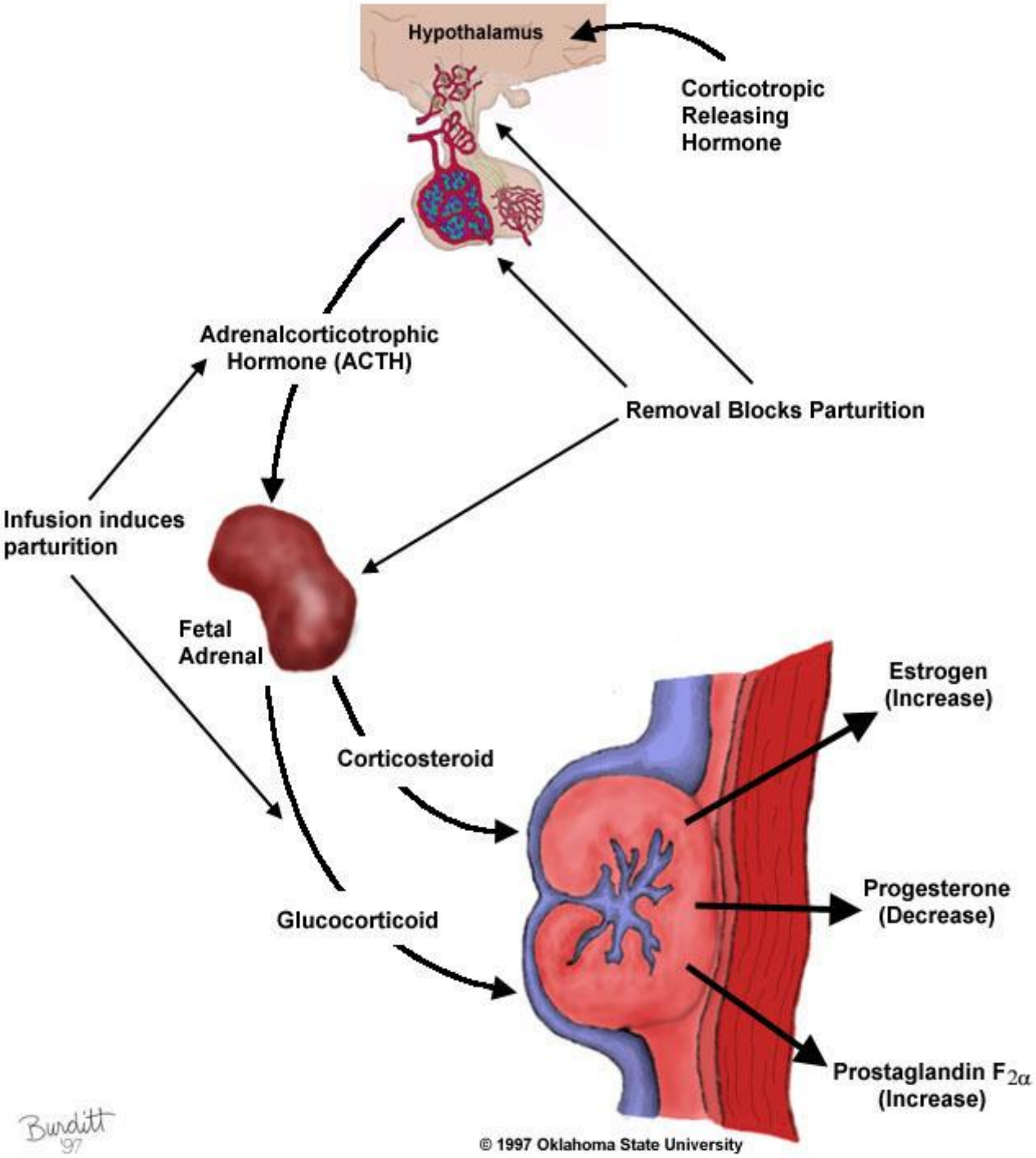
Parturition

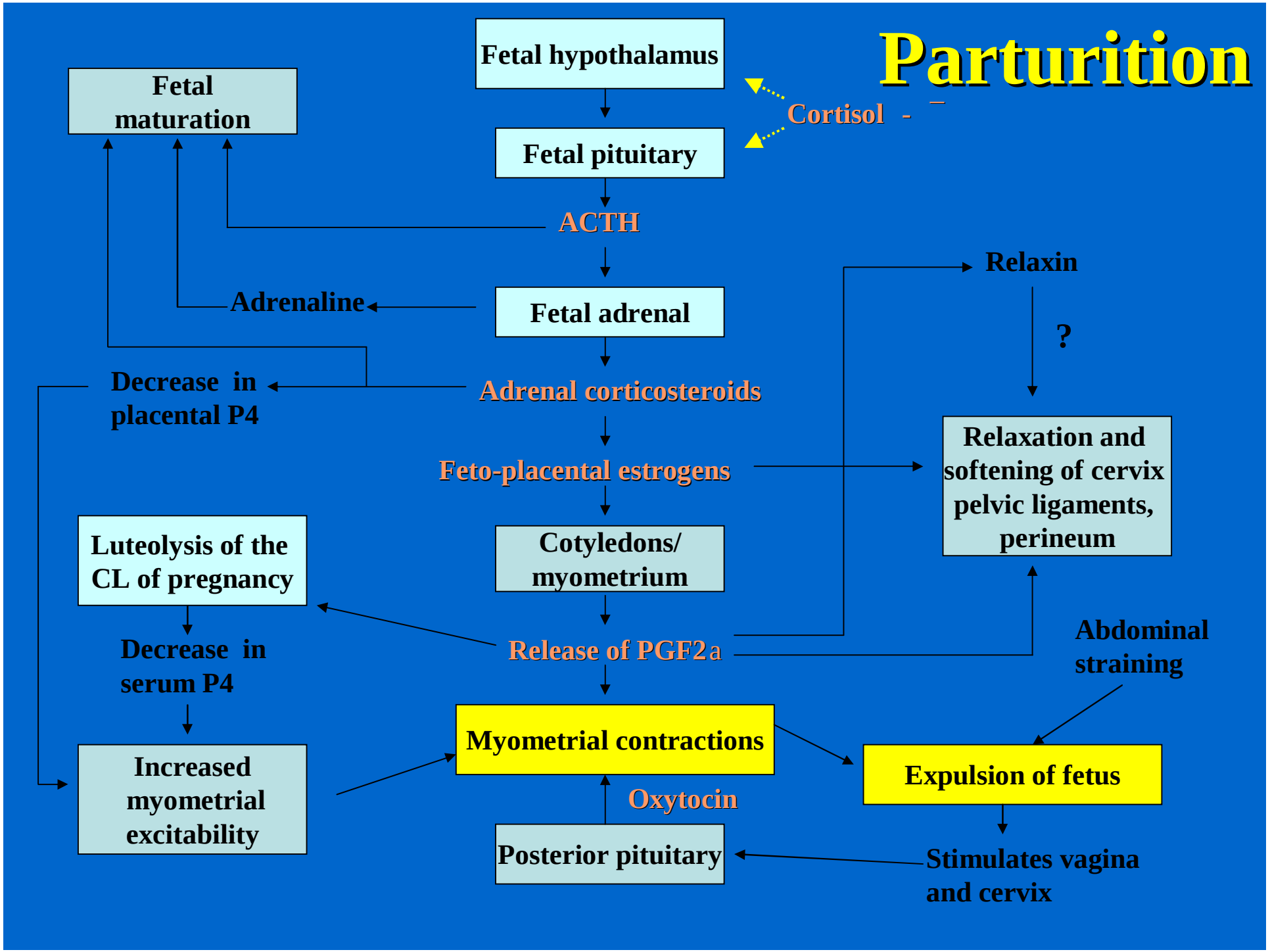


Progesterone ⁻



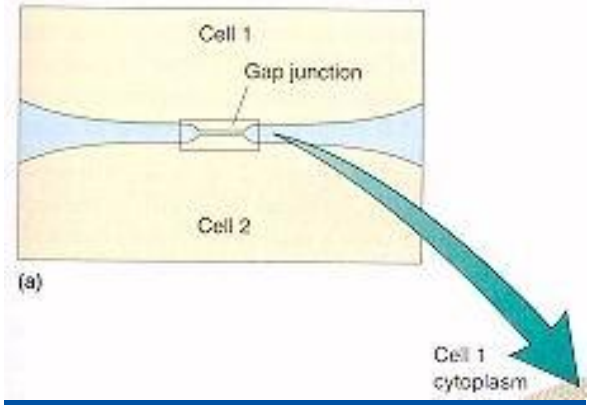
Parturition



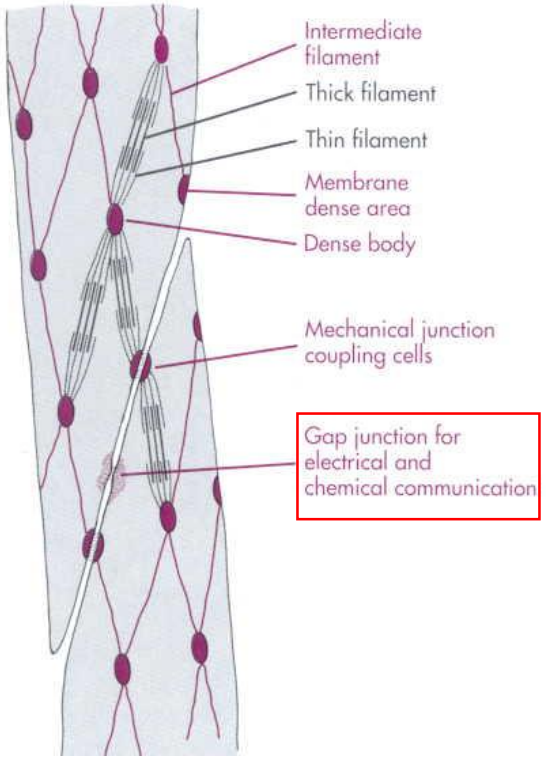

Parturition
Fetal hypothalamus
Fetal maturation
Cortisol -
Fetal pituitary
ACTH
Relaxin
Adrenaline
Fetal adrenal
?
Decrease in placental P4
Adrenal corticosteroids
Feto-placental estrogens
Relaxation and softening of cervix pelvic ligaments, perineum
Luteolysis of the CL of pregnancy
Cotyledons/ myometrium
Abdominal straining
Decrease in serum P4
Release of PGF2a
Myometrial contractions
Expulsion of fetus
Increased myometrial excitability
Oxytocin
Posterior pituitary
Stimulates vagina and cervix

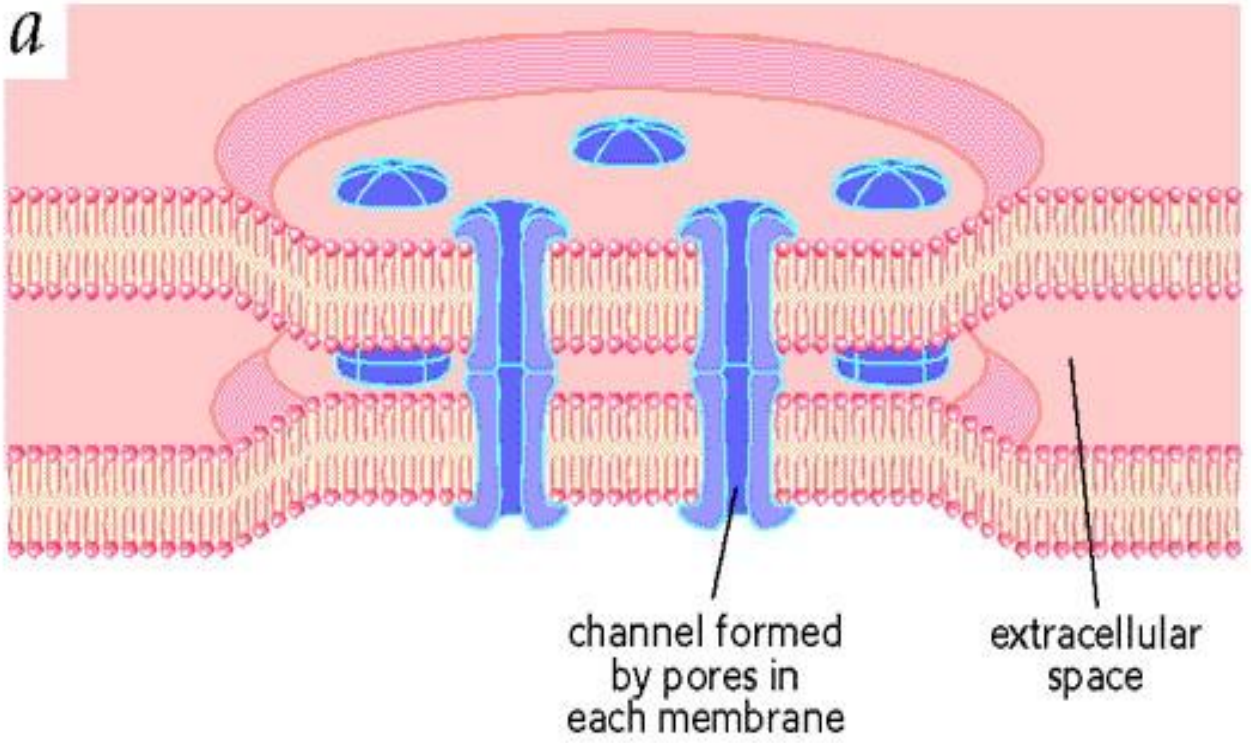
Parturition

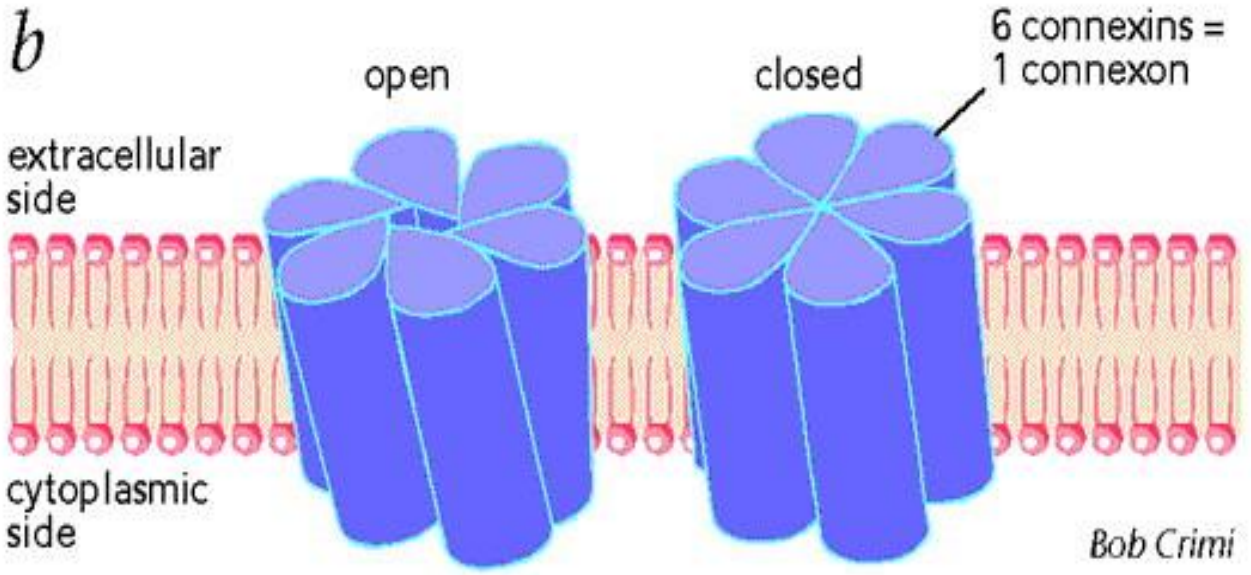
- **Prostaglandins are soluble in fat and water,**
  Prostaglandins → pass from cell to cell
- **Actions:**
  - **smooth muscle contraction**
  - **luteolysis**
  - **softening of cervical collagen**
  - **developing gap junctions**
  - **movement of Ca^{2+} between the myofibrils**
    - **$PGF_{2\alpha}$, PGE ↑, PGI_2 ↓**

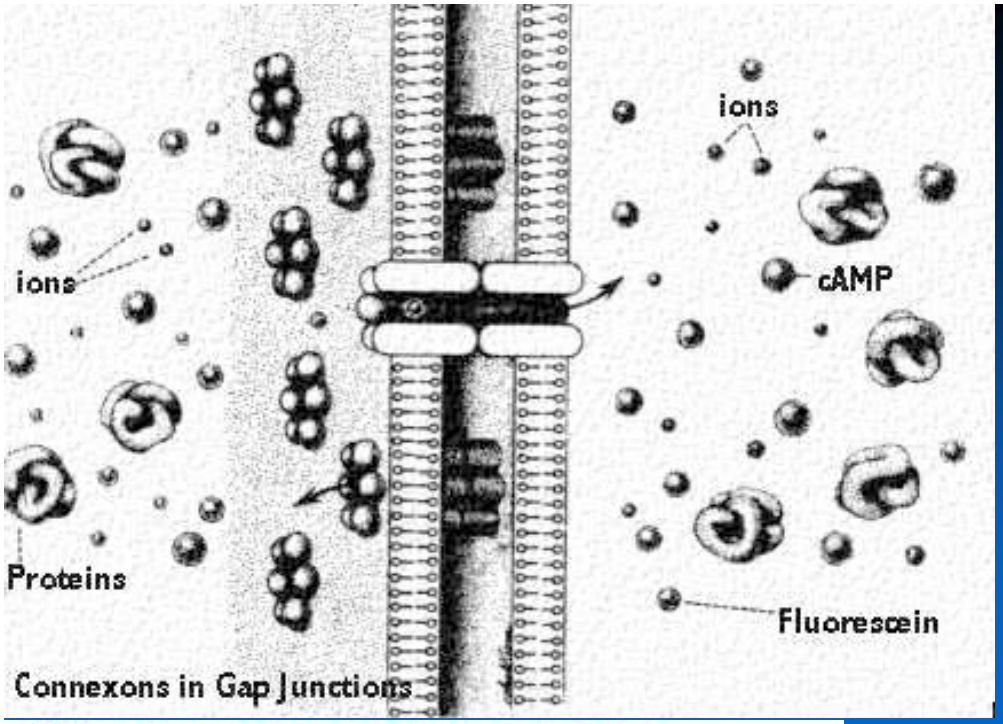


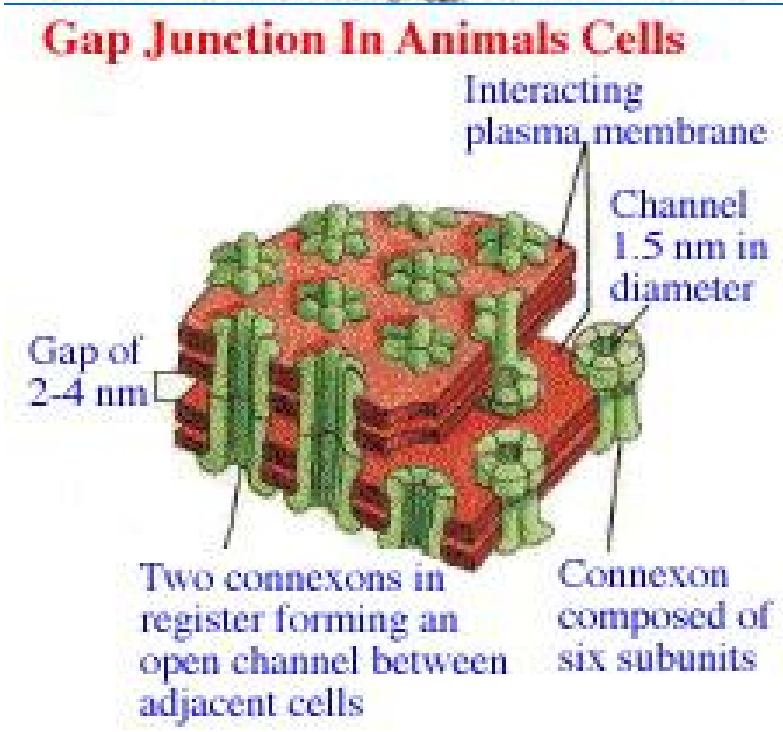
Smooth muscle cells











molecules (< 1000 daltons) that can pass include calcium ions, cAMP, ATP, glutathione, and large molecules like amino acids and sugars etc.

Smooth muscle contraction

- **Muscle protein: myosin and actin**
  - **Calmodulin activates MLC kinase**

Calcium-Calmodulin → MLC kinase

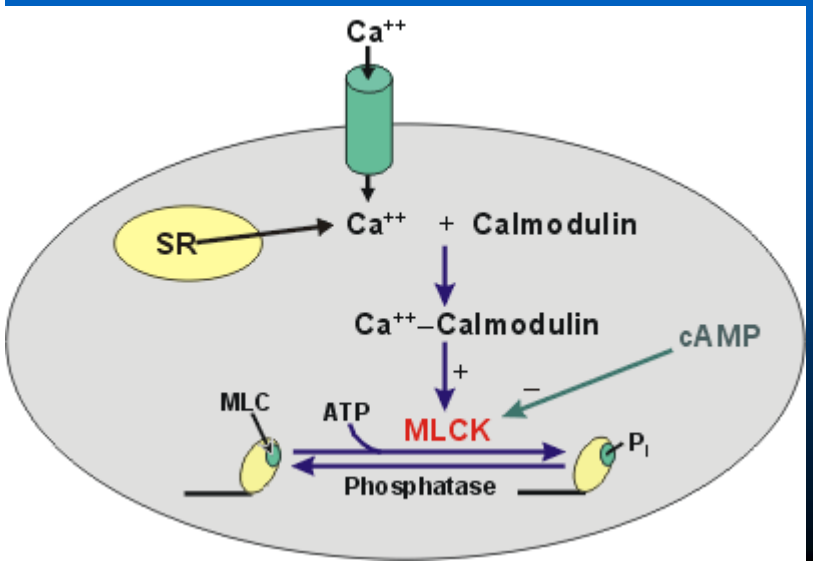
inactive myosin light chain

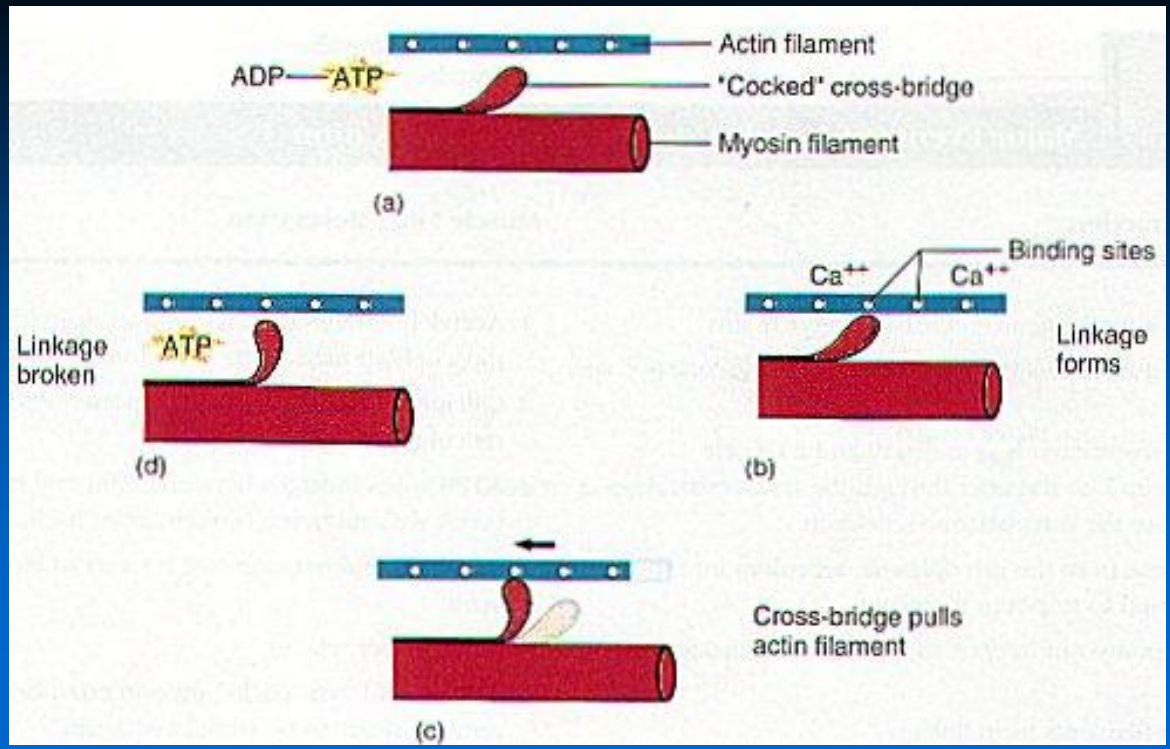
(M*-ATP) + A <----> (M*-ADP-A) + Pi

(M*-ADP-A) <-----> (M-A) + ADP

actomyosin complex

contractions

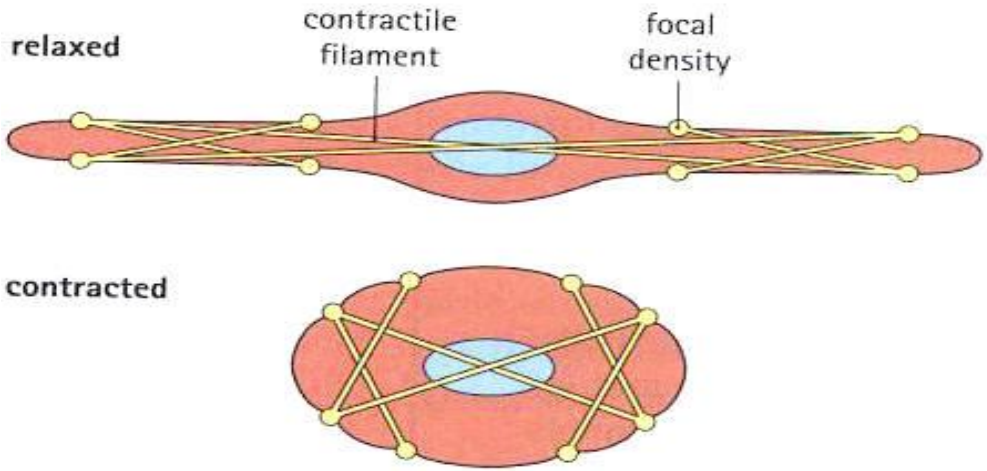


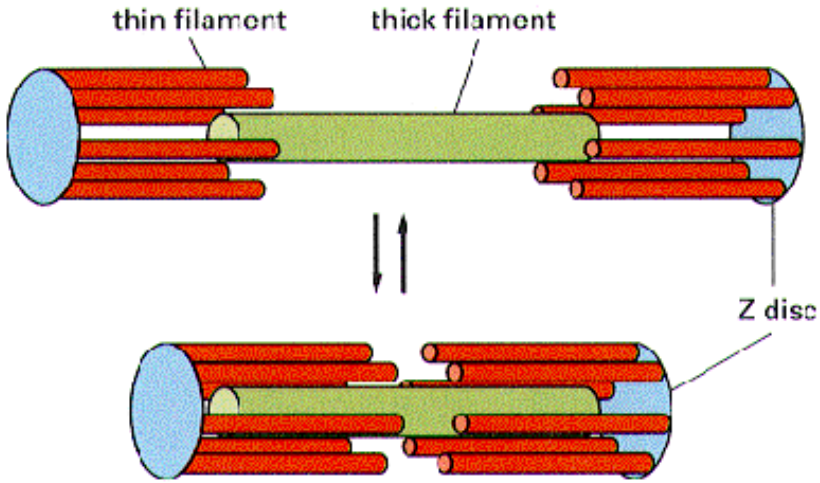


Smooth muscle contraction

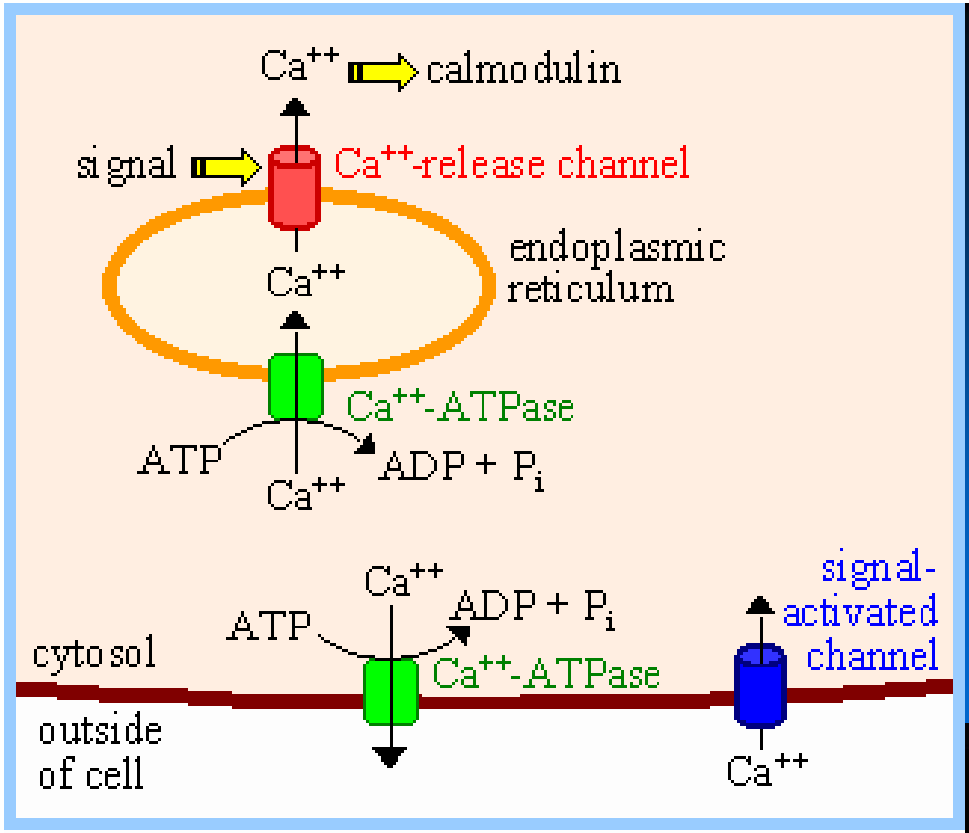
1. Calcium ions come from outside of the cell.
2. Calcium ions bind to calmodulin-MLCK complex on myosin.
3. The enzyme complex breaks up ATP into ADP and transfers the Pi directly to myosin.
4. This Pi transfer activates myosin.
5. Myosin forms cross-bridges with actin.
6. When calcium is pumped out of the cell, the Pi gets removed from myosin by phosphatase.
7. The myosin becomes inactive, and the muscle relaxes.

Smooth muscle contraction



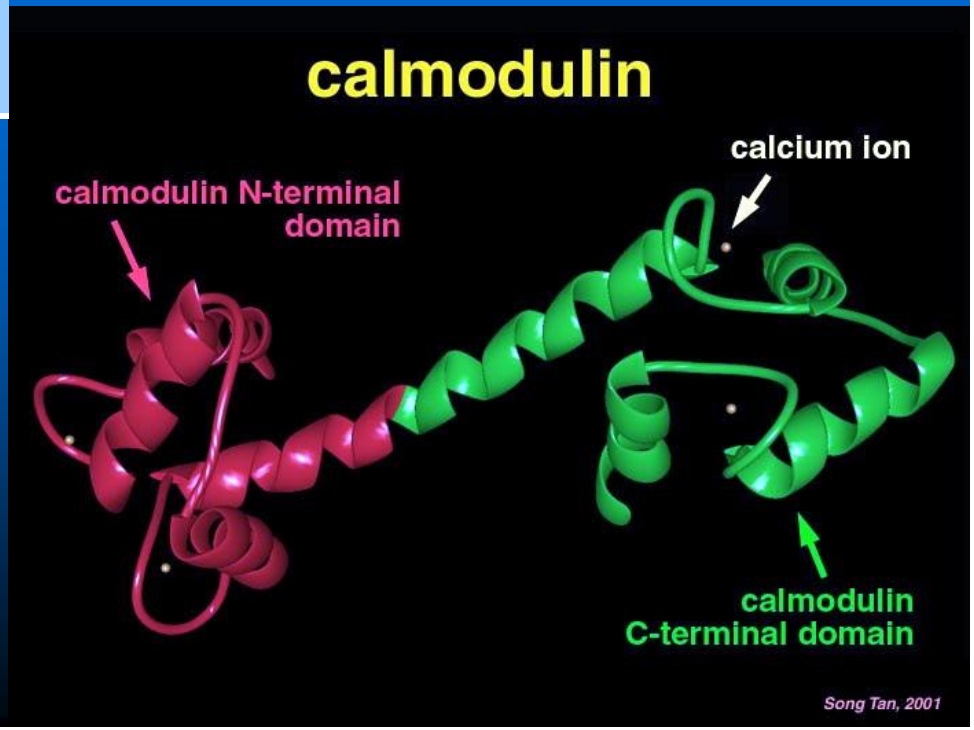


The top view shows a relaxed smooth muscle cell. Note the focal densities and the network of actin and myosin filaments.
When contracted, the filaments slide together and pull the cell to a more rounded appearance.



Calmodulin is one of the primary receptors of intracellular calcium. 4 Ca^{2+} bind to calmodulin. At each binding site, Ca interacts with oxygen atoms, mainly glutamate and aspartase side-chain carboxyl group.

Calmodulin mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, nerve growth, immune response….



Parturition

- **Actions of estrogens**
  - **contractile protein synthesis ↑**
  - **receptors of oxytocin and $PGF_{2\alpha}$ ↑**
  - **calmodulin synthesis ↑**
  - **MLC kinase activity ↑**
  - **gap junctions ↑**

Parturition

- **Actions of progesterone**
  - **gap junctions ↓**
  - **receptors of oxytocin and $PGF_{2\alpha}$ ↓**
  - **synthesis of oxytocin and $PGF_{2\alpha}$ ↓**
  - **calmodulin binding ↑**

Parturition

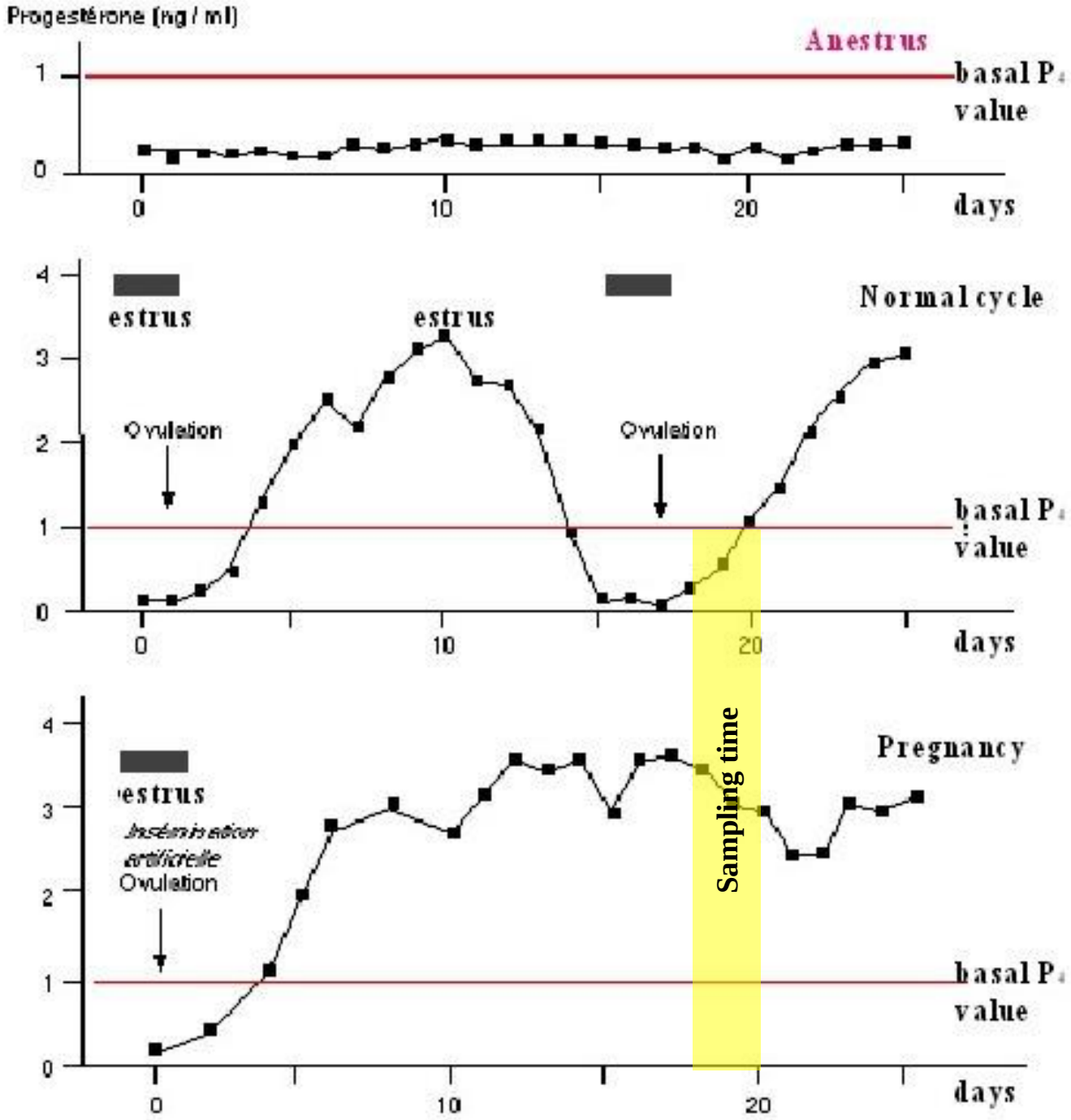
- **Actions of oxytocin**
  - **dependent on progesterone ↓ , estrogens ↑**
  - **stimulation of receptors in the anterior vagina and cervix ⟶ oxytocin release**
    - **increasing PG release**
    - **increasing Ca^{2+} release**

Clinical aspects of reproductive endocrinology

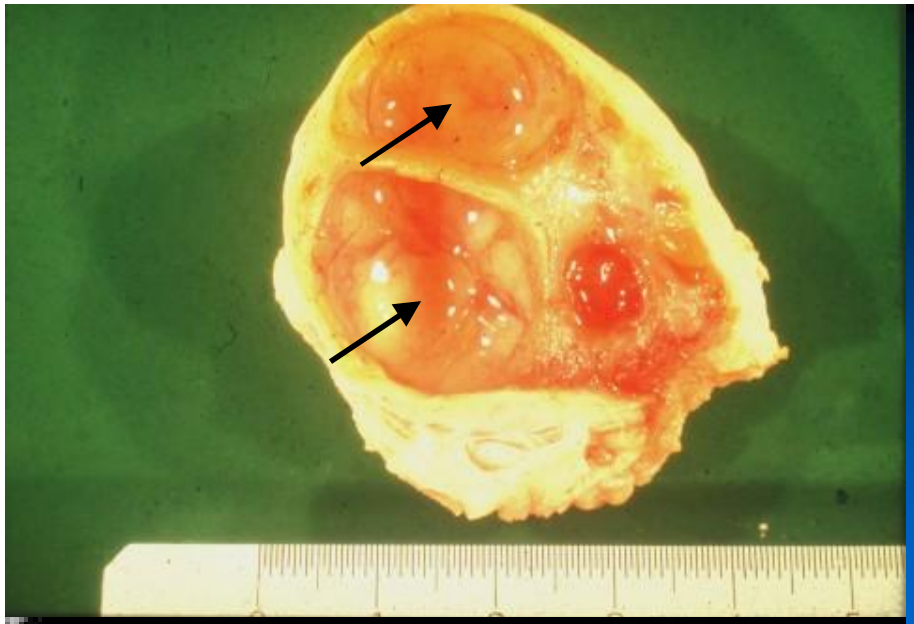
- **Steroid hormone concentrations are lower in animals than in human. (estrogens < 1/10)**
- **Progesterone analysis give the most useful information on the reproductive status.**
- **Blood, milk, urine, saliva and feces are sources for endocrine information.**

Clinical aspects of reproductive endocrinology

- **Cow:**
  - **P4 for early diagnosis of pregnancy**
    - **Negative forecast (98%) is better than positive forecast (67-90%).**
  - **P4 for postpartum ovarian activity**
  - **Estrone sulfate for diagnosis of pregnancy**
    - **from Day 120, 96% accuracy rate**
  - **Bovine pregnancy specific protein B for early diagnosis of pregnancy on Day 30**

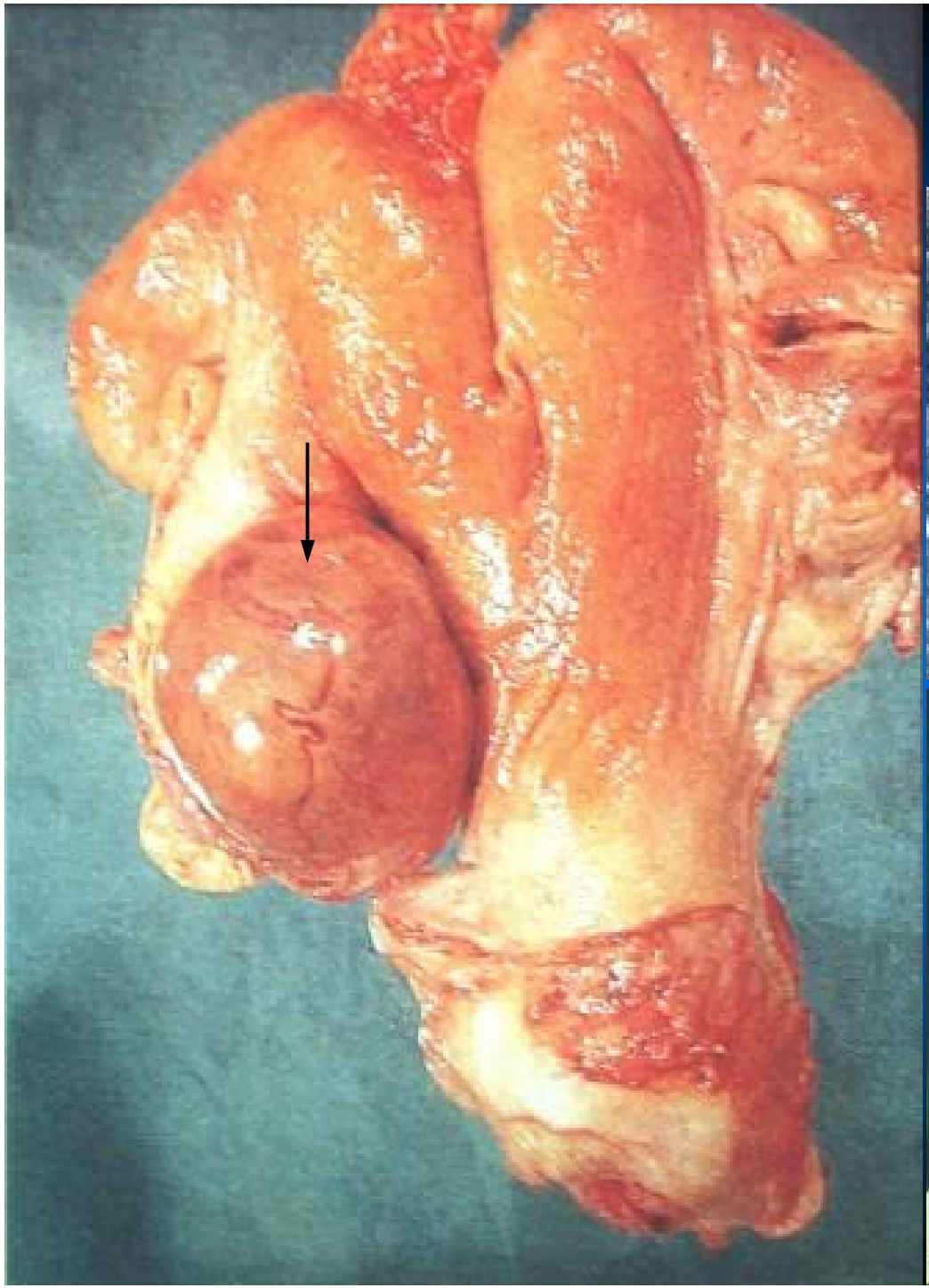


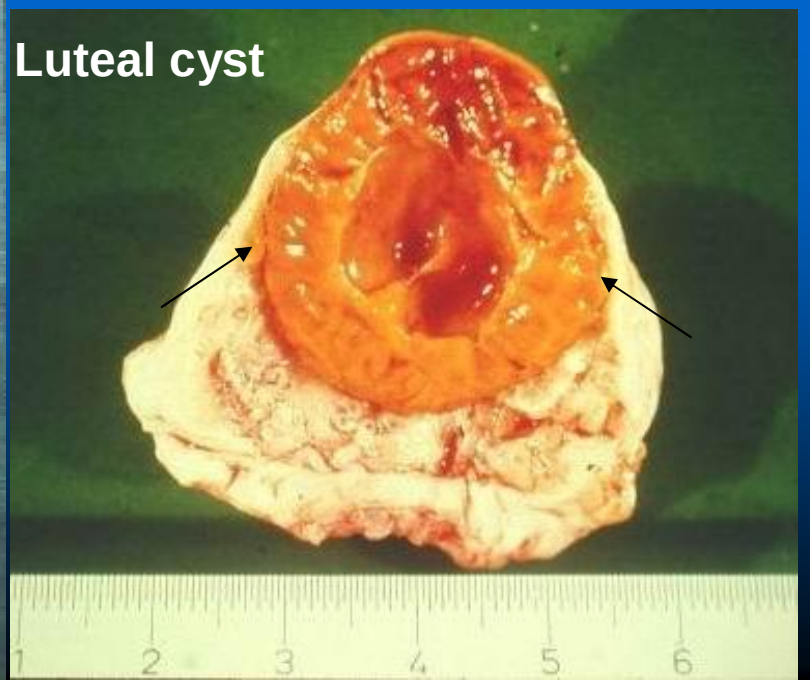
P_4 for postpartum ovarian activity or early diagnosis of pregnancy



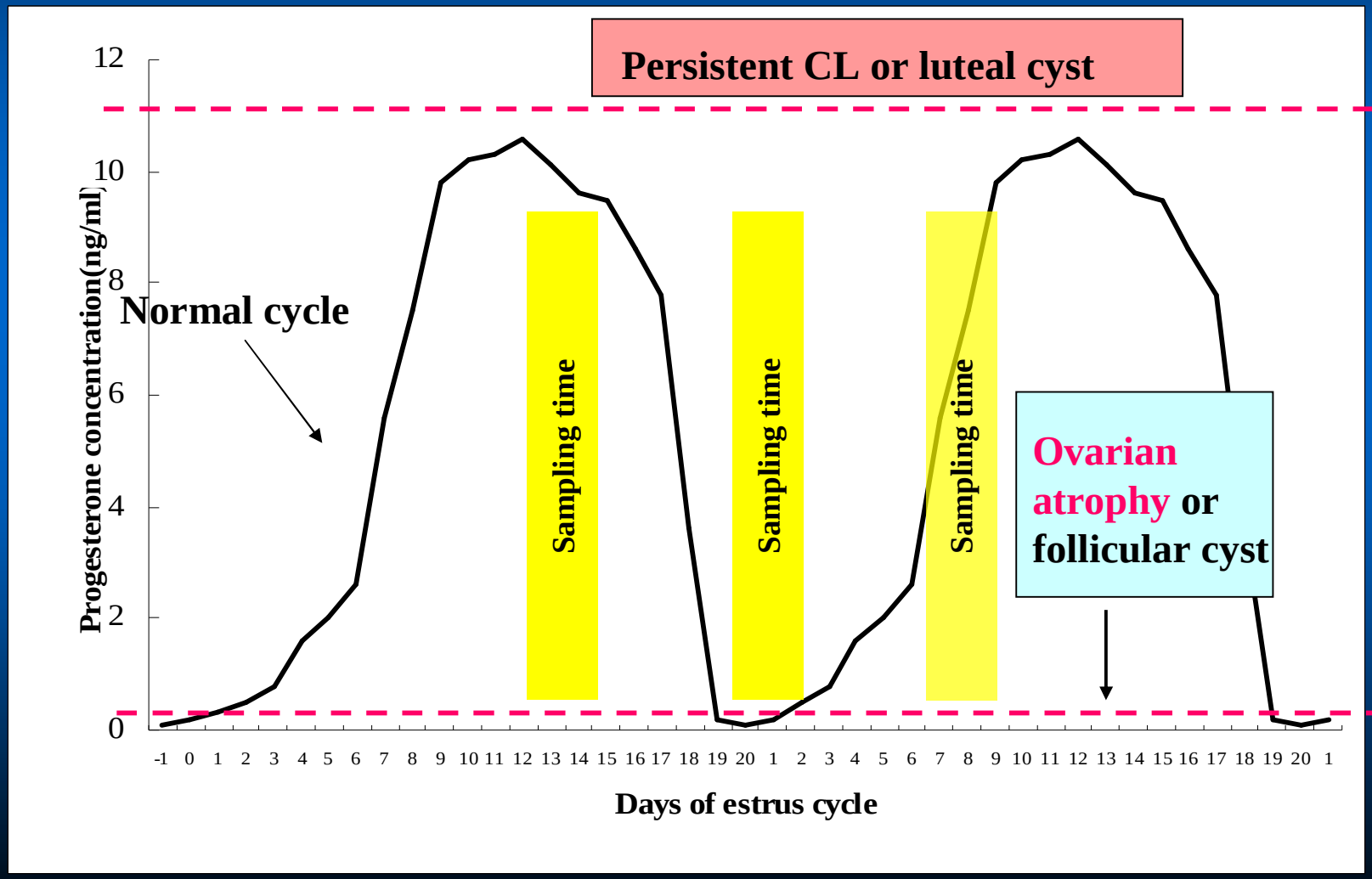
P_4 for postpartum ovarian activity

P_4 for postpartum ovarian activity

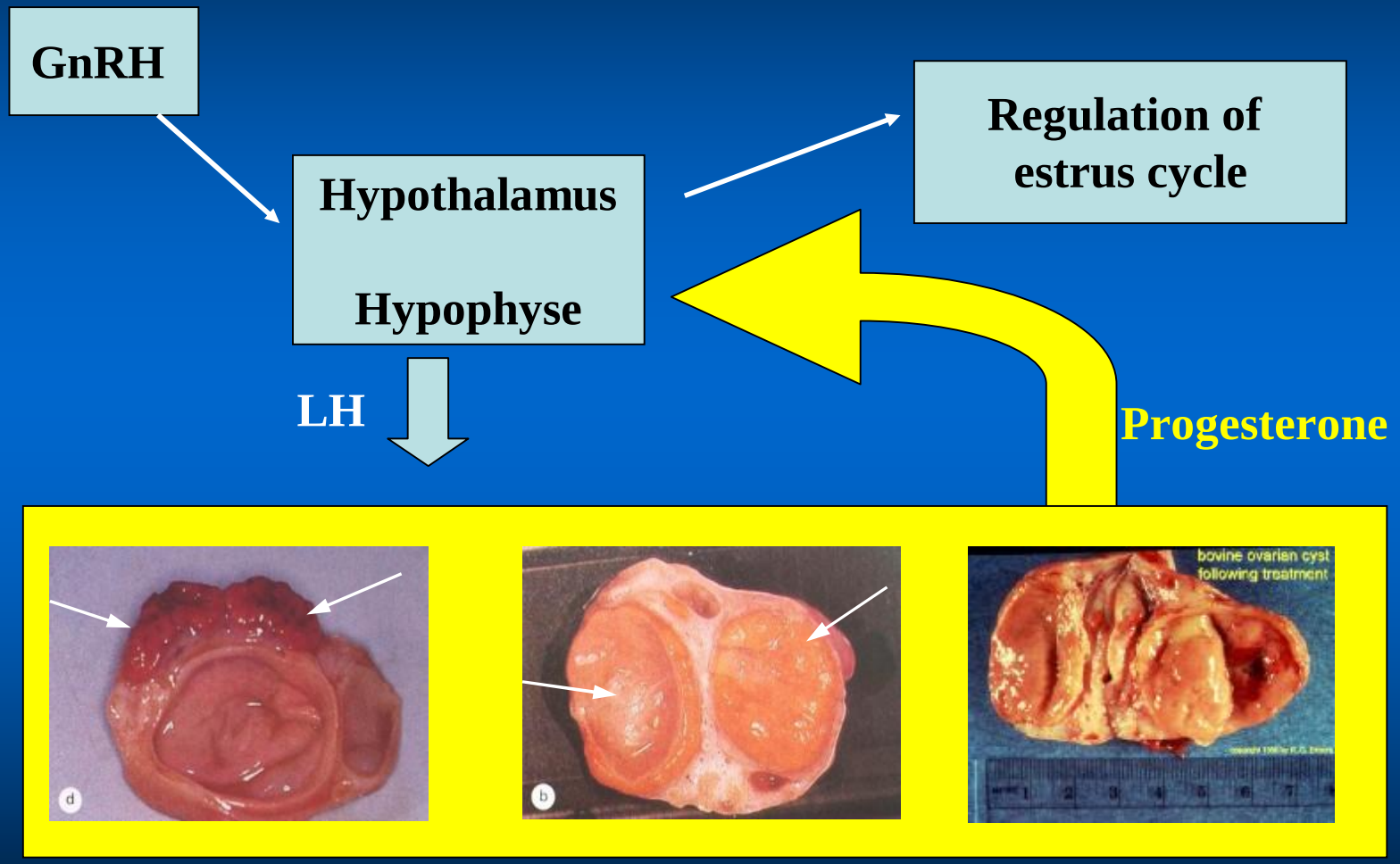




Diagnosis of the ovarian diseases in cattle

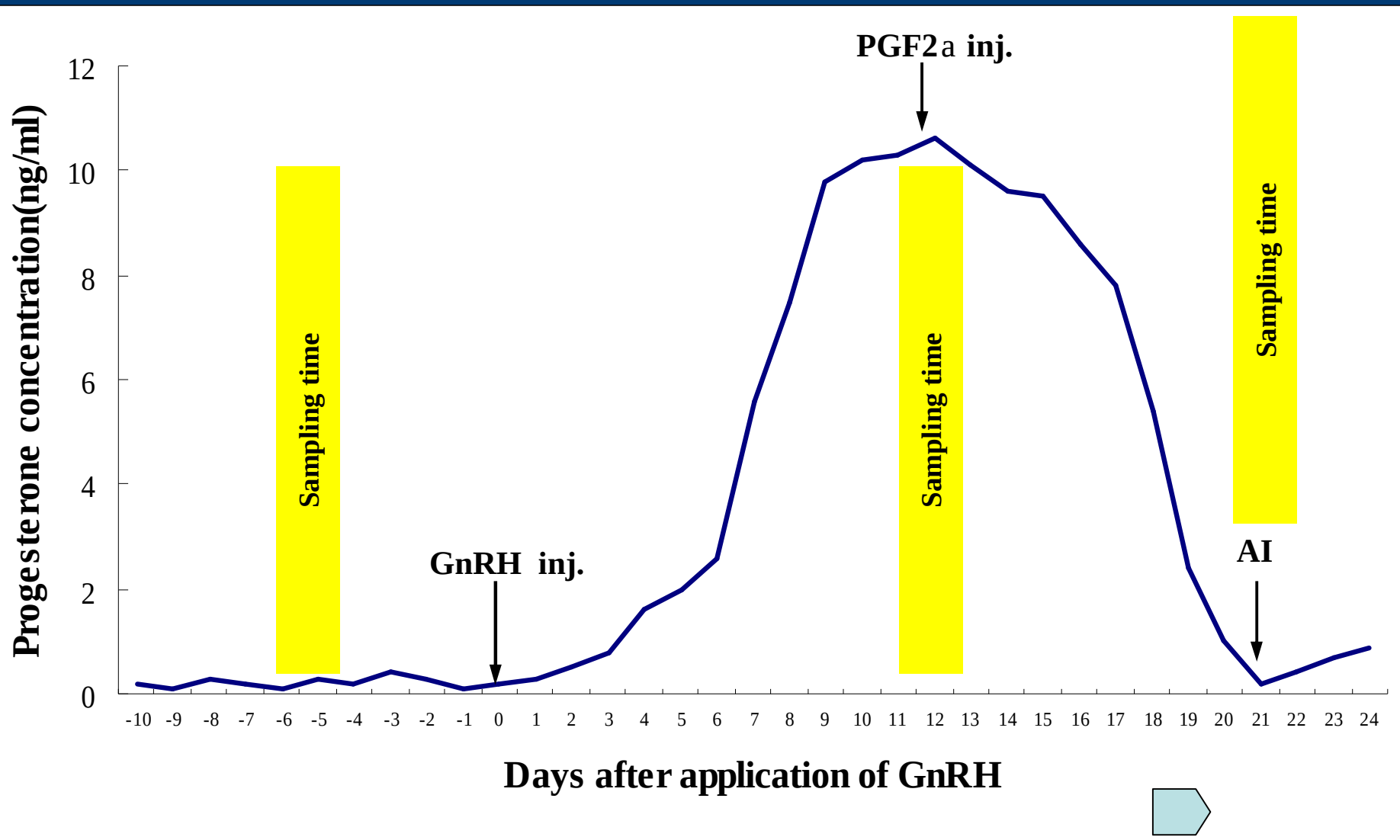


Treatment of bovine follicular cyst



ovulation stimulation or cyst luteinization or ovulation of cyst

Monitoring the treatment of bovine follicular cyst



Clinical aspects of reproductive endocrinology

- **Sheep:**
  - **P_4 for early diagnosis of pregnancy**
    - **P_4 in estrus: 0.15-0.25 ng/ml, in CL phase: 2-4 ng/ml, in pregnancy from 60 days: 12-20 ng/ml**
  - **Estrone sulfate (fetoplacenta) for diagnosis of pregnancy from 70 days (≒ 88%)**
  - **Pregnancy specific protein, ovine placental lactogen for diagnosis of pregnancy**

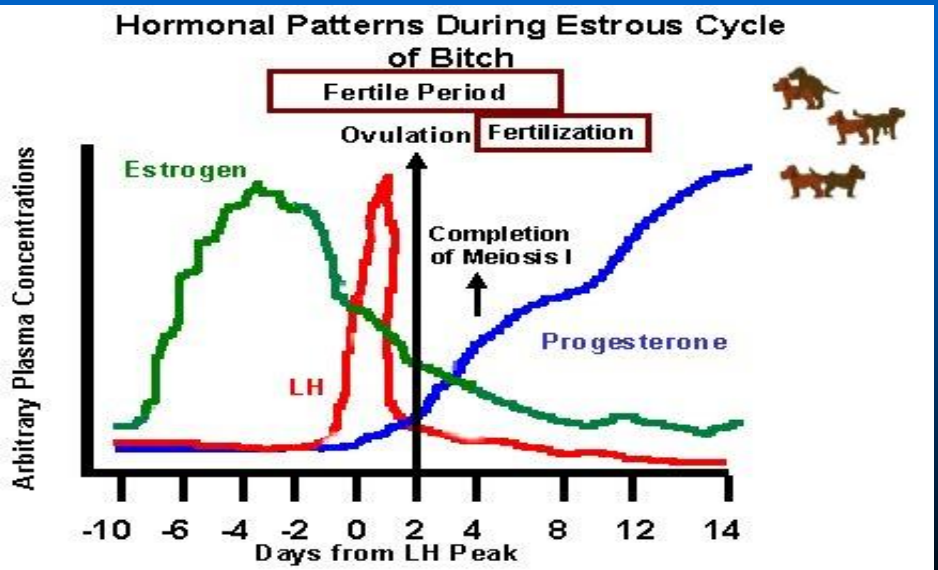
Clinical aspects of reproductive endocrinology

- **Sow:**
  - **P_4 for early diagnosis of pregnancy**
    - **P_4 in pregnancy: 20-50 ng/ml**
  - **Estrone sulfate for early diagnosis of pregnancy from Day 20- 26, 98% accuracy rate**

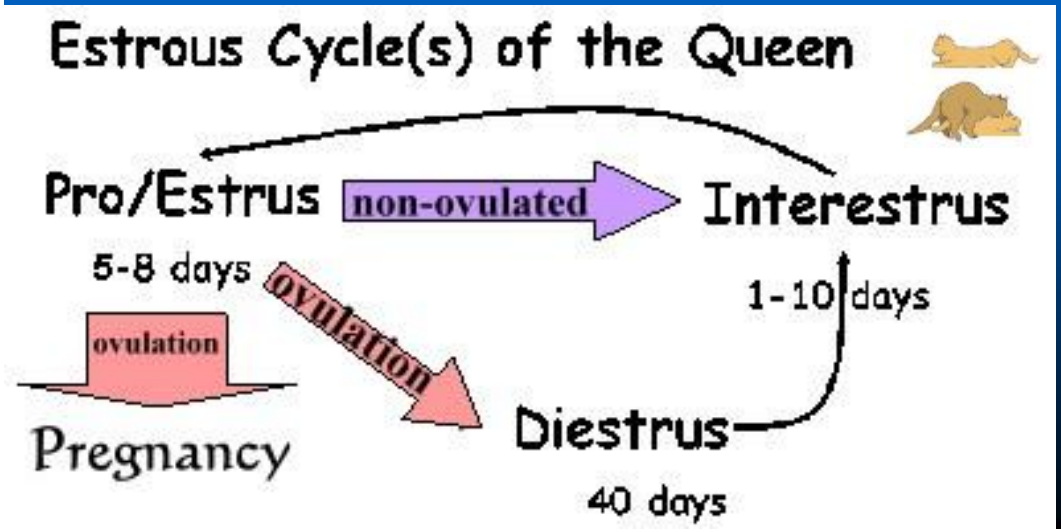
Clinical aspects of reproductive endocrinology

- **Horse:**
  - **eCG for diagnosis of pregnancy**
  - **P_4 for ovarian activity**
    - **Persistent CL exhibits low P_4 value**
    - **Presence of cyclic ovarian activity**
    - **Ovulation prediction (88%)**
  - **Estrone sulfate for diagnosis of pregnancy**
  - **Testosterone for diagnosis of granulosa cell tumor, cryptorchidism**

- **Dog:**
  - **P_4 for preovulatory breeding**
  - **P_4 for diagnosis of remaining ovarian remnant**
  - **Testosterone for checking the secretory status of the Ledig cells**
  - **Confirmation of sertoli cell tumors: LH ↓ , T ↓ (Cryptorchidism)**
  - **Relaxin for confirmation of pregnancy status**

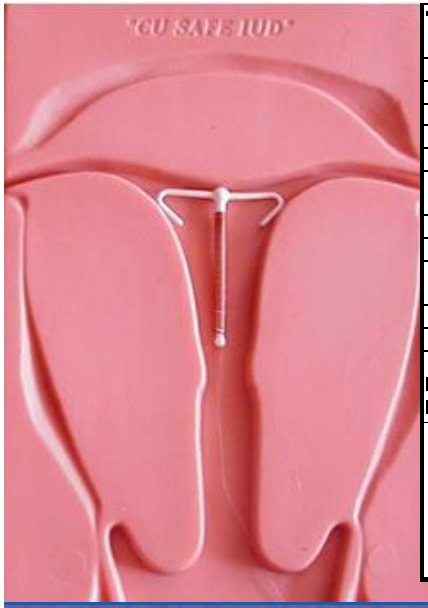


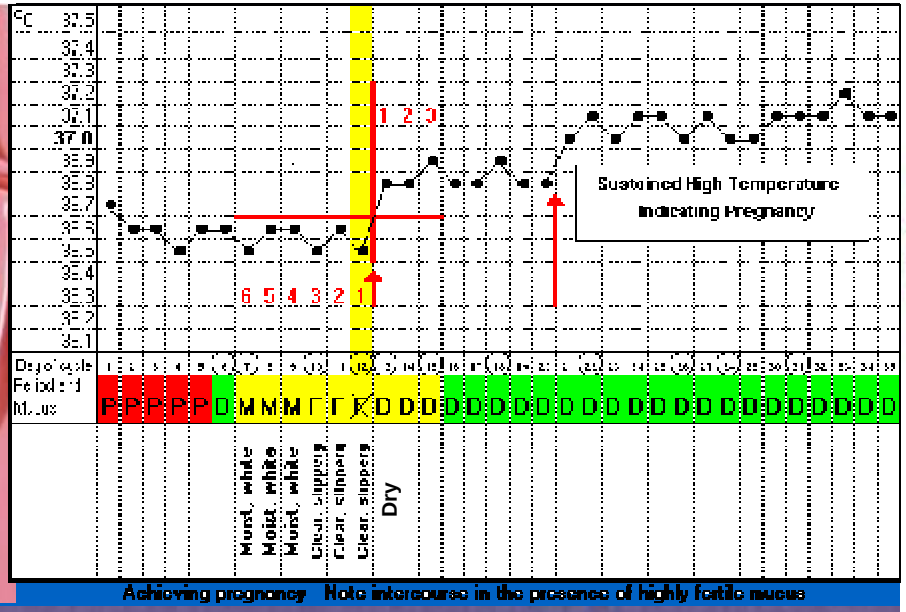
- **Cat:**
  - **P_4 for confirmation of ovulatory failure, P_4 ↓ 10 days postbreeding (P_4 < 0.15 ng/ml)**
  - **Estrogen analysis (from feces) for ovarian follicular activity, completeness of an OHE**
  - **Testosterone for evaluation of Ledig cell function in the testis**
  - **Relaxin for confirmation of pregnancy status**

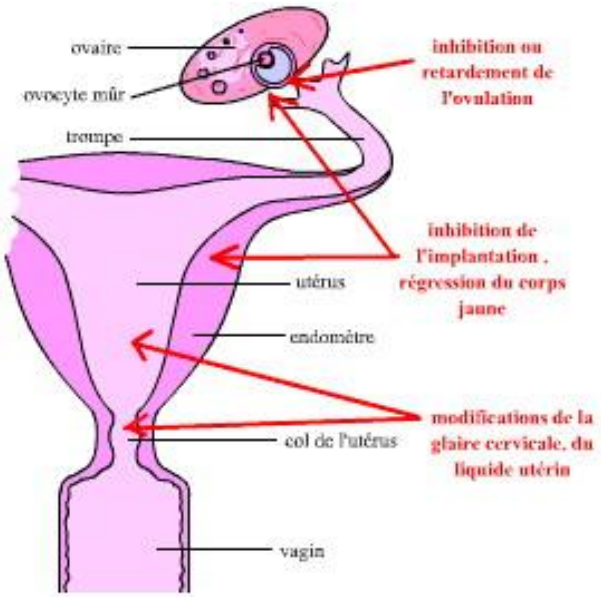


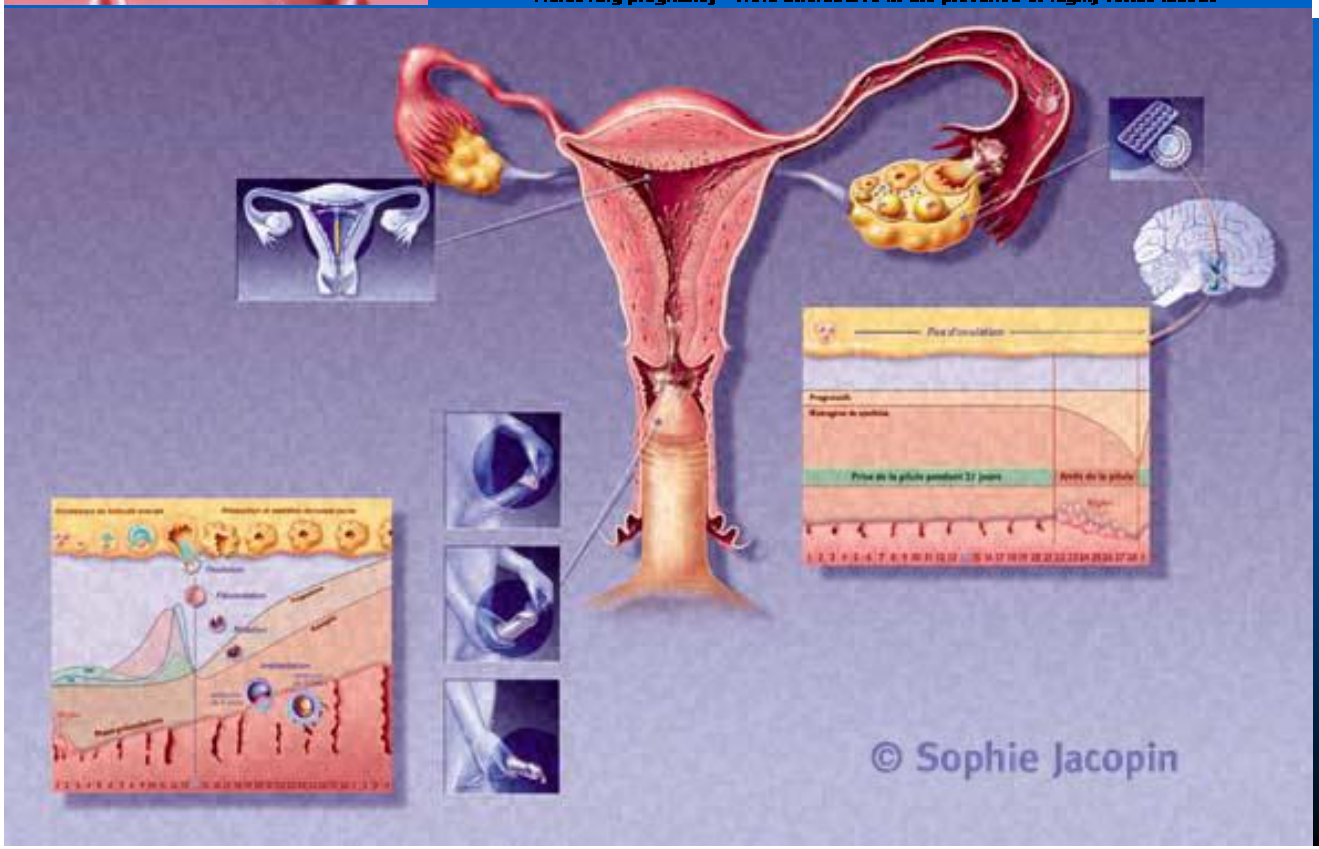
General comments

- **Reference values developed by laboratories are depend on the type of assay used.**
  - **1 ng/ml as the basal value of P_4**
- **Harvesting serum soon after collection**
  - **RBC can metabolize P_4 to other steroids**
  - **Temperature ↓ , P_4 decline slowly**
  - **Skim milk for the immunoassay of P_4**









contraception