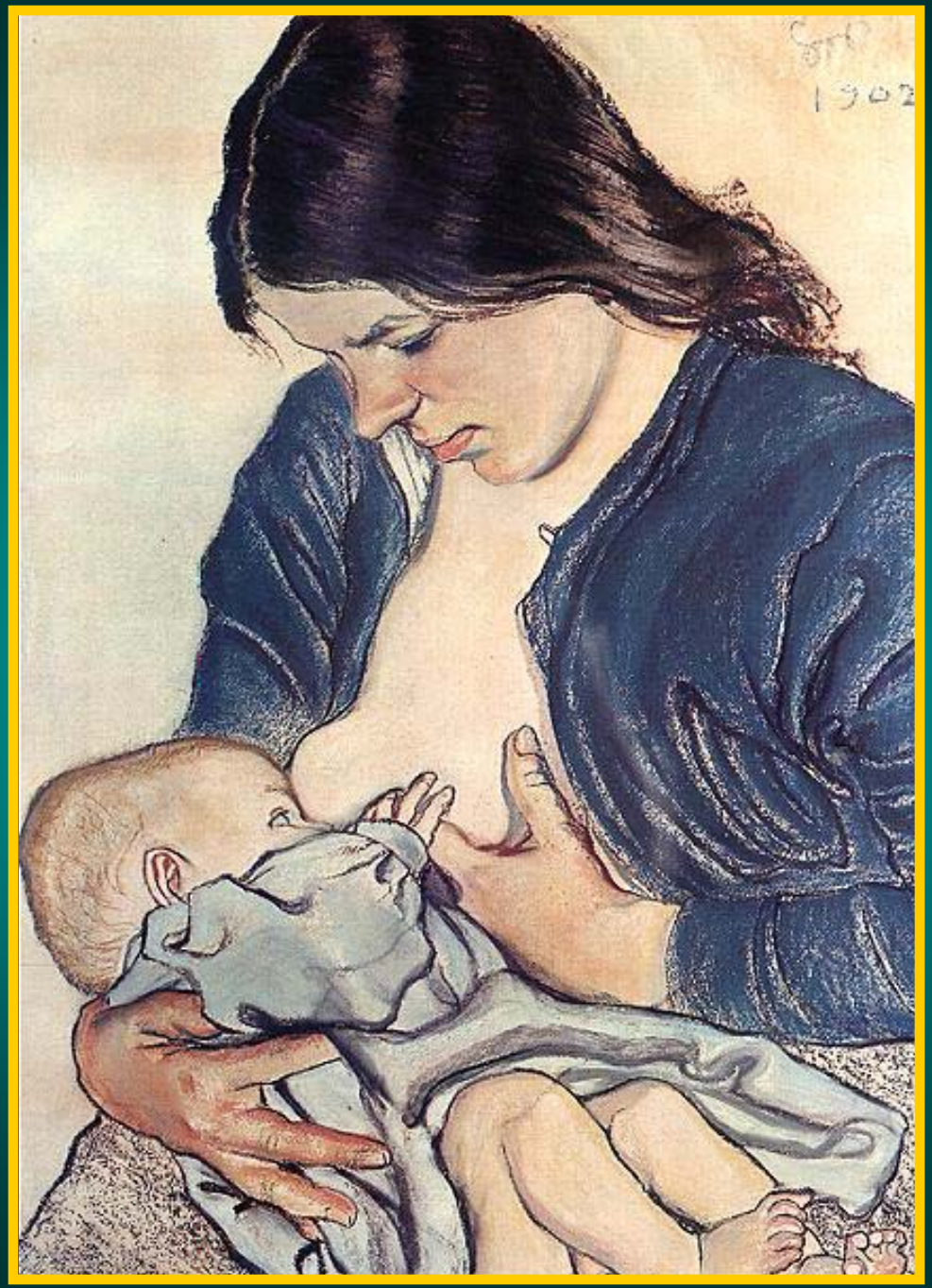
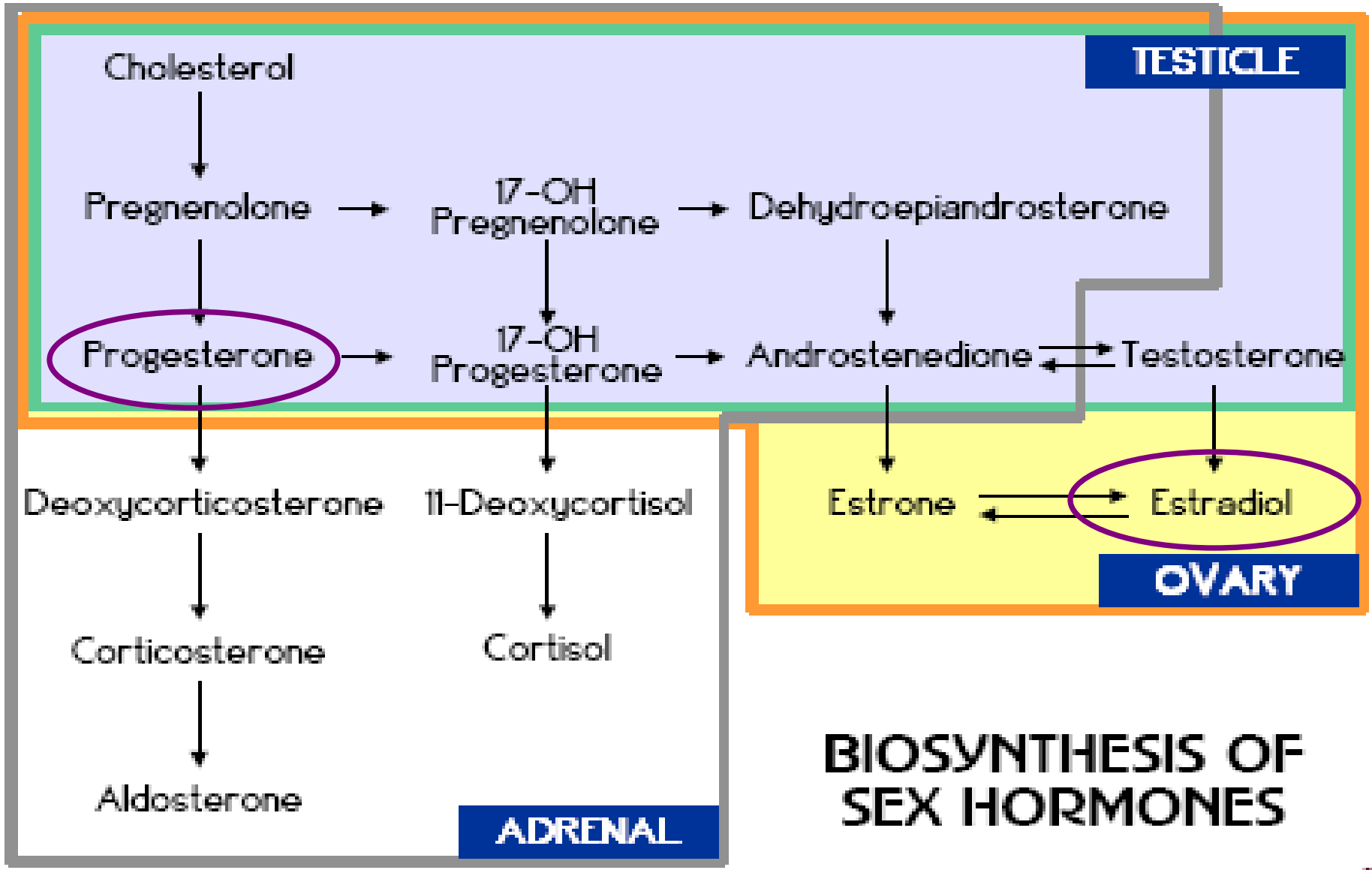
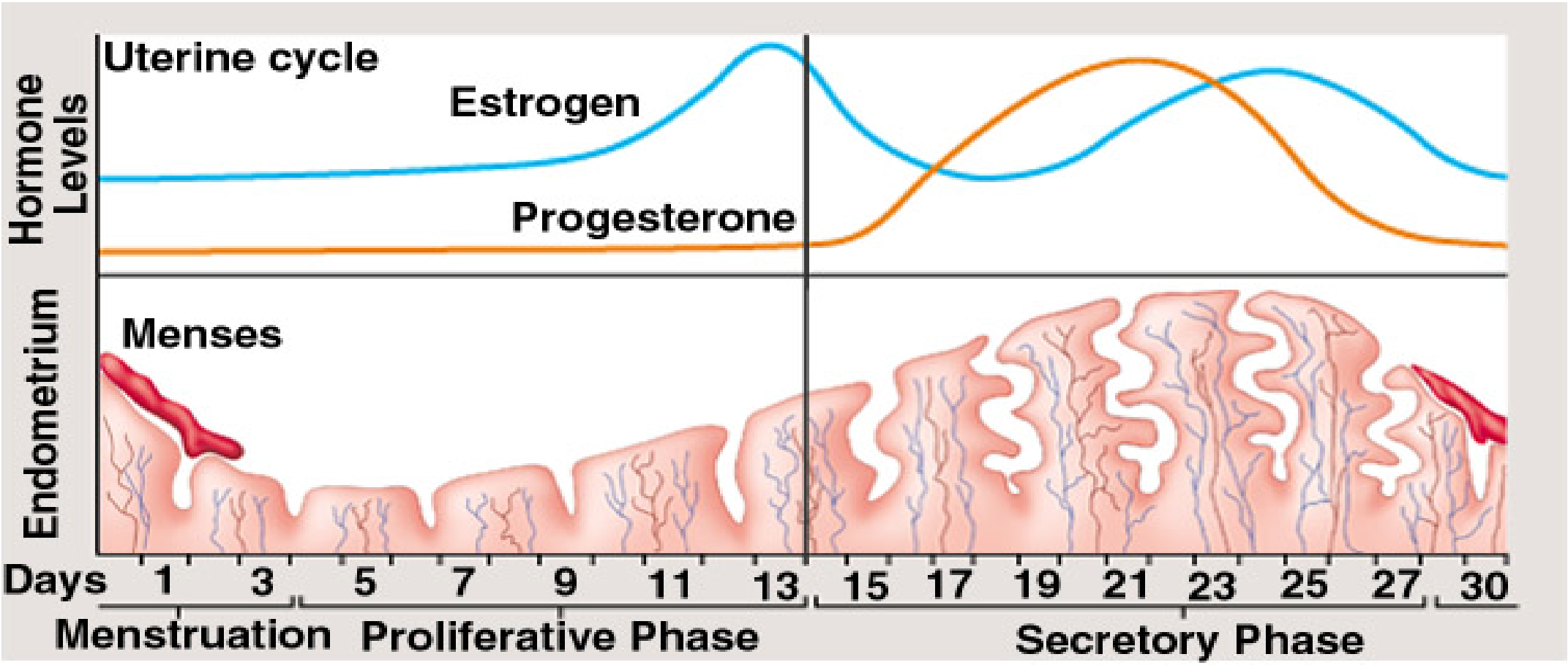


ER and PR

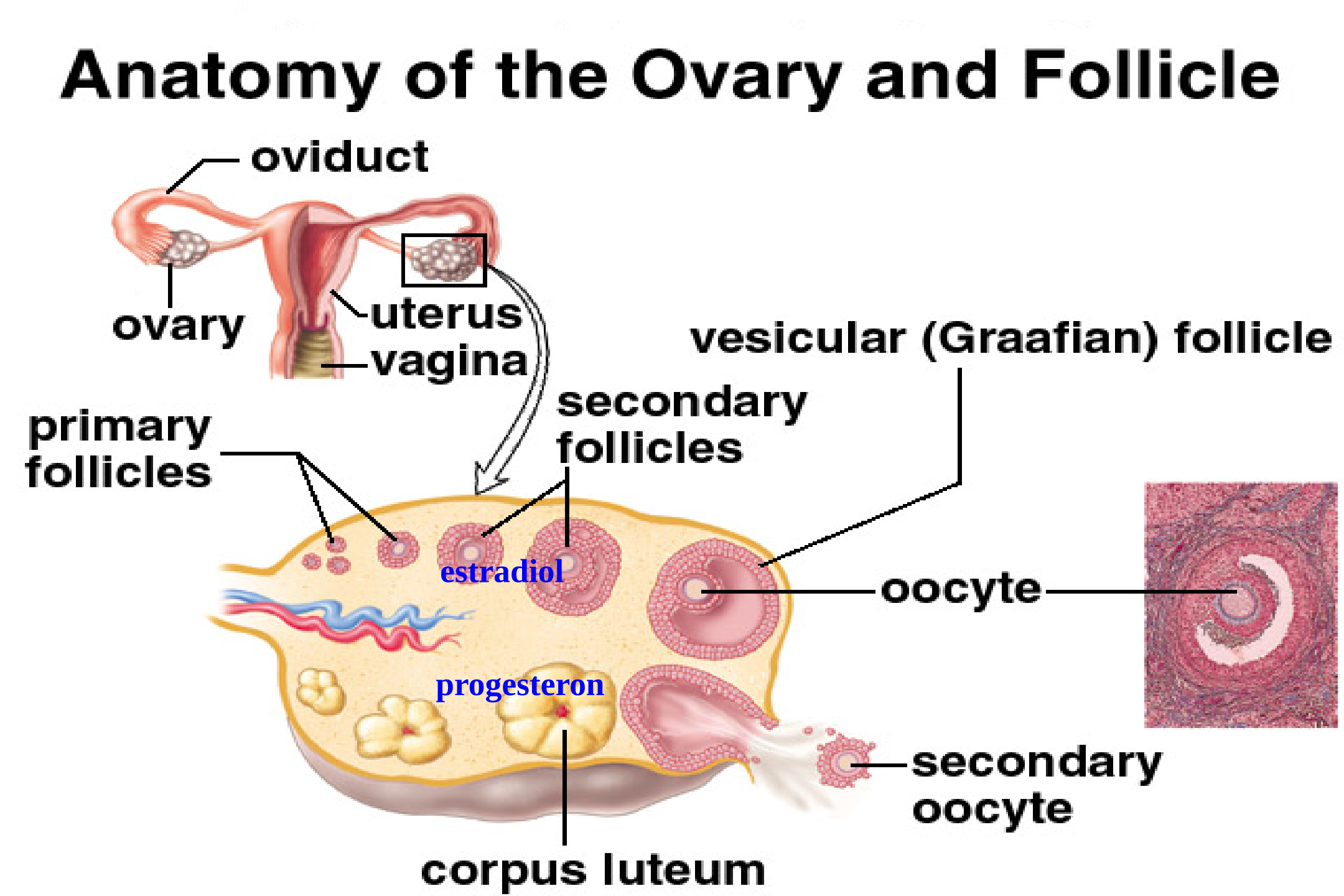


BIOSYNTHESIS OF SEX HORMONES

Female
Hormonal Levels



Anatomy of the Ovary and Follicle

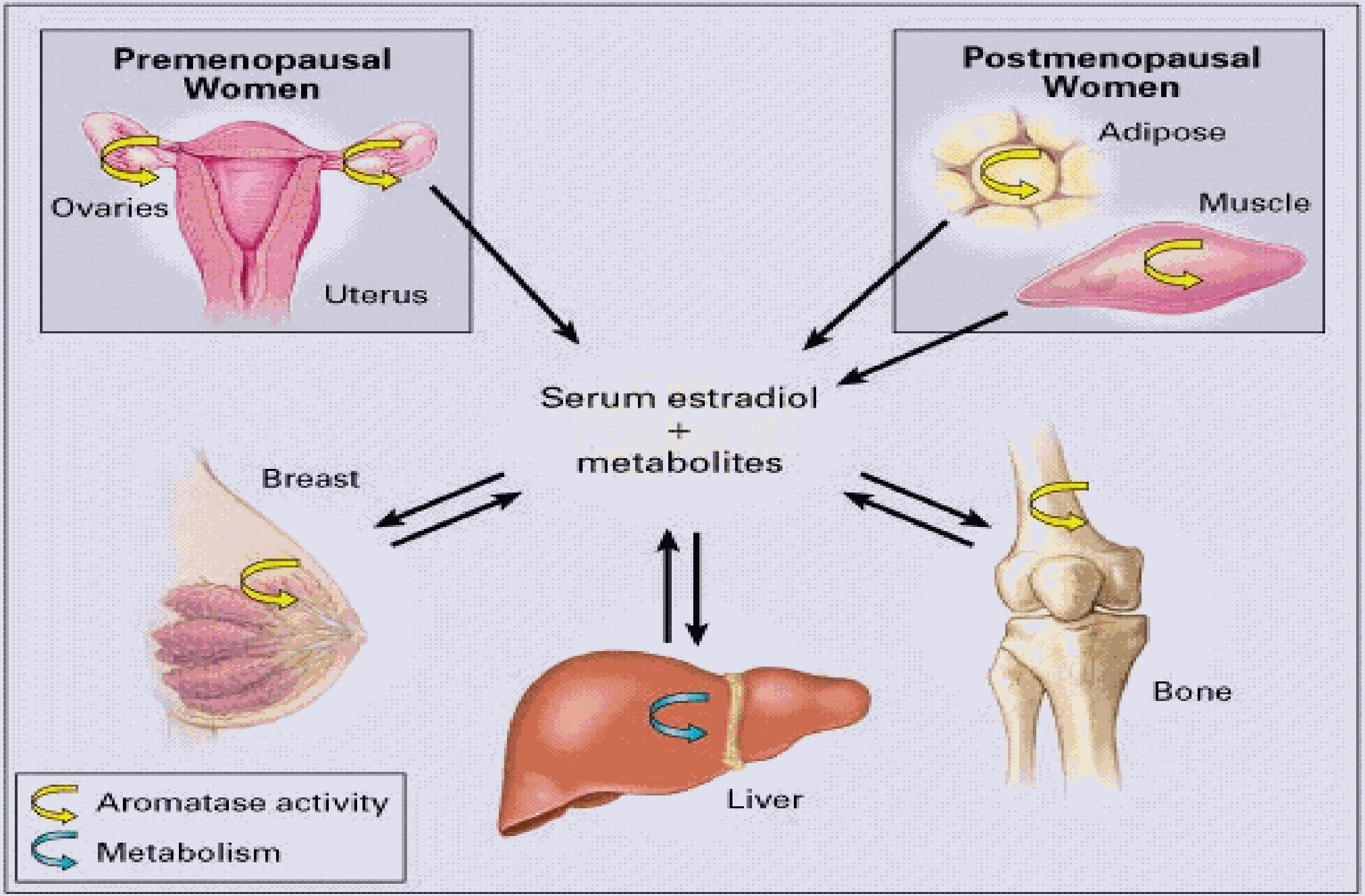


Sites of estrogen syntheses

- Before menopause estrogens are produced by enzyme aromatase mostly in:
  * ovary (granulosa cells)

and in other tissues, such as:
  * subcatenous adipocytes (especially estradiol)
  * skeletal muscle (especially estradiol)
  * stroma cells in breast (especially estrone)
  * osteocytes (especially estrone)
  * placenta (especially estriol)

- After menopause estrogens are not produced by ovary but aromatase is still active in other tissues.

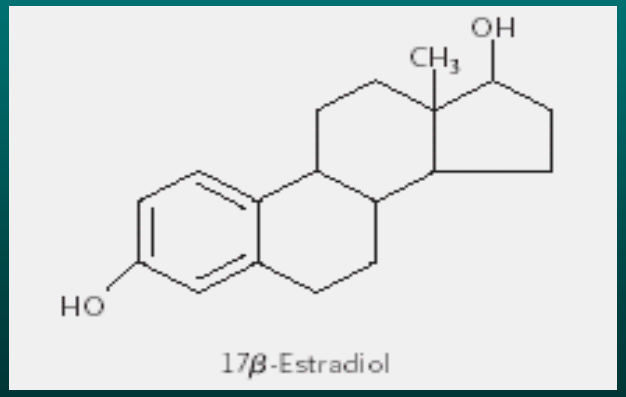

Premenopausal Women
Ovaries
Uterus
Postmenopausal Women
Adipose
Muscle
Serum estradiol
+
metabolites
Breast
Liver
Bone
Aromatase activity
Metabolism

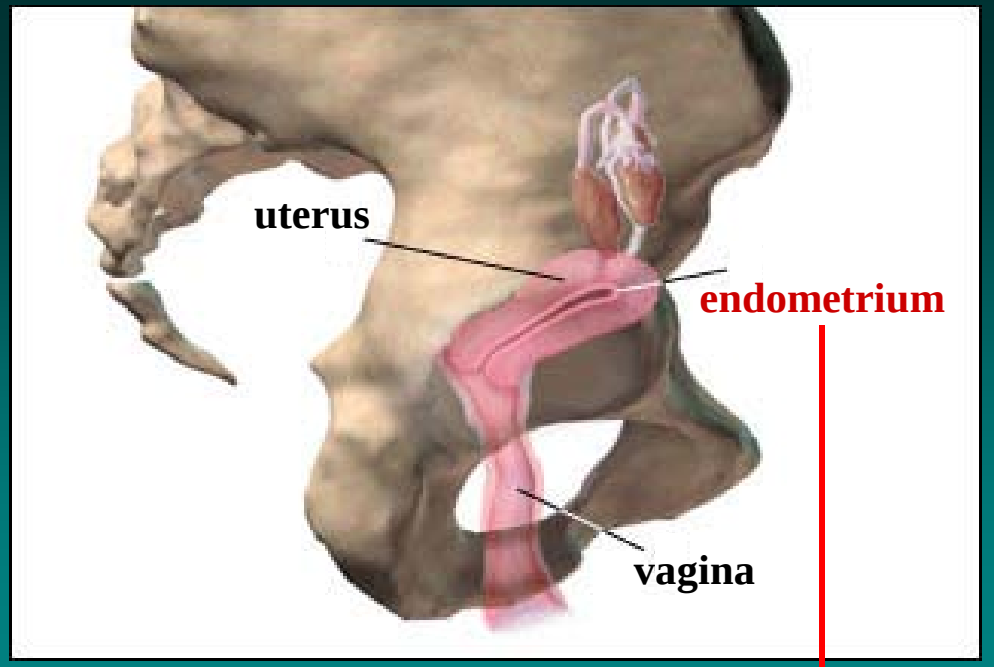
Estradiol

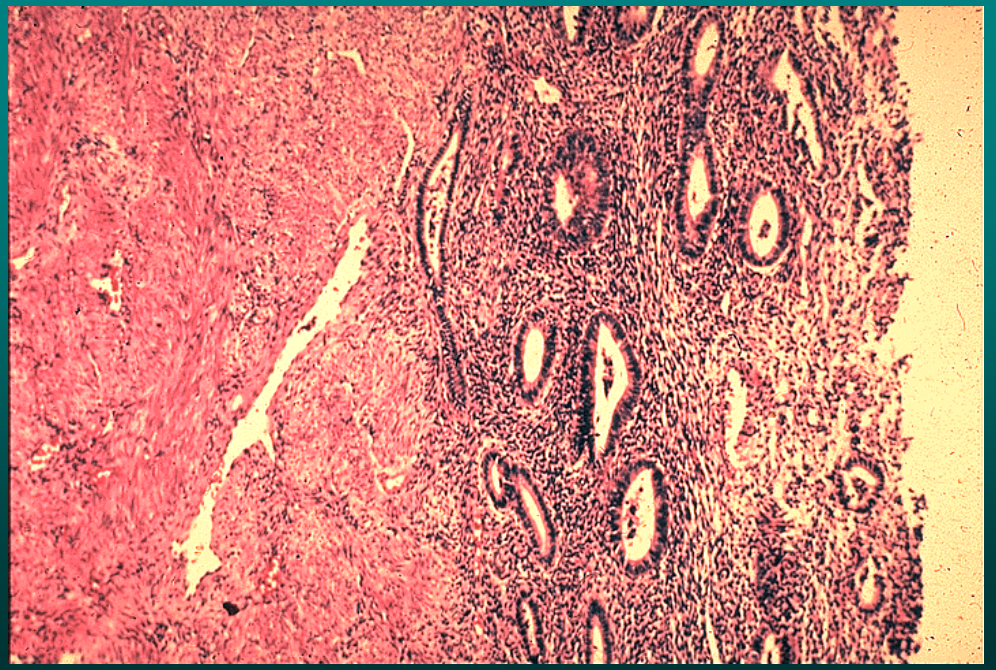
- In the female reproductive system it acts mostly on endometrium.

- Stimulates the epithelial cells of the basal layer of the endometrium to proliferate, forming a thick mucosa as well as numerous endometrial glands (proliferative phase of endometrial cycle, 6-14 days).

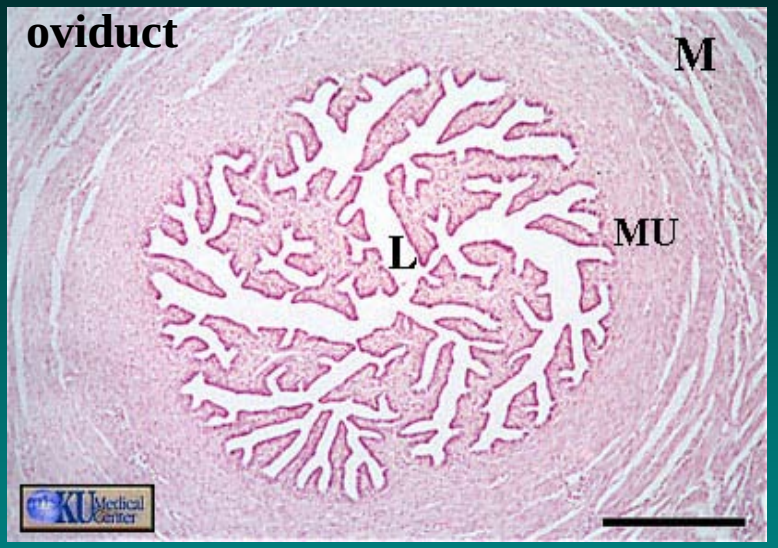
- Increases the expression of progesterone receptors in endometrium.







Estradiol



• Stimulates the development of extensive mucosal folds of the oviduct as well as the formation of cilia on these epithelial cells.

• Promotes growth of uterus, vagina, and oviducts, as well as mammary glands.

• Triggers estrus in females of different mammals (supported by progesteron).

Estradiol

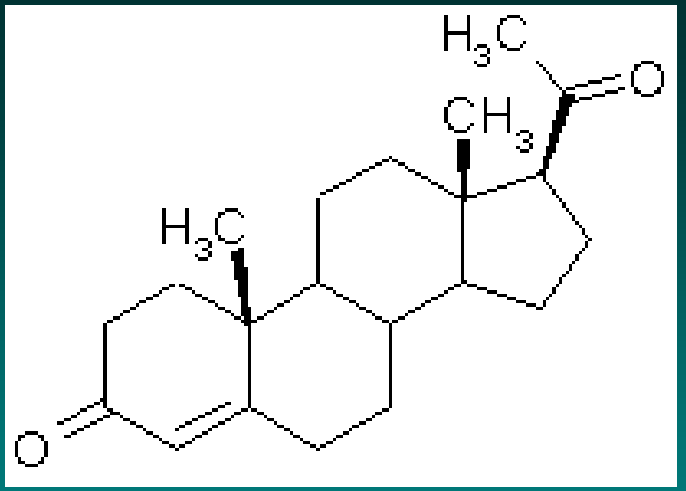
- Estradiol increases development of secondary sex characteristics: promotes growth of wider pelvic bones as well as the closure of epiphysal plates in long bones. However many of female secondary sex characteristics are due to the absence of androgens.

- Allows development of softer skin and promotes deposition of fat in subcatenous zones, particularly in breast and buttocks, leading to mature female shape.

- Estradiol enhances calcium deposition in bone and stimulates bone tissue formation.

- Functions as a neuroprotectant and cardioprotectant.

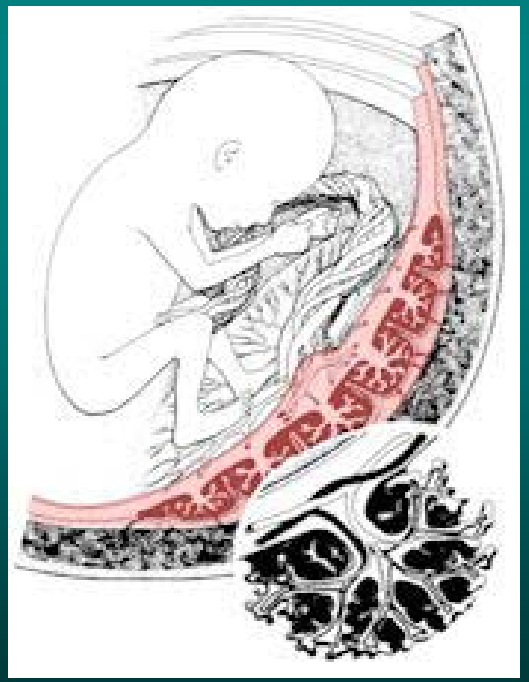
Progesterone



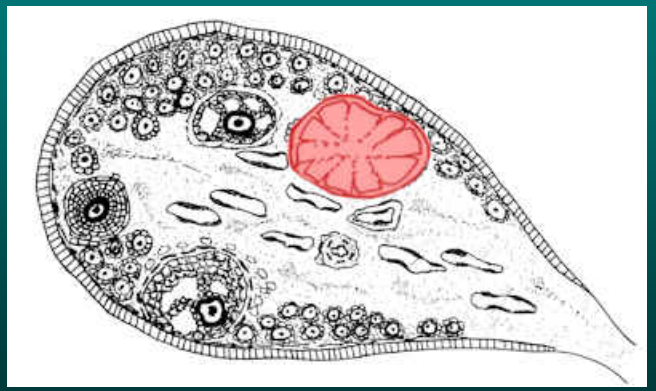
- Is involved in maintenance of pregnancy.

- Progesteron is absent in the blood during the follicular phase and appears only after ovulation.

- In the female reproductive system it acts mostly on the glands in the endometrium to promote their secretory activity (secretory phase of endometrial cycle) and prepare endometrium for pregnancy.



Produced by the ovaries and placenta (in nonpregnant women in corpus luteum)

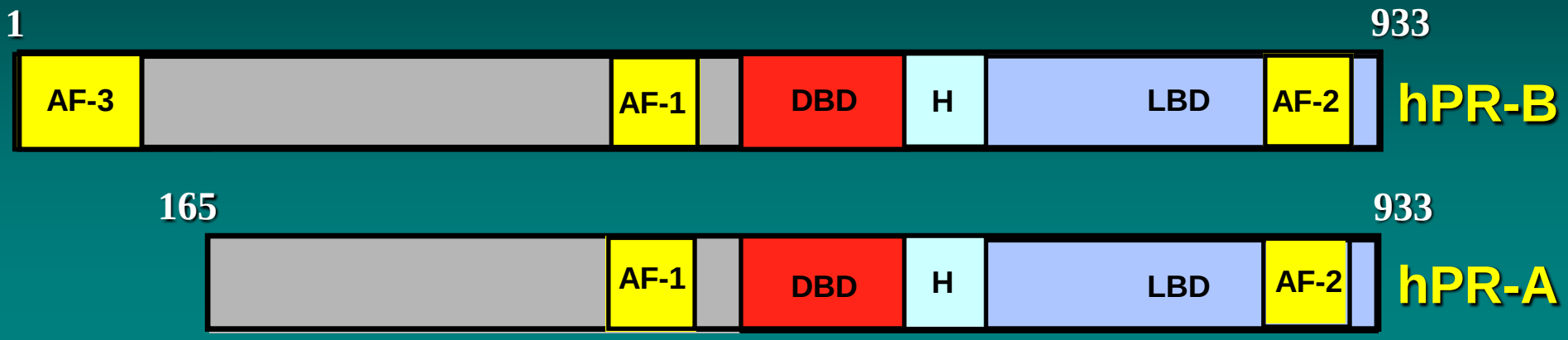


Progesterone during the pregnancy:

- Increases mammary gland alveolar-lobular formation and growth of breast.
- Inhibits new follicular development.
- Activates the endometrial gland to secrete fluids.
- Maintains the functions of the placenta.
- Keeps the endometrium in a thickened condition.
- Stops the uterus contractions.
- Prevents lactation until after the birth (with estrogen)
- Strengthens the mucus plug covering the cervix to prevent infection.
- Strengthens the pelvic walls in preparation for labour.

At the end of the pregnancy, the levels of progesterone secreted by the placenta drop off. It is this action that stimulates the beginning of the contractions that will lead to birth.

Progesterone Receptor: A and B Forms

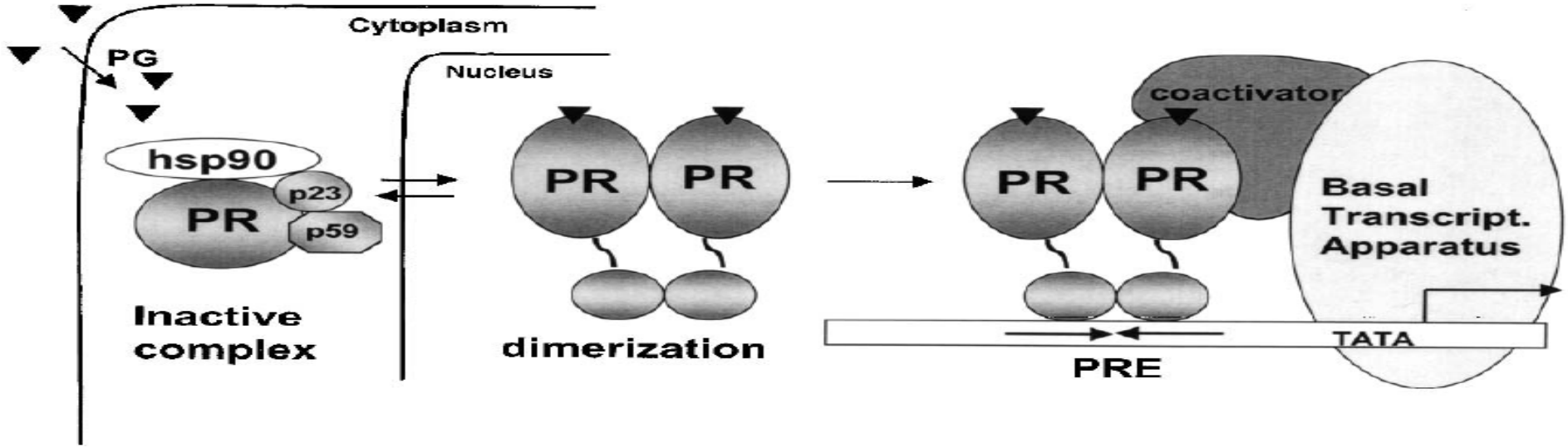


- From single gene by alternate transcription initiation (different promoters).
- Isoforms differs in activities.
- PR-B is highly expressed in endometrium during proliferative phase, but not during secretory phase.
- In humans PR-A acts as a repressor of activity of PR-B, GR, ER, AR, and MR.

Progesterone Receptor: A and B Forms

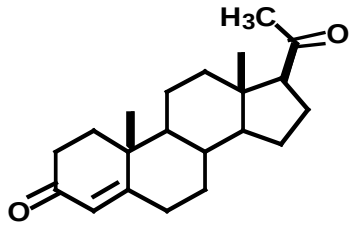
Components of female reproductive system and phenotype identified in progesterone receptor knockout mouse models.

Component	Genotype	Phenotype
Ovaries	PRKO	Inability to ovulate
	PRAKO	Severely impaired ovulation
	PRBKO	Normal ovulation
Uterus	PRKO	Impaired implantation/decidualization/infertility
	PRAKO	Impaired implantation/decidualization/infertility
	PRBKO	Normal implantation/decidualization
Mammary gland	PRKO	Impaired mammary gland development
	PRAKO	Normal progesterone-induced mammary gland morphogenesis
	PRBKO	Reduced pregnancy-associated side-branching and lobuloalveolar development

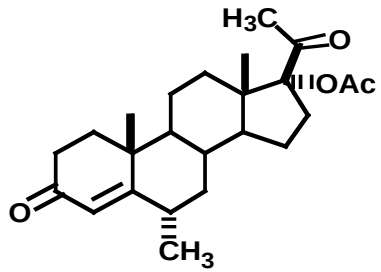


Ligands for Progesterone Receptor

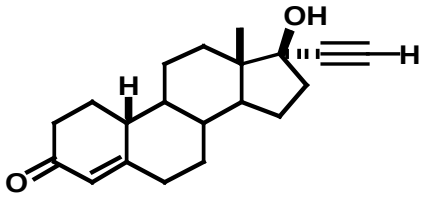
Progestins



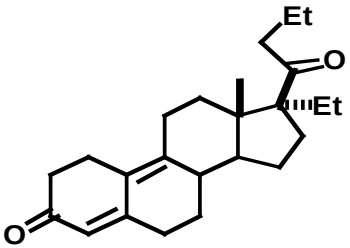
Progesterone
[natural]



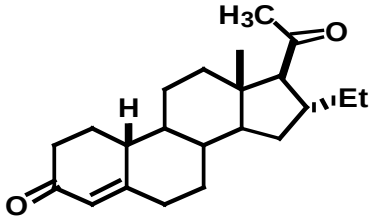
Medroxyprogesterone
Acetate (MPA)
[HRT]



Norethindrone
[oral contraceptives]

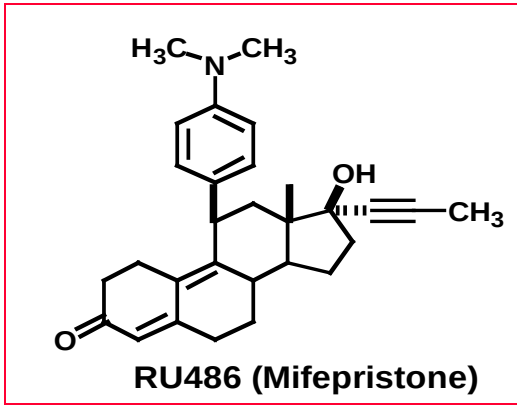


R5020
(Promegestone)

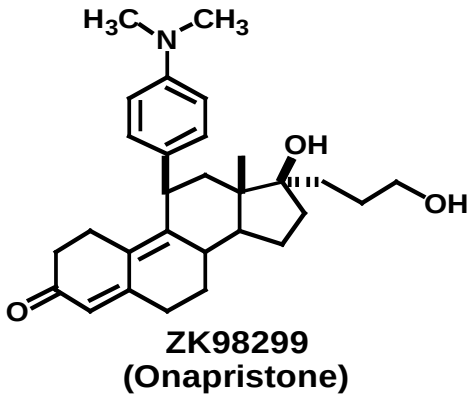


ORG2058

Antiprogestins



RU486 (Mifepristone)



ZK98299
(Onapristone)

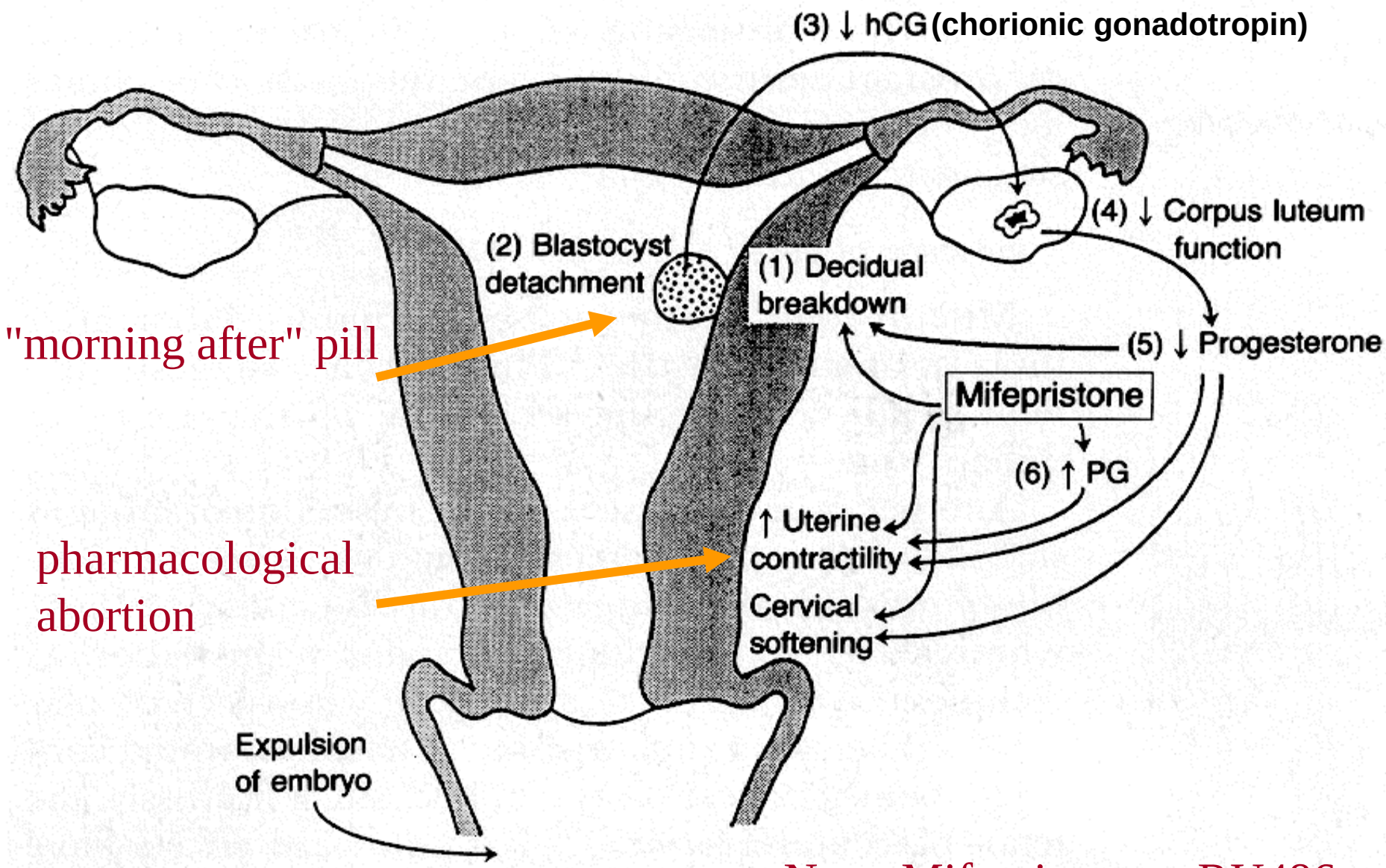
RU 486 – PR antagonist

Ru486 as a ligand of PR:

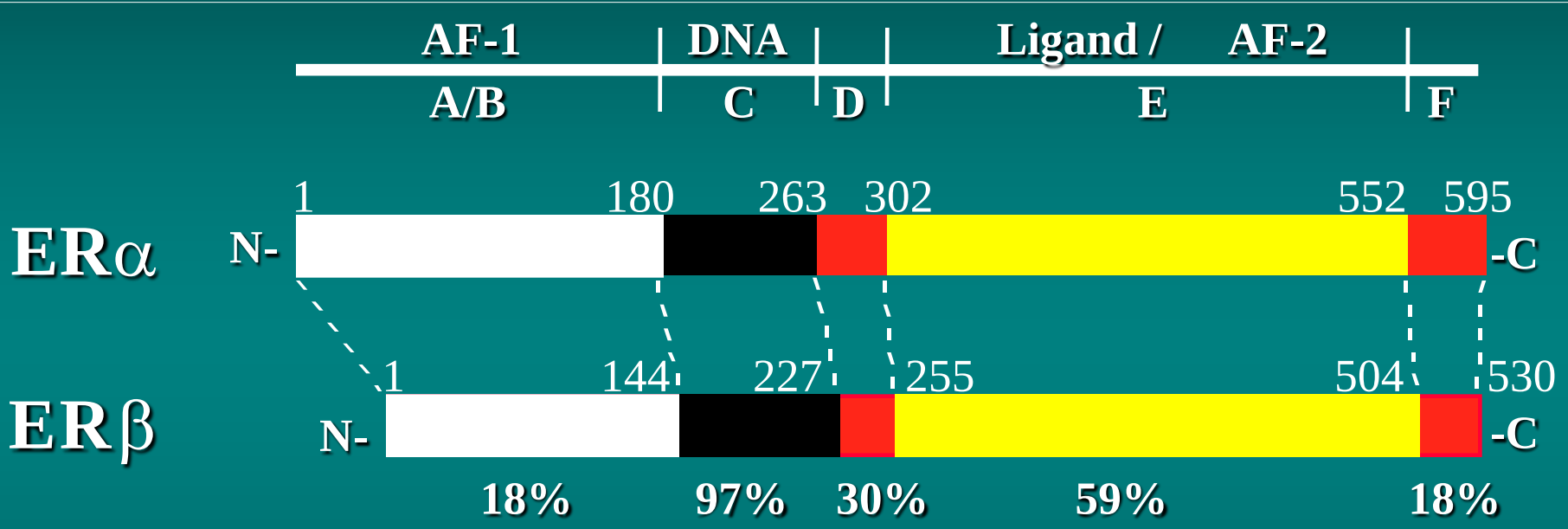
- RU486 binds to PR 3 times stronger than progesterone
- RU486 promotes a high affinity interaction of PR with DNA, thus antagonists-bound PR can effectively compete with binding of agonist-bound PR to PREs.
- In the presence of RU486 PR recruits corepressors to promoters.
- PR liganded with RU486 has the ability to heterodimerize with PR bound agonist. Such heterodimers had a significantly reduced ability to bind to PREs. Heterodimerization could potentially sequester a portion of cellular PR bound to agonist in an inactive form, without requiring direct binding of RU486 to PR.

RU486 action:

1) initiates breakdown of endometrium - prevents embryo implantation
2) promotes contraction of uterine wall and dilatation of cervix, increases sensitivity to $PGF_{2\alpha}$ - expulsion of embryo (early pregnancy termination)

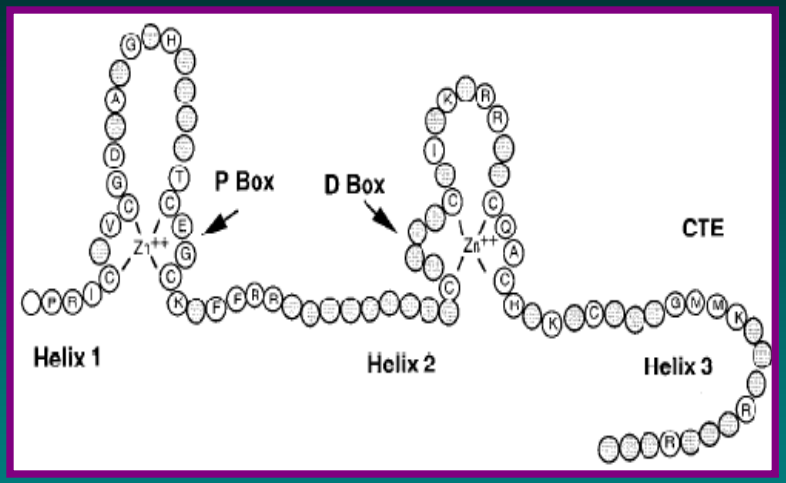

(3) ↓ hCG (chorionic gonadotropin)
(4) ↓ Corpus luteum function
(2) Blastocyst detachment
(1) Decidual breakdown
(5) ↓ Progesterone
"morning after" pill
Mifepristone
(6) ↑ PG
pharmacological abortion
↑ Uterine contractility
Cervical softening
Expulsion of embryo
Note: Mifepristone = RU486

Estrogen Receptors α and β genes



- Two separate genes
- Several splicing isoforms
- Different tissue/cell distributions
- AF1 is very active in ERα but not in ERβ.

ERα and ERβ:



- ERα is much better characterized. Role of ERβ is not fully recognized. One of its splicing forms (503 aa) was reported to act as a dominant negative regulator.

- ERβ is strongly expressed in male reproductive system.

- ERα and ERβ have the same DNA binding domain (identical P-box), thus they bind to the same consensus sequence

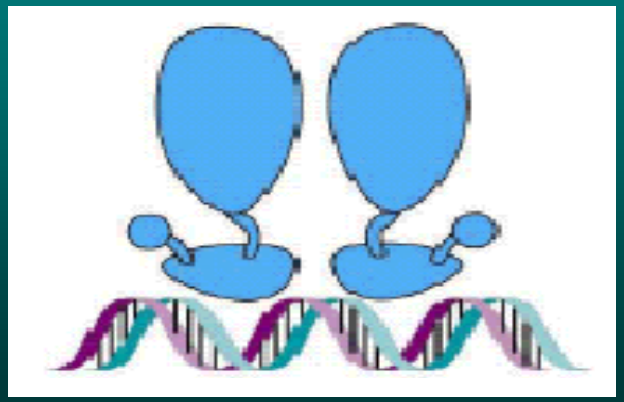
- ER form homodimers, both ERα/ERα, ERβ/ERβ, or ERα/ERβ.

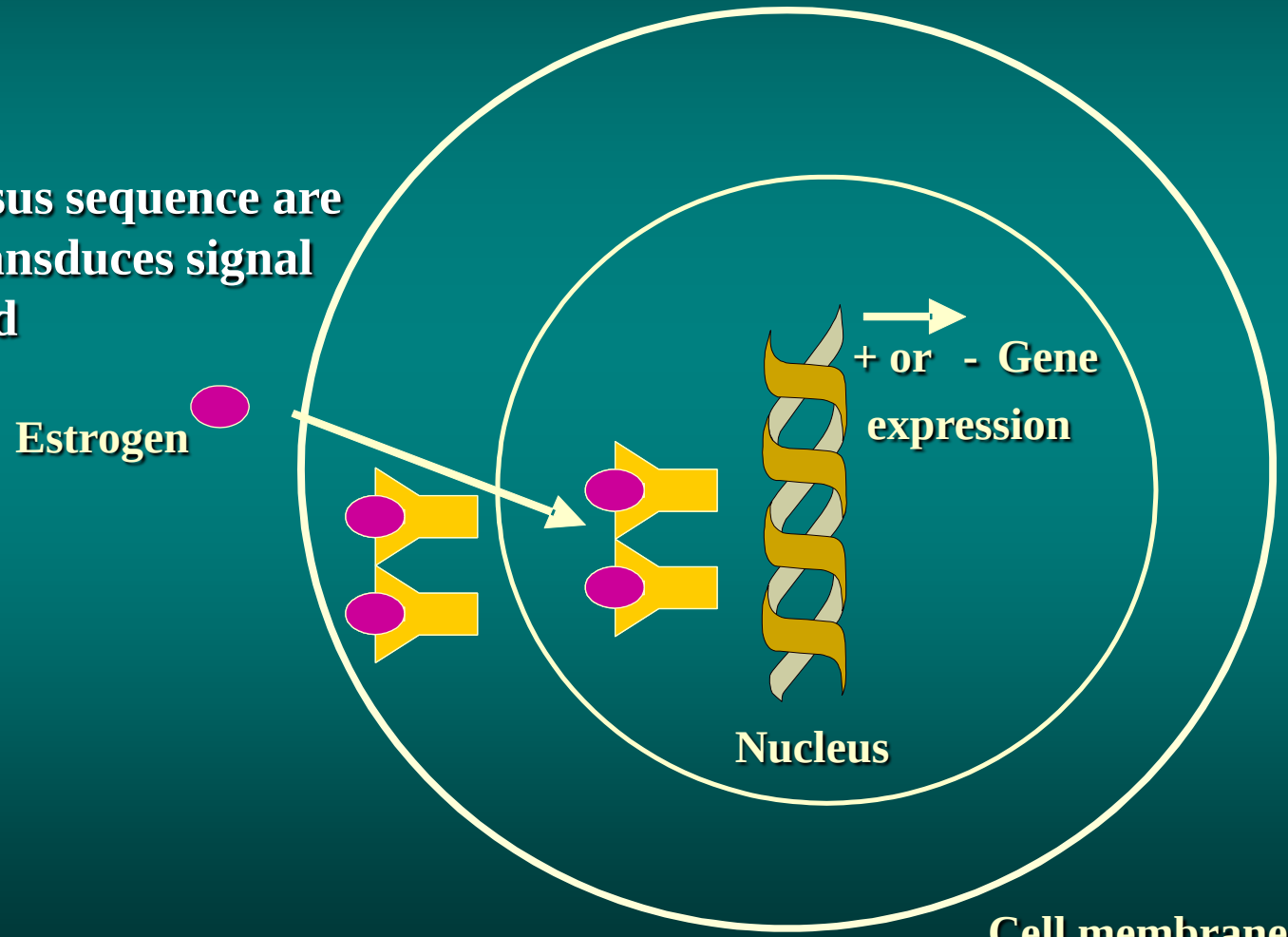
- There are no drugs which act as a selective ligands for one type of ER receptor. Some compounds, however, bind with much higher affinity (120x) to one of them. Some are agonists of one receptor and antagonists of the second receptor.

Estrogen receptors (ERs):

* ERs are localized in cell plasma and nucleus and are bound to heat shock proteins. After ligand binding they form homodimer and bind to consensus sequences in the promoters of target genes.

* ERs after binding to consensus sequence are ubiquitinated. Possibly ER transduces signal only once and then is degraded by proteasomes.



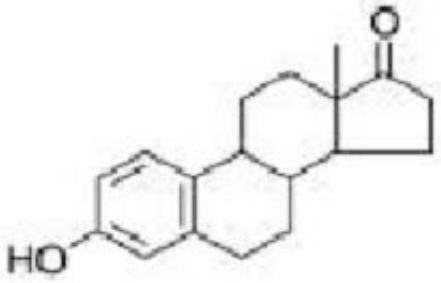


Activation of ER:

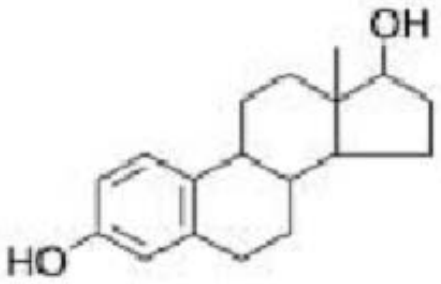
* Classical way of ER activation is binding of ligand (e.g. estradiol).

* ER may be activated without binding the ligands e.g. as a result of phosphorylation. ER can be phosphorylated e.g. by PKA (protein kinase-A), PKC (protein kinase-C), cycline-dependent kinases, MAP kinases. Phosphorylation can take place both at AF-1 and AF-2.

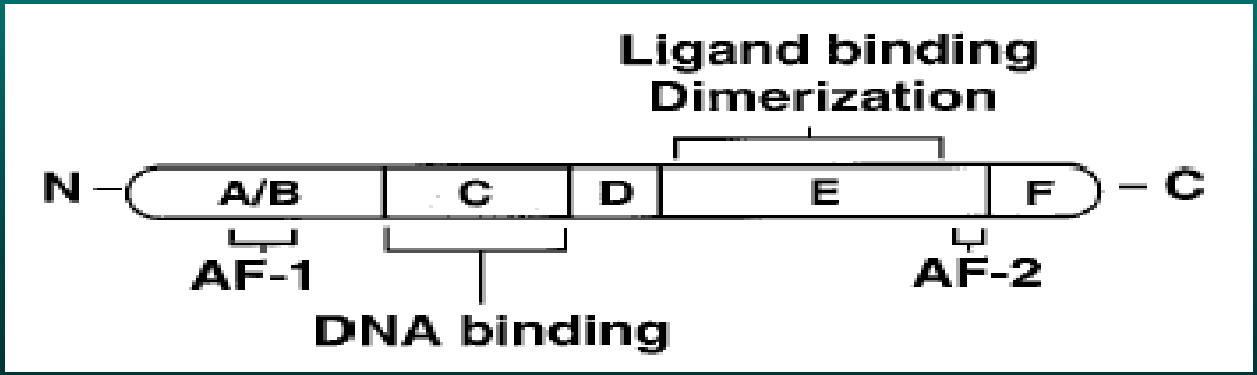
* Phosphorylation may influence the ligand binding, dimerization, binding to DNA and interaction with co-factors. Possibly, even if phosphorylation may occur without ligand binding, the binding of ligand increases it.

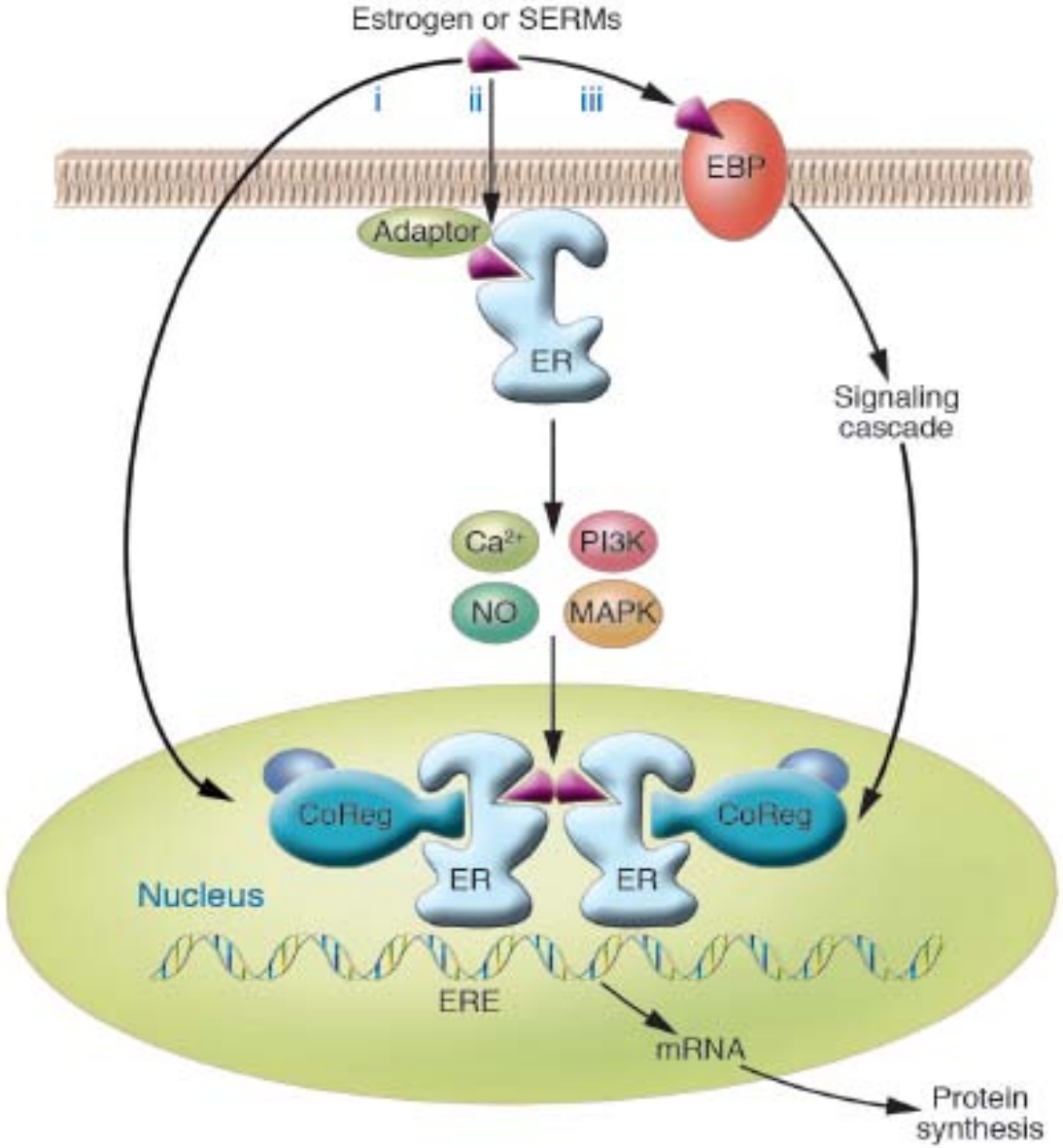


Estrone



17β-Estradiol





Estrogen action:

i) classical activation of transcription through nuclear ER

ii) activation of membrane ER and stimulation of kinase pathways

iii) activation of non-ER membrane associated estrogen binding proteins (EBPs) and activation of kinase pathways

Influence of ER on gene transcription:

ER may affect gene transcription by:

*** Direct binding to consensus sequence.**

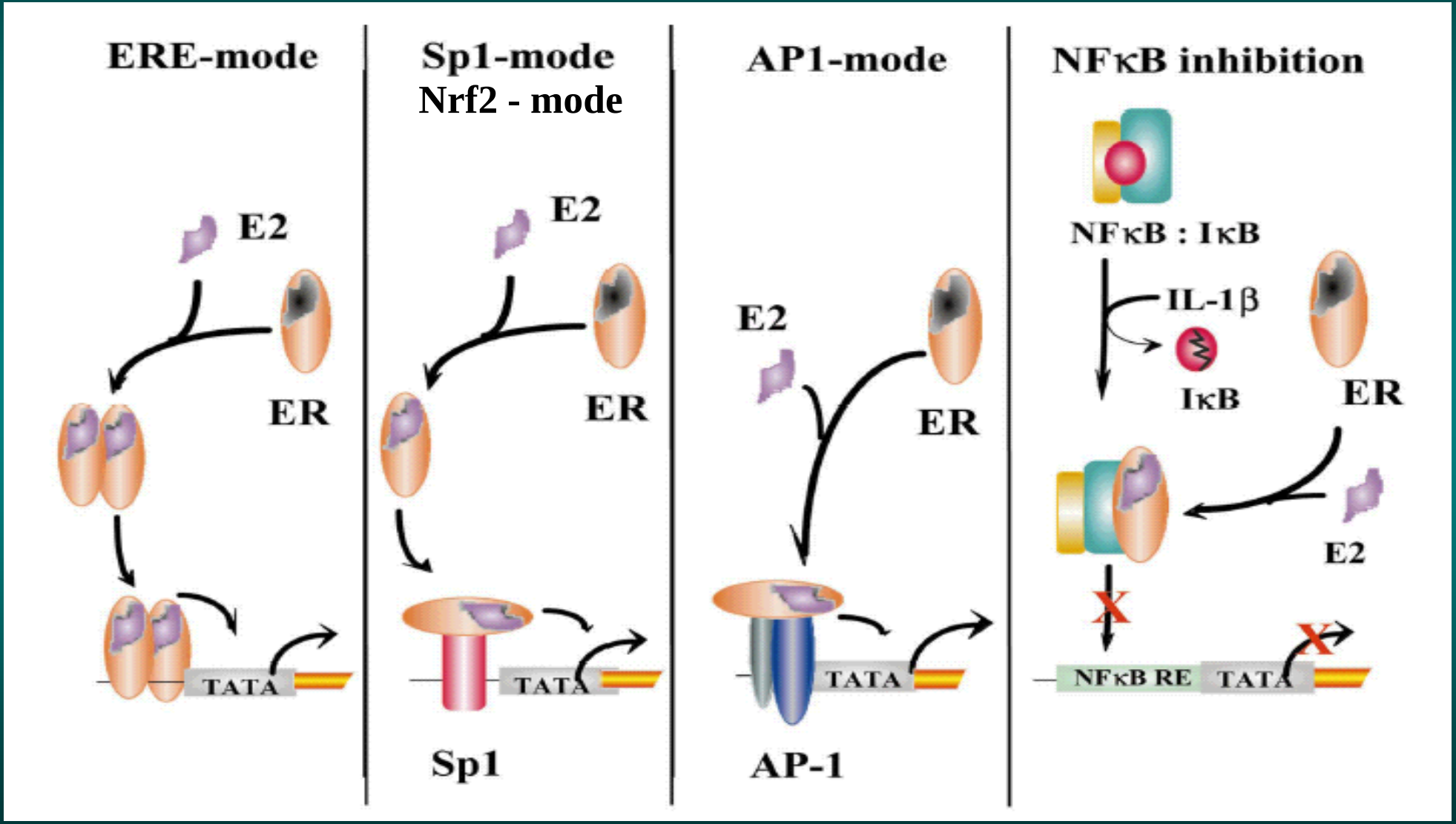
*** Influence on NFκB (formation of complex with c-rel subunit) – it inhibits activity of NFκB. Therefore, estrogens may have antiinflammatory activity, inhibiting e.g. IL-6, IL-1β, TNFα, M-CSF .**

*** Influence on Sp1 (formation of complex with Sp1) – increases activity of Sp1, leading e.g. to induction of RARα and eNOS and nNOS expression.**

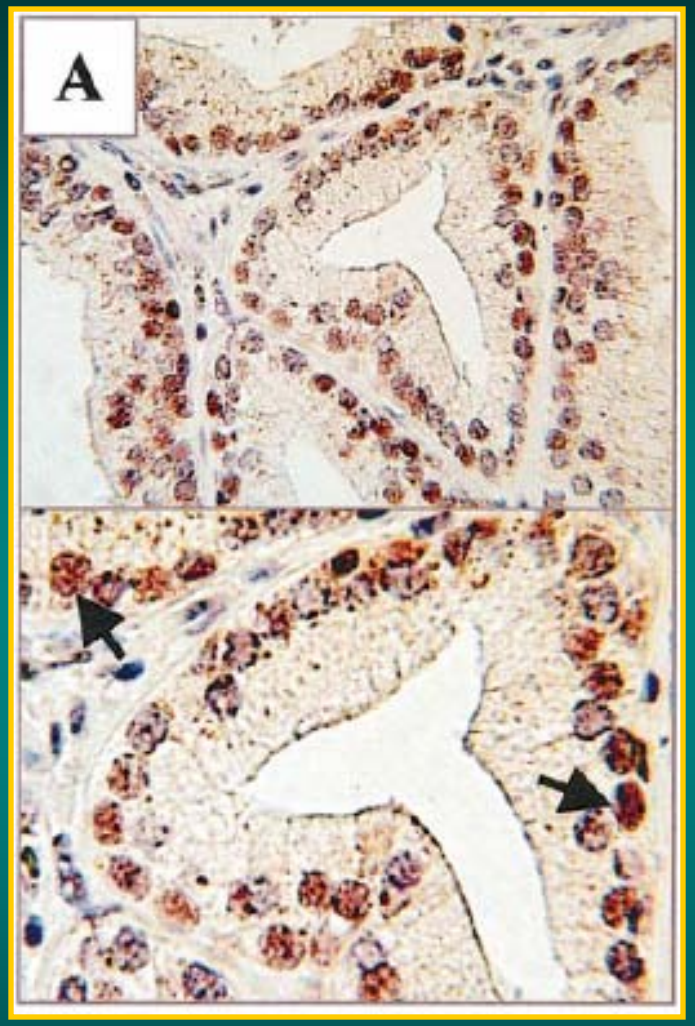
*** Influence on AP-1 (through interaction with p160 coactivators) – usually increases activity of AP-1, but it may depend on type of ER receptors and type of ligand.**

*** Influence on Nrf2 (which bind to the antioxidant response element) – in most cases it may increase the activity of promoters of genes involved in protection against oxidative stress.**

Influence of ER on gene transcription:

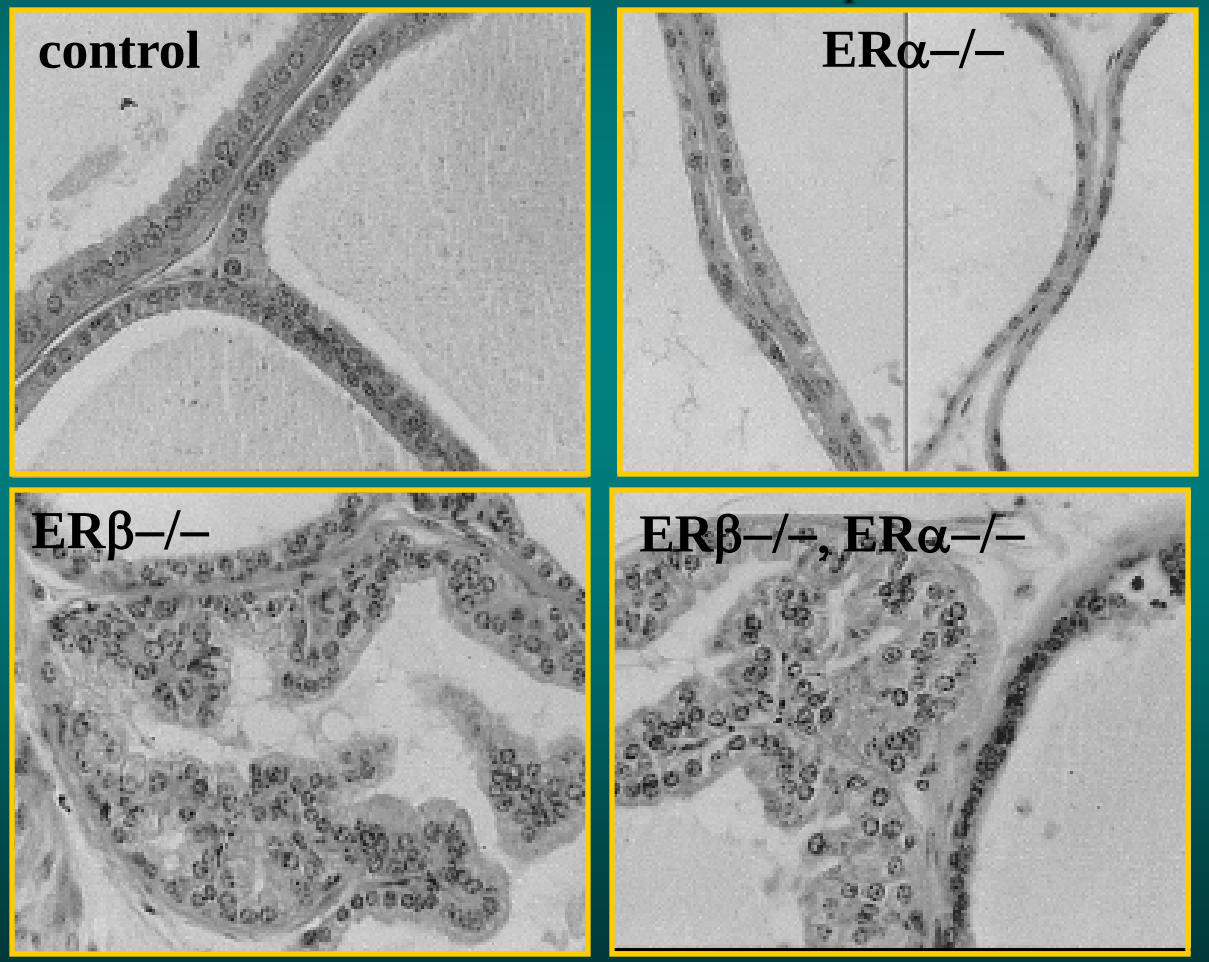


ERα and ERβ in the prostate



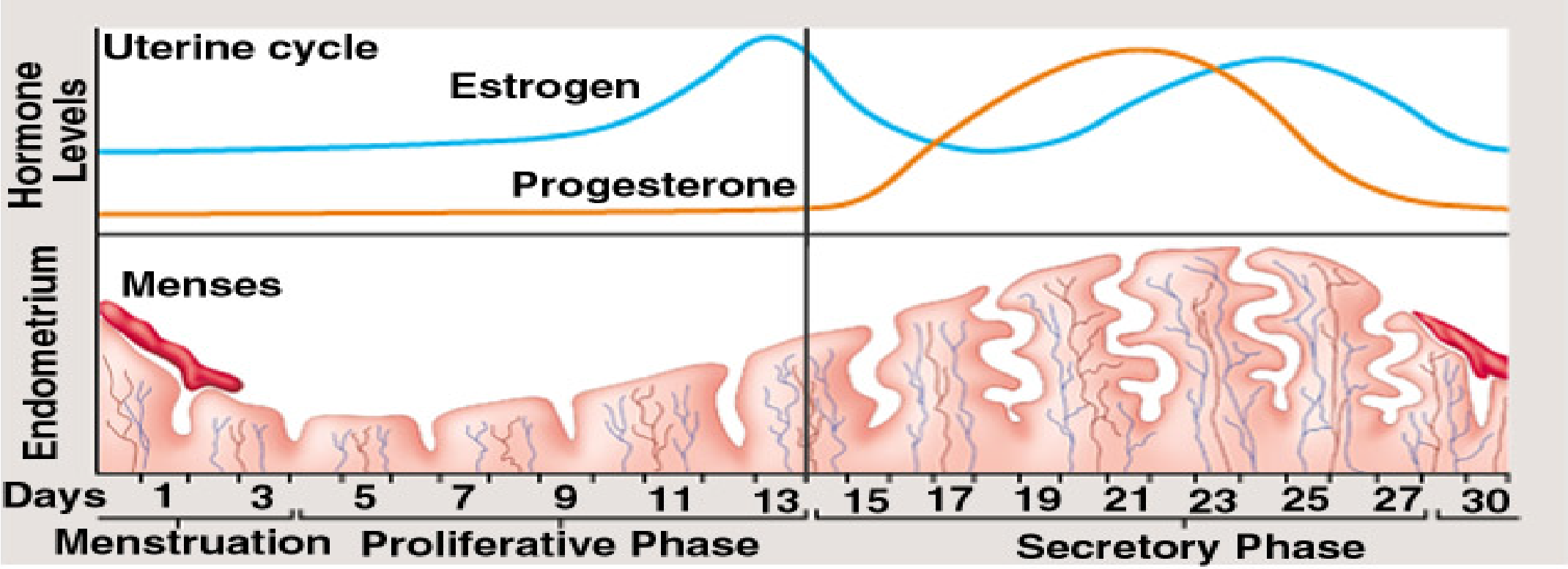
Majority of epithelial cell nuclei in the prostate express ERβ (ERα are present only on some stromal cells)

prostate acinae



Nilsson et al. 2001

Female Hormonal Levels



and finally stops ☹

Menopause

- Menopause (cessation of ovulation) is unique to human. Females of other species show a decline in but not complete termination of reproductive functions.

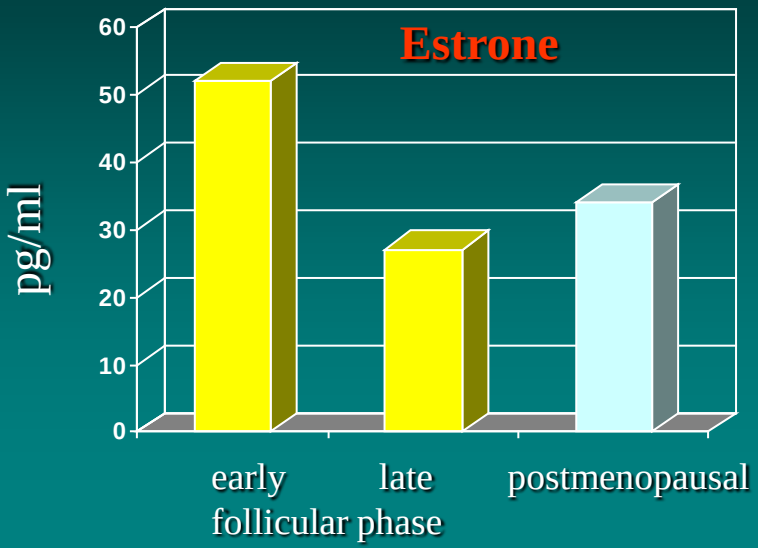
- Menopause occurs usually between the ages of 48-52. This has not changed considerably throughout history, although menarche occurs at yanger ages today than 100 years ago and life expectancy has increased.

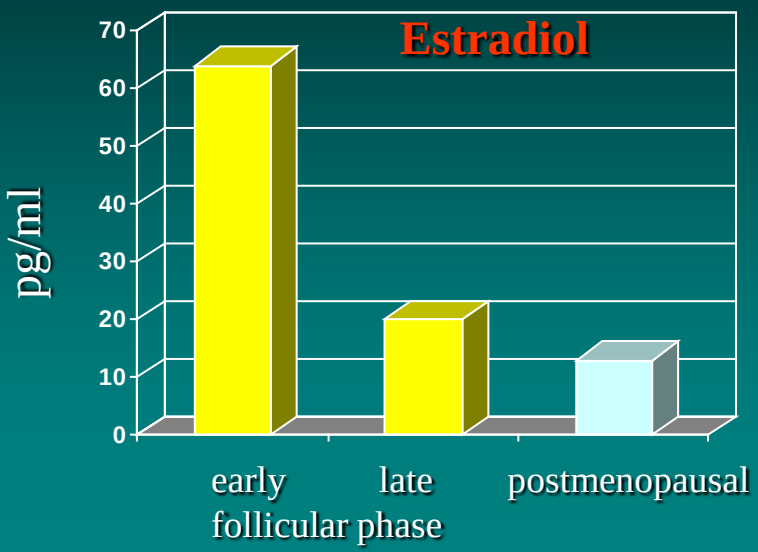
- Menopause is associated with decrease in estradiol production and in consequency an elevation in plasma gonadotropin levels. It stimulates ovarian stroma cells to continue producing androstenedione.

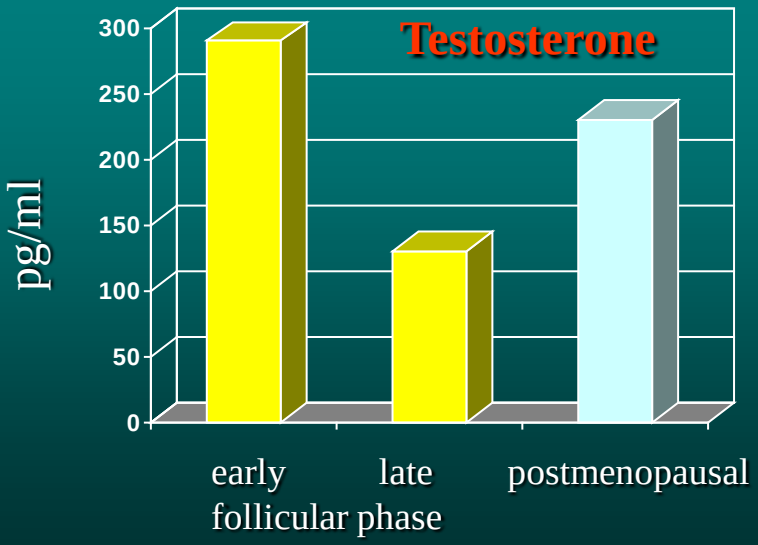
- **Estrone**, derived almost entirely from peripheral conversion of adrenal and ovarian androstenedion, becomes the dominant estrogen.

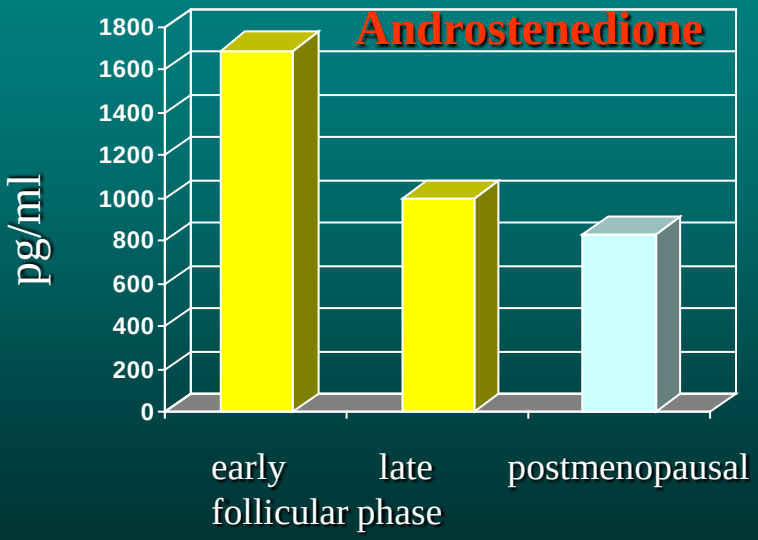
- Because the ratio of estrogens to androgens decreases, some women have hirsuitism due to androgen access (e.g. muostache...).

Hormonal changes during menopause









Osteoporosis

Osteoporosis

- One of the most frequent chronic diseases. 80% of patients are women.

- Develops both in male and female, but in male this process is slower (as they have more dense bones, smaller decrease in sex hormones and are more active).

- In USA ~90% fractures (hip and backbone) in old women and 70% in old men results from osteoporosis.

Bone density (hip) in women older than 65:
>833 mg/cm^2 - norm - 40%
833-648 mg/cm^2 - osteopenia - 40%
<648 mg/cm^2 - osteoporosis - 13% (in older than > 80 years - 27%)
<648 mg/cm^2 and fractures – strong osteoporosis - 7%, (in older than 80 - 27%)

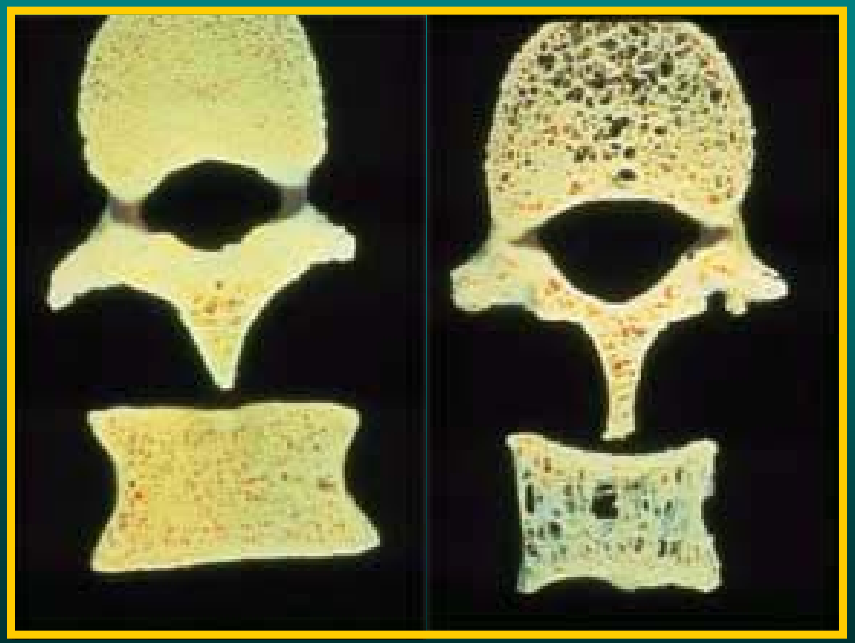
- Similarity in bone density is higher in monozygotic than in dizygotic twins. Thus there is a significant influence of genetic factor(s) controlling bone metabolism.

Estrogens and bones

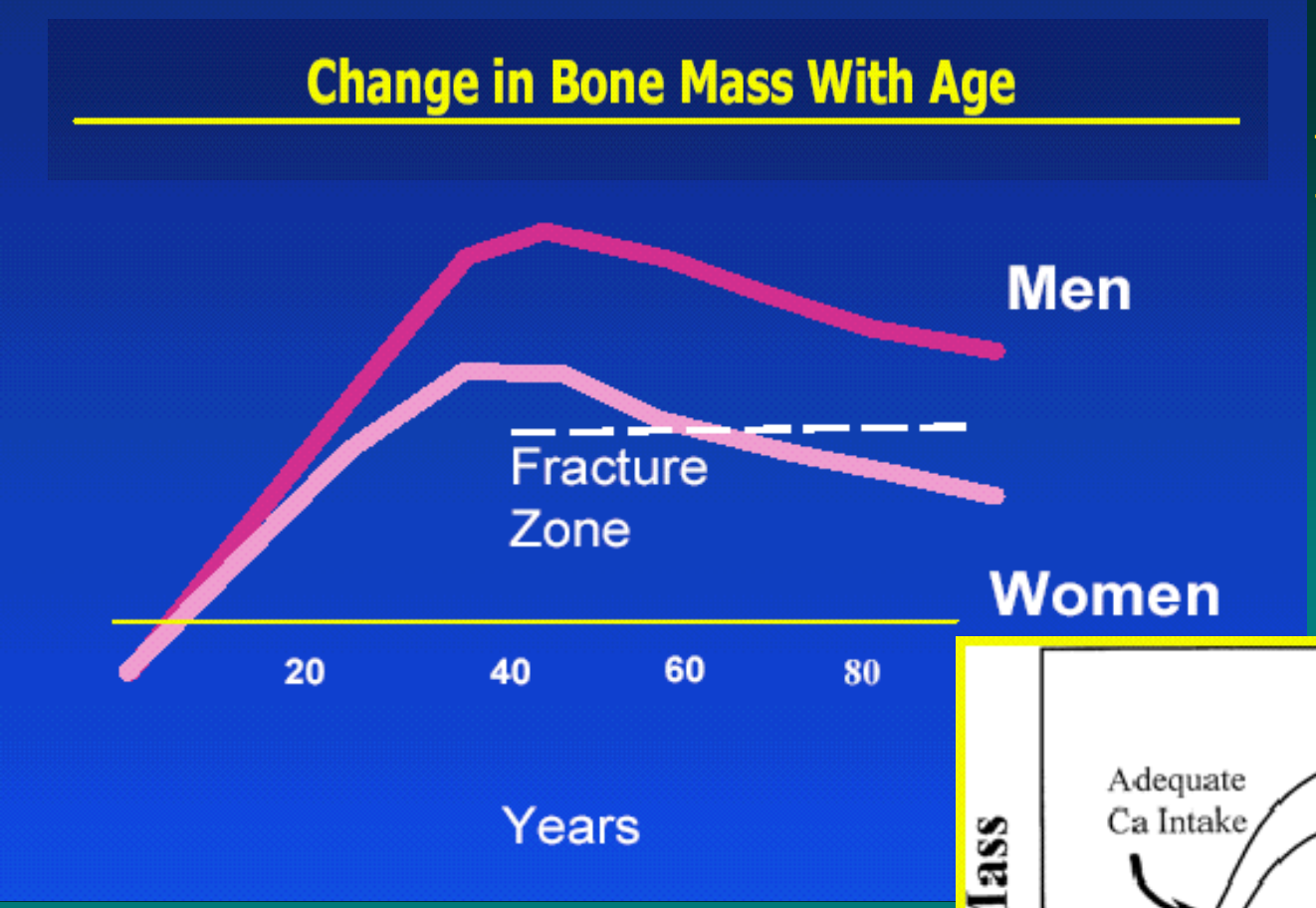
* Development of bones during maturition depends mostly on estrogens both in males and females.

* In both sexes, estrogen deficiency leads to osteoporosis: in the only known case of complete insensivity to estrogens (point mutation of ER receptor resulting in formation of stop-codon) the ill man had increased bone metabolism, osteoporosis, immature epiphysal plates and continous growth of bones also during adulthood. Similar effects are observed in men with unfuctional aromatase.

* Estradiol increases proliferation, migration, maturition and secretory activity of chondrocytes, leading to maturition of epiphysal plates and ceases the growth of long bones.

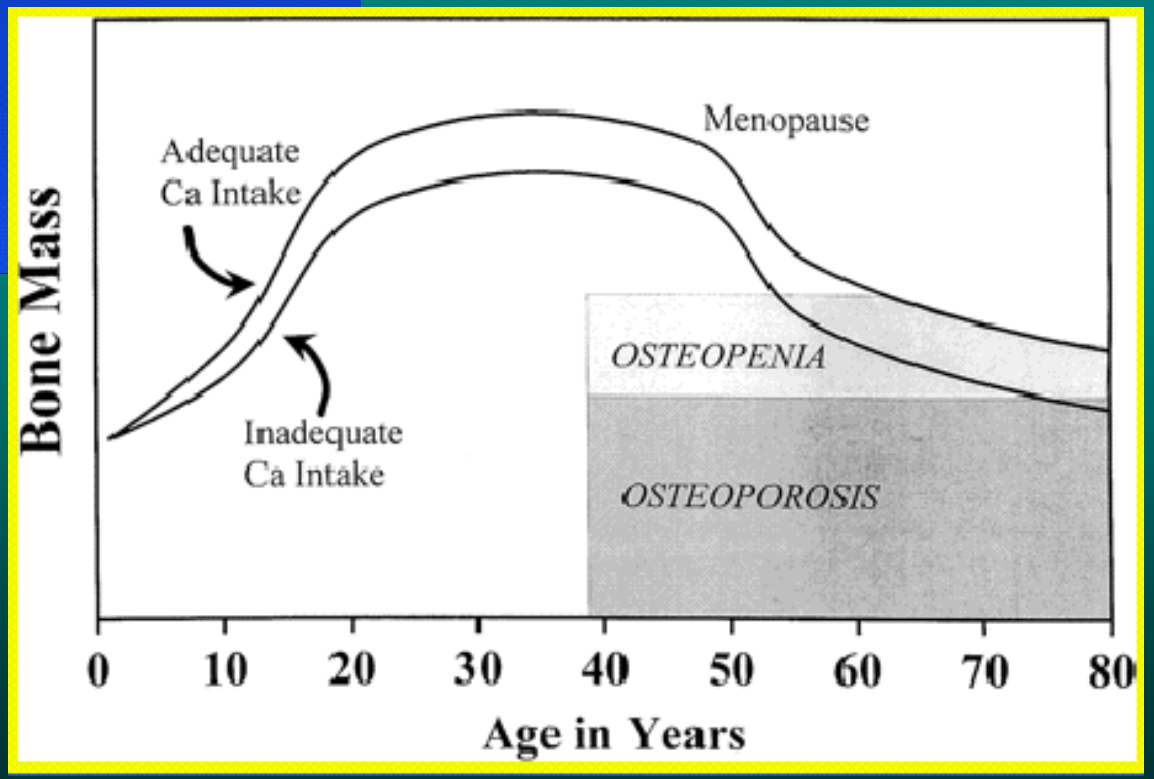


Risk of osteoporosis



sex

nutrition



J. Z. Ilich, and J. E. Kerstetter (2000).

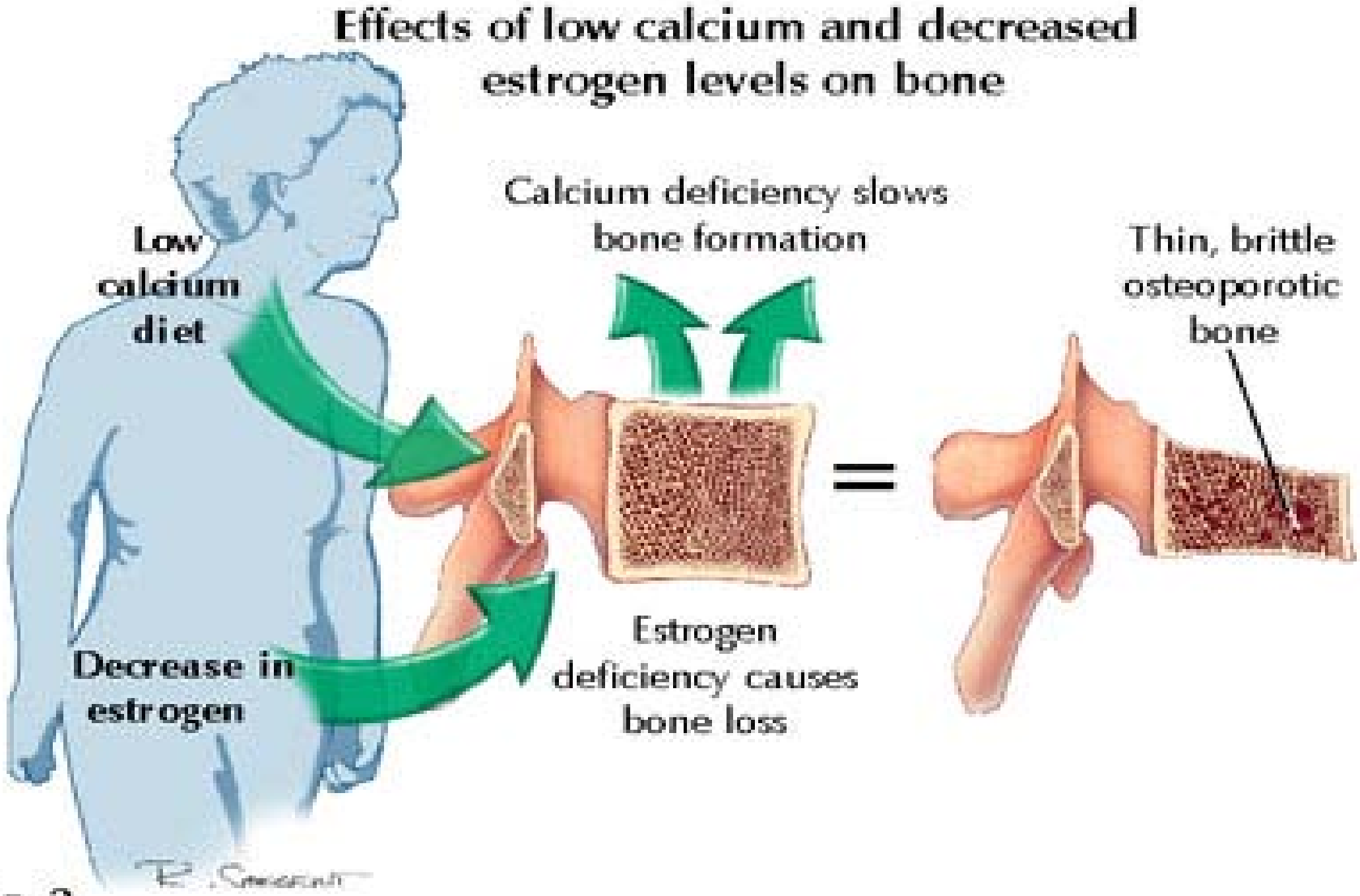
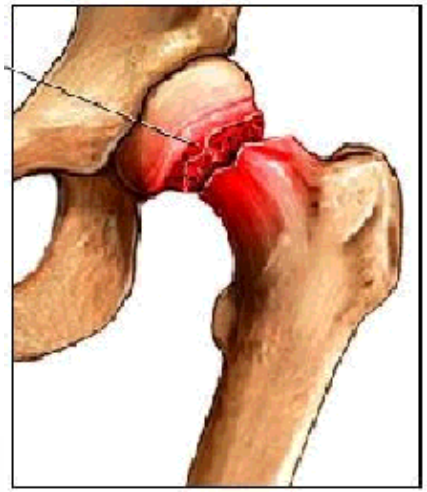
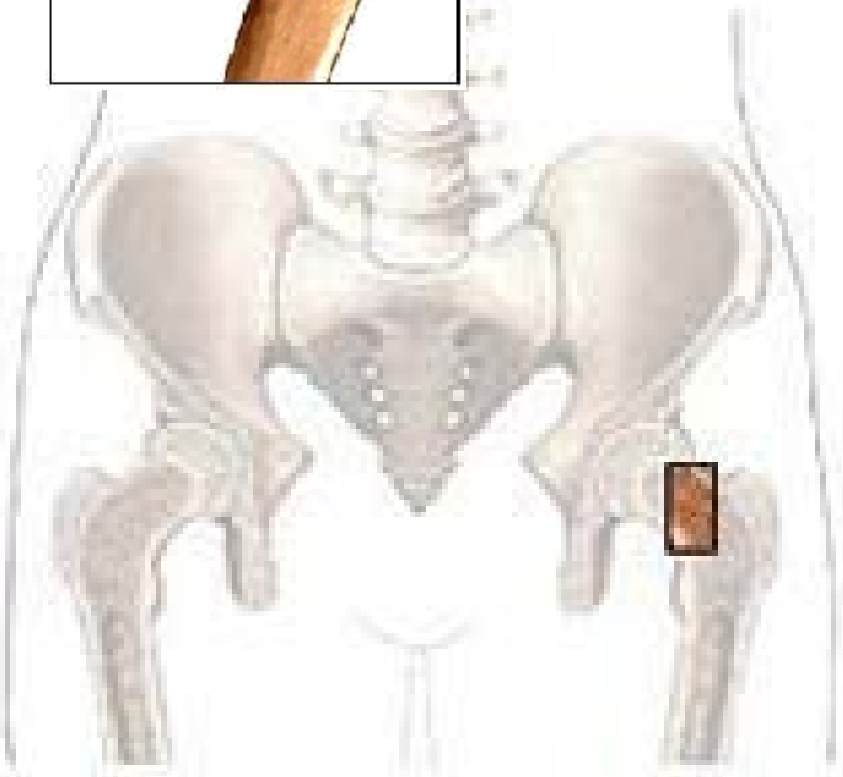

Effects of low calcium and decreased estrogen levels on bone
Low calcium diet
Calcium deficiency slows bone formation
Thin, brittle osteoporotic bone
Decrease in estrogen
Estrogen deficiency causes bone loss

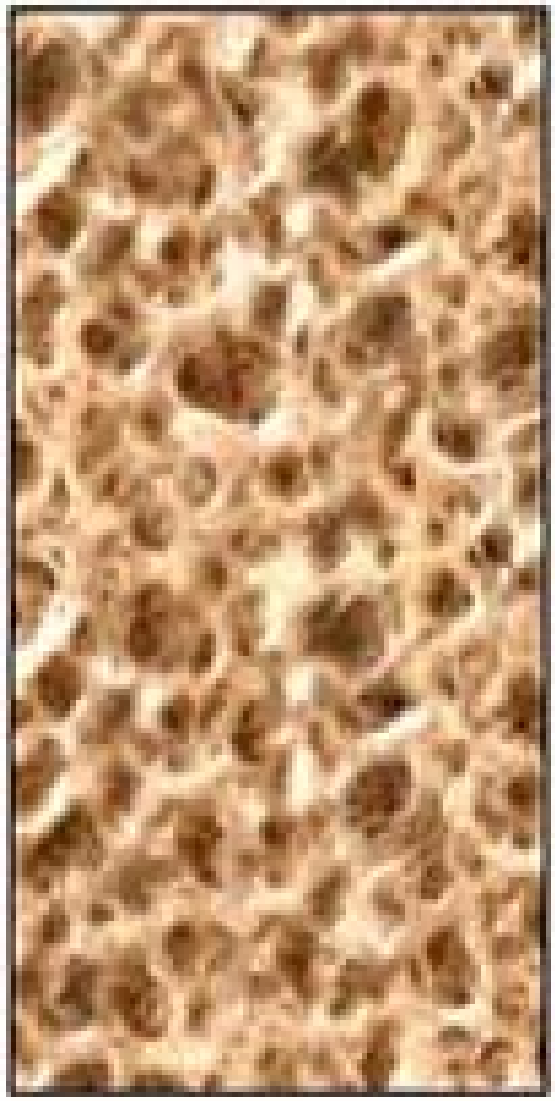
Fig. 2



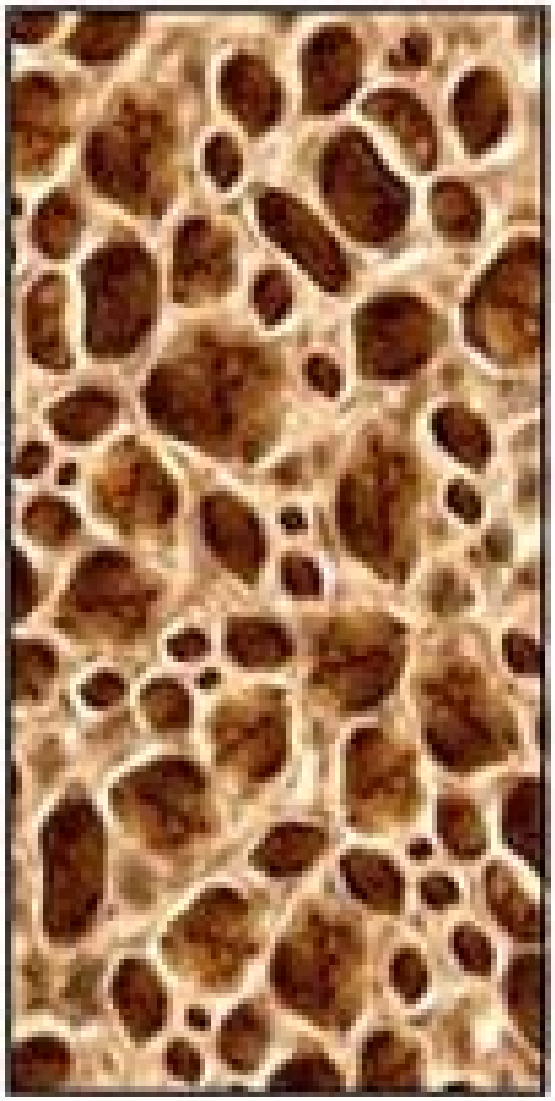


Bone section through hip

Normal Bone



Bone with Osteoporosis



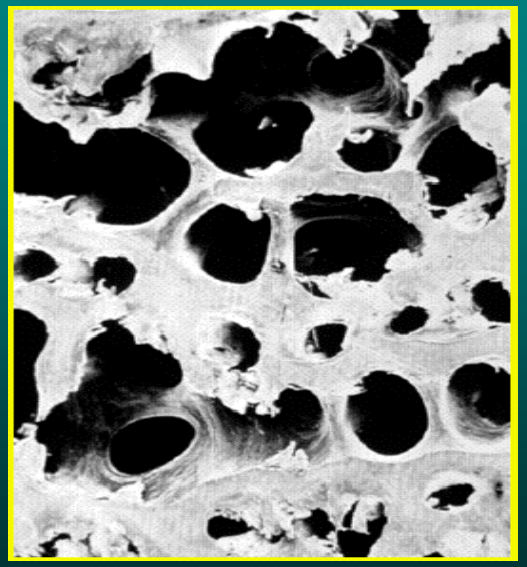
Osteoporosis

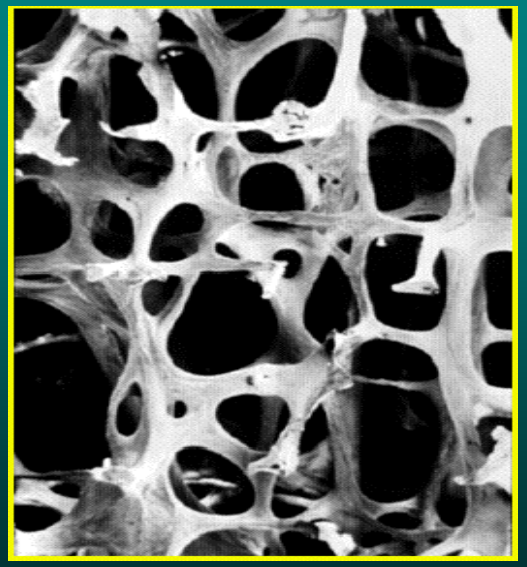
- Higher risk of osteoporosis is noticed in women with familial history of osteoporosis. Frequency of osteoporosis is increased by:

* inactive life style,
* low calcium diet (especially during maturition),
* high alcohol intake,
* slimness

- Risk is increased in patients treated with corticosteroids and thyroid hormones.

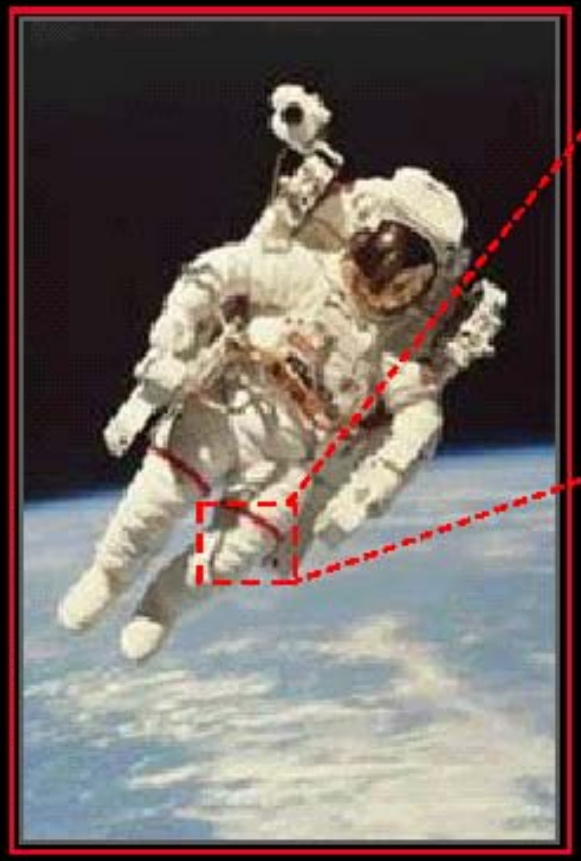
Normal trabecular bone

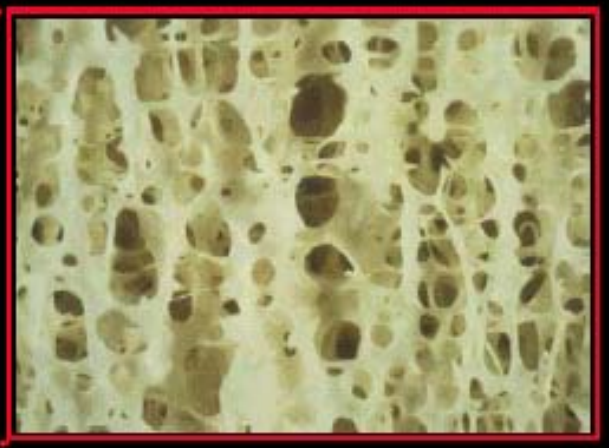


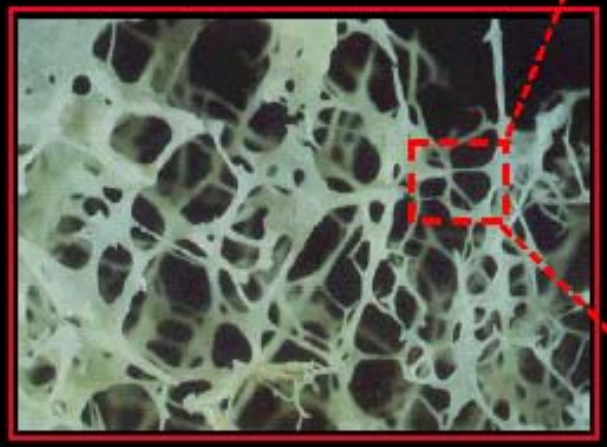


Osteoporotic bone

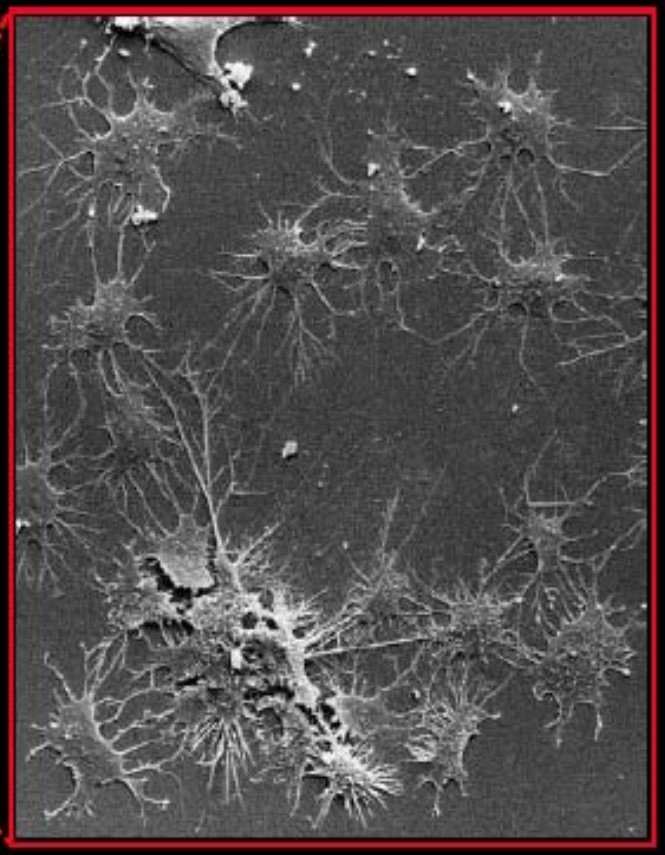
Space flight induces bone loss







Are osteoblasts responding to altered gravity?



Osteoporosis

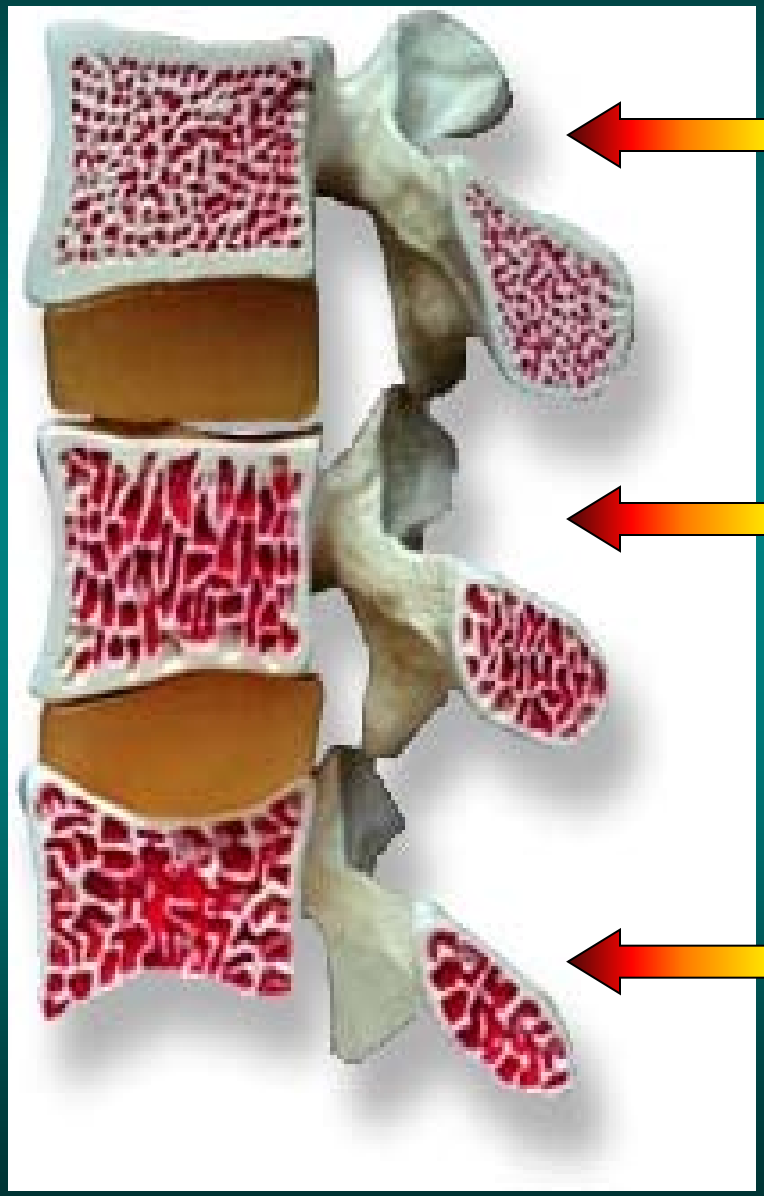
- Fractures are usually a consequences of a fall, but sometimes they occur spontaneously (~ 5%).

- They can concern all bones, but usually they occur in:
    * backbones,
    * upper part of femur (and hip)
    * wrist (as a consequence of fall)

- Fractures in backbones are painful, but (as often they do not result from strong trauma) are often ignored. They are also commonly diagnosed as osteoarthritis. Usually they are recognized during routine X-ray of lungs.

- If there are several fractures, they may lead to kyphosis (”widow hump”), resulting in disturbed functions of lungs and heart and difficulties in deeper breathing.

Model of vertebrae

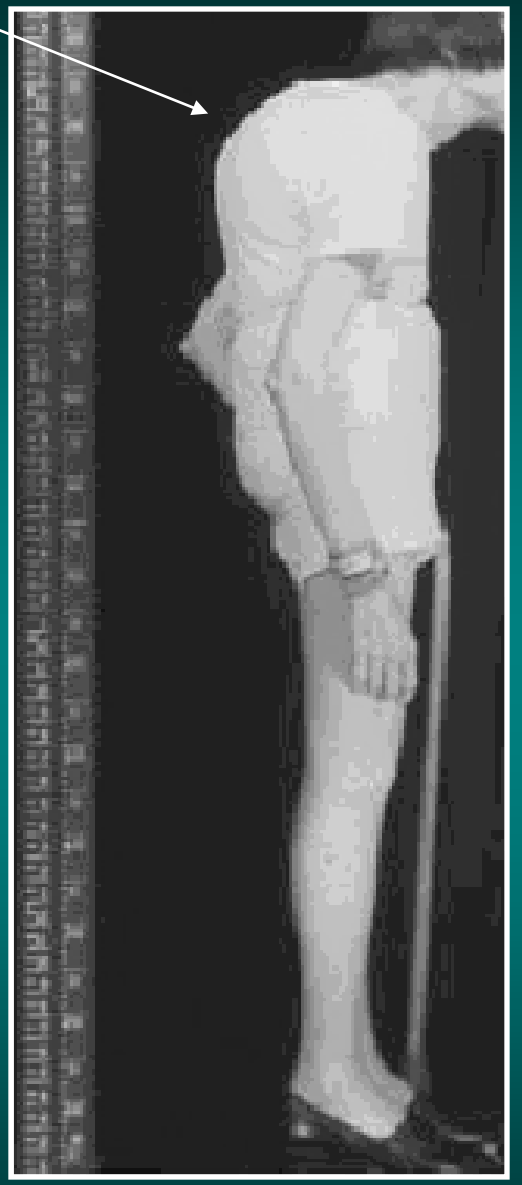


a normal bone;

a bone affected by osteopenia showing the beginning of a lack of calcium and low bone density,

a bone showing a severe case of osteoporosis

kyphosis



University of Arizona Extension Center

Estrogens and bones

* Perhaps, in bone cells both ERα and ERβ are expressed. The level of expression is, however, relatively low.

* Estrogens act both on osteoblasts and osteoclasts. In osteoblasts they upregulate the expression of alkalic phosphatase, osteopontin, osteocalcin, osteonectin. It is associated with increased cell differentiation, production of extracellular matrix, and stronger mineralization of matrix.

* Estrogens inhibit the activation of osteoclasts and increase their apoptosis. It increases expression of osteoprotegrin, the decoy receptor for osteoclasts differentiation factor.

* Possibly, estrogens affect bones mostly through regulation of cytokine and growth factors expressions: increased synthesis of IGF-I and reduced synthesis of IL-6, IL-1β, TNF, M-CSF, TGFβ.

* Estrogens upregulate the expression of 1α-hydroxylase which produces the active form of vitamin D.

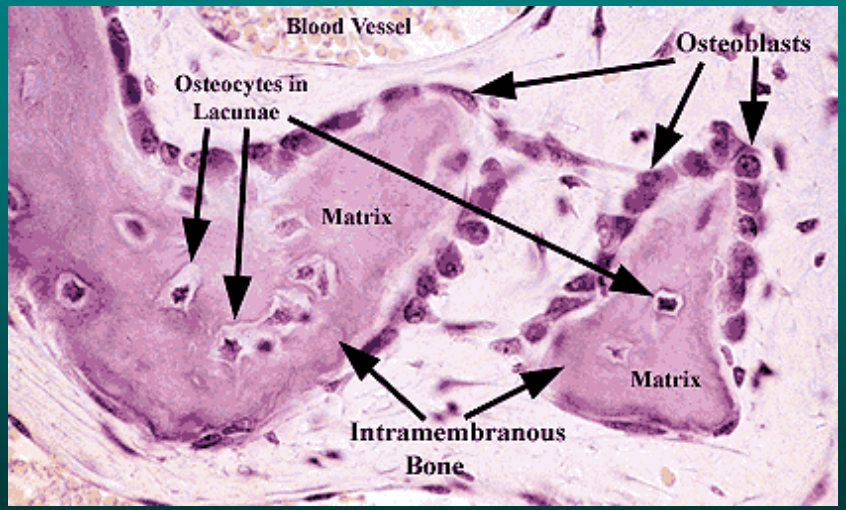
Progesterone and bones

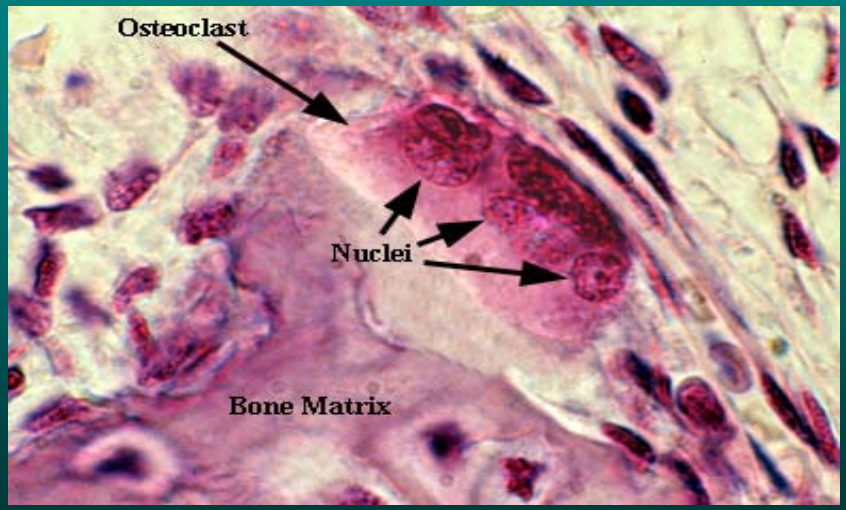
* Progesterone protects against osteoporosis, but its efficacy is lower than that of estrogen.

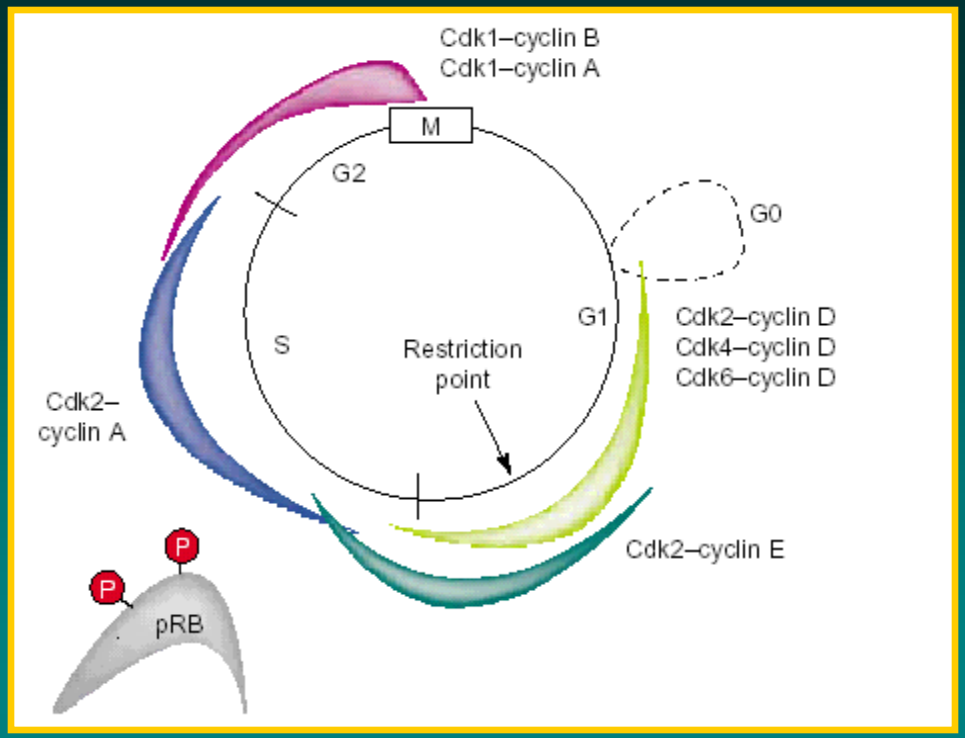
* Progesterone receptors are present both in osteoblasts and osteoclasts.

* Increases proliferation and differentiation of osteoblasts in vitro.

* Progesterone increases expression of 1α-hydroxylase

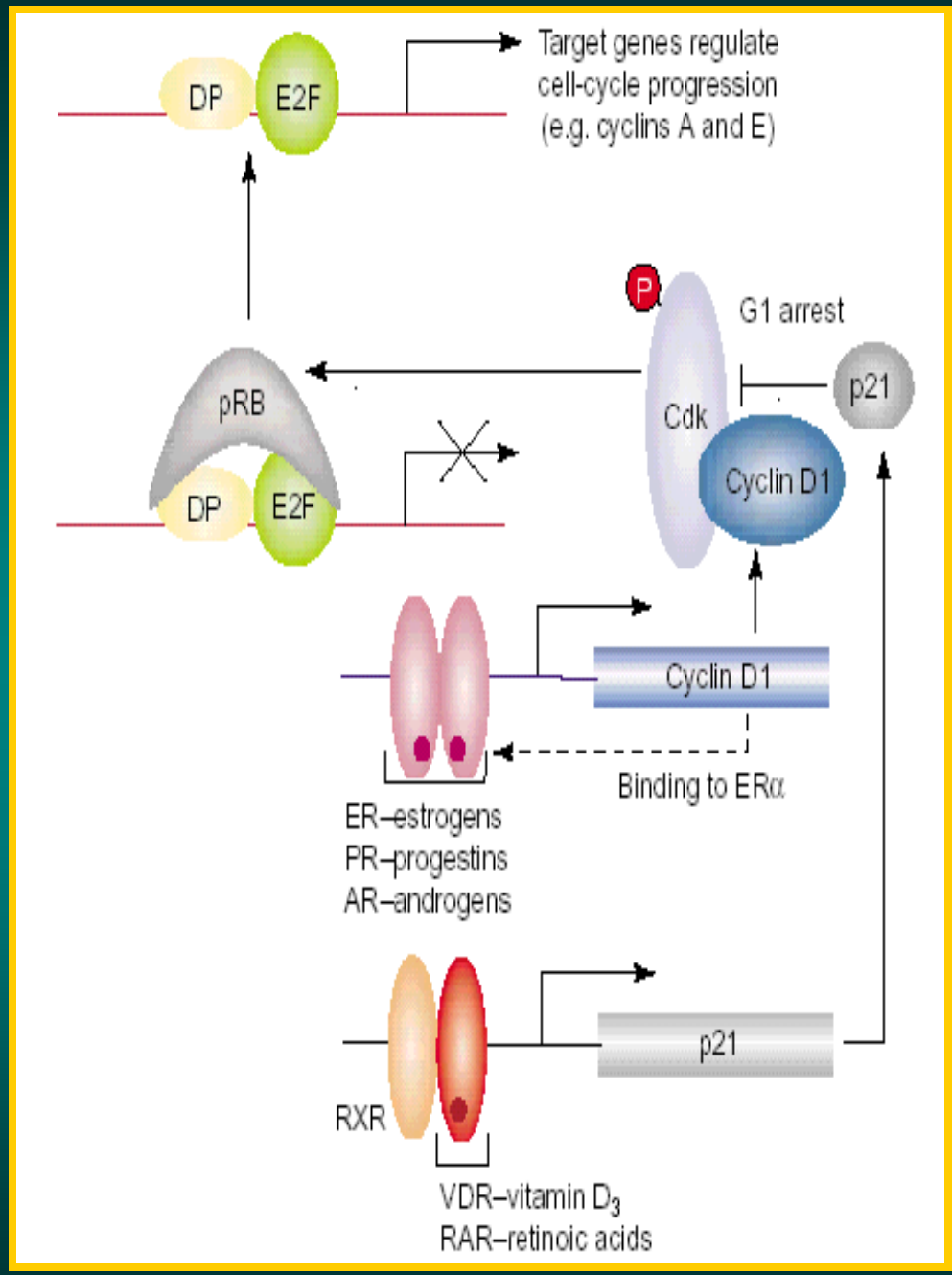






Some nuclear receptors (ER, AR, PR) stimulate expression of cyclin D, which activates Cdk4. It leads to phosphorylation of pRB, and increases transcription of genes increasing proliferation.

Others receptors (VDR, RAR) increase p21 expression, thus block Cdk activity, which keeps cells at G1 phase.



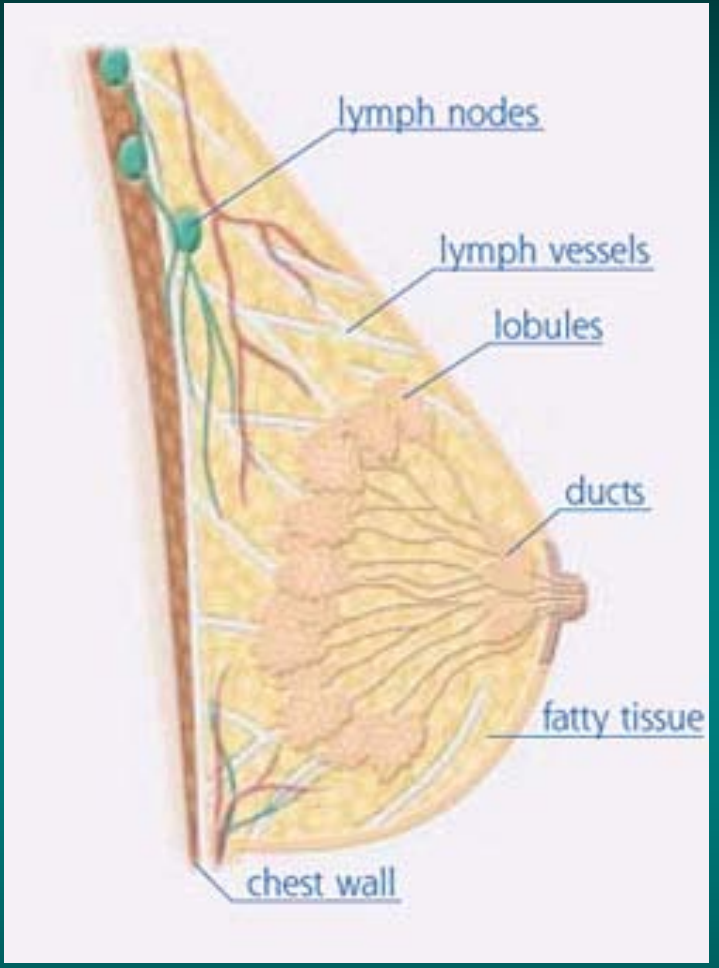
ER in breast

Estrogens are necessary for normal breast development.

Postnatal mammary gland development involves two distinct phases:

At puberty, estrogen promotes ductal elongation and dichotomous branching. At adulthood, the virgin gland becomes relatively quiescent with the exception of side branching and alveolar budding that occur as a result of the cyclic rise of ovarian steroids.

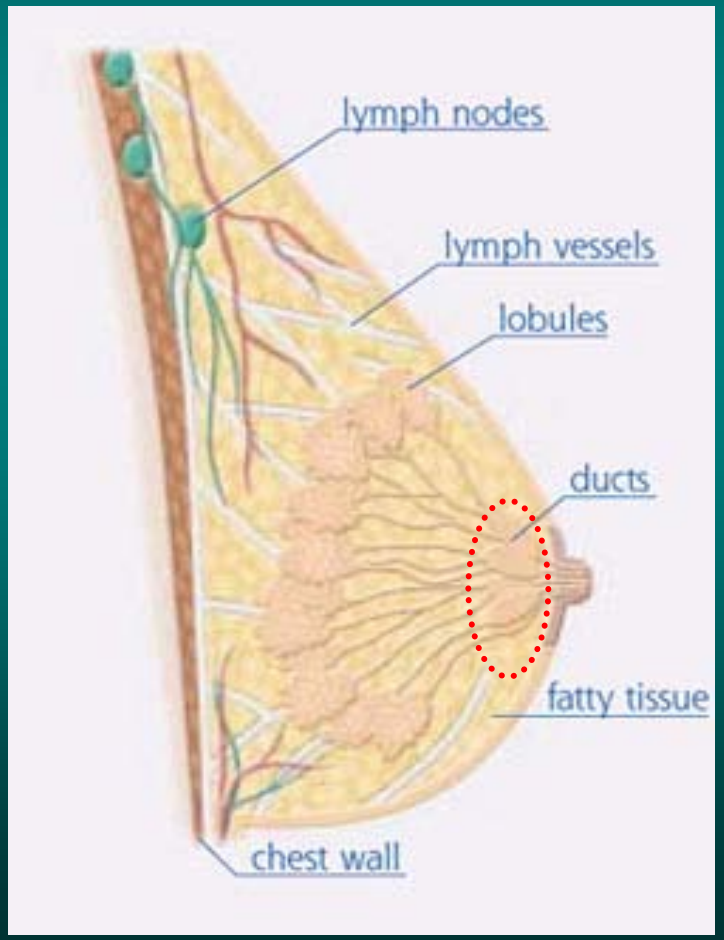
At pregnancy, exposure to progesterone and prolactin results in extensive epithelial proliferation, increased dichotomous side branching and differentiation of milk-filled alveolar lobules.



Relatively small proportion (under 10%) of luminal epithelial cells express ERα in the normal breast, whereas myoepithelial and stromal cells do not. Proportion of positive cells declined in luteal phase in naturally cycling women. However, in women with breast cancer, this luteal phase decrease was not observed. Expression of ERα is higher in women of European than of Asian or African origin.

BREAST CANCER

- One out of every 12 women will develop breast cancer.
- Breast cancer is the second leading cause of death in women (commonest cause of cancer death in women, 6% of all deaths)
- Worldwide, 600,000 cases of breast cancer are diagnosed each year. In UK there are 24 000 new cases and 12 800 deaths annually.
- About 80% of invasive breast cancer occurs in women over age 50.



WHO Classification of breast cancers

* Epithelial
  * Ductal (85%)
  * Lobular (1%)
  * Papillary (<5%)

* Mixed Connective tissue and Epithelial

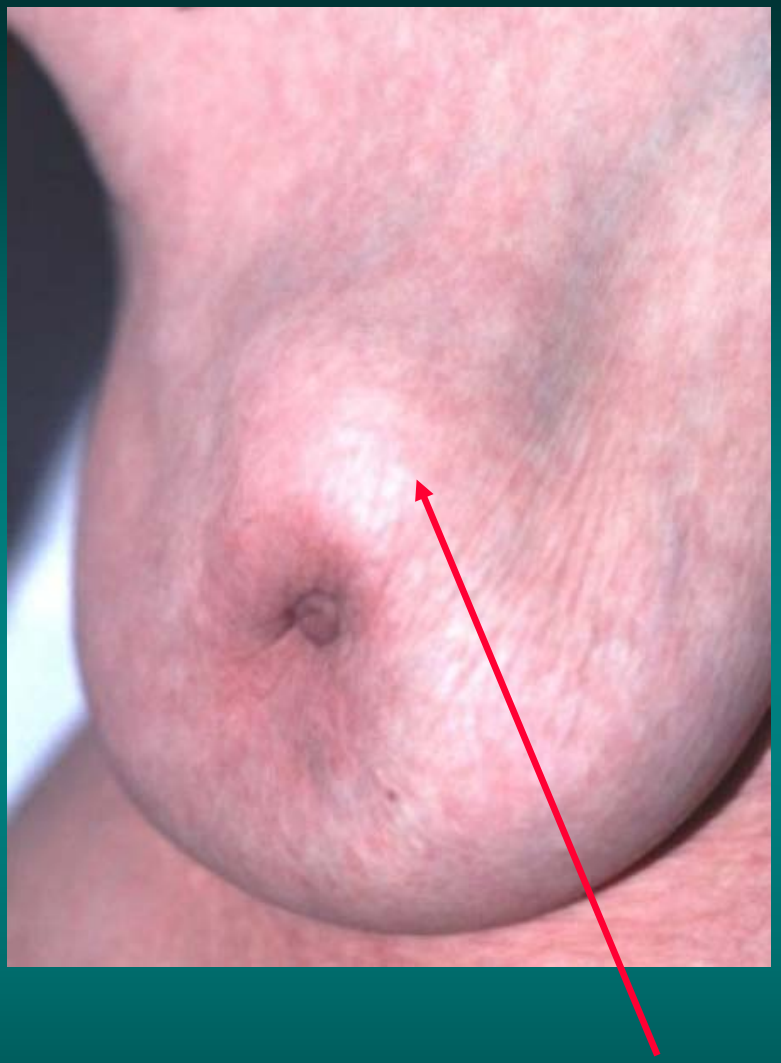
Prognostic factors in breast cancer

- **Age**
  Younger women have poorer prognosis of equivalent stage

- **Tumour size**
  Diameter of tumour correlates directly with survival

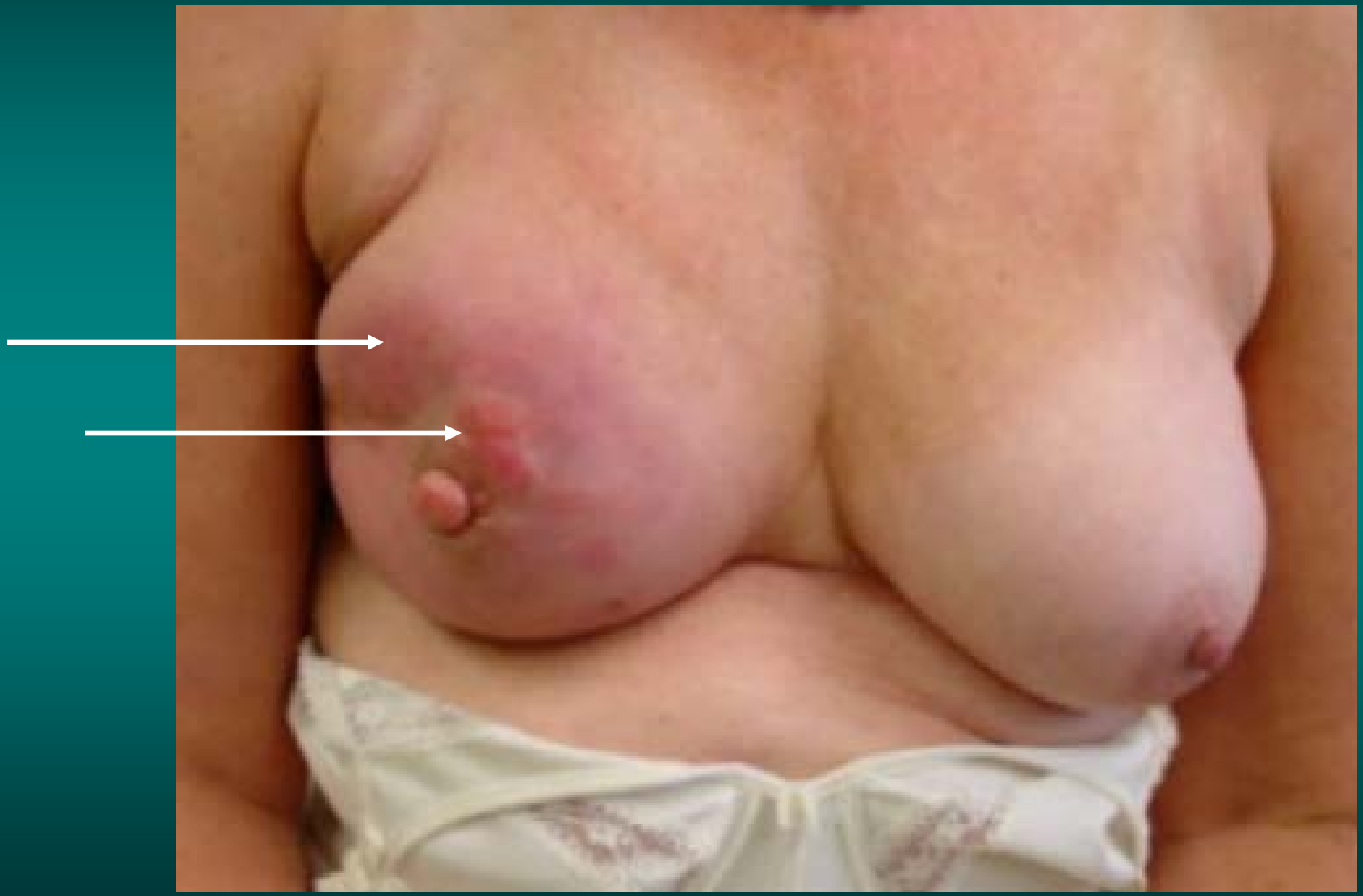
- **Lymph node status**
  Single best prognostic factor
  Direct correlation between number and level of nodes involved and survival

- **Metastases**
  Distant metastases worsen survival

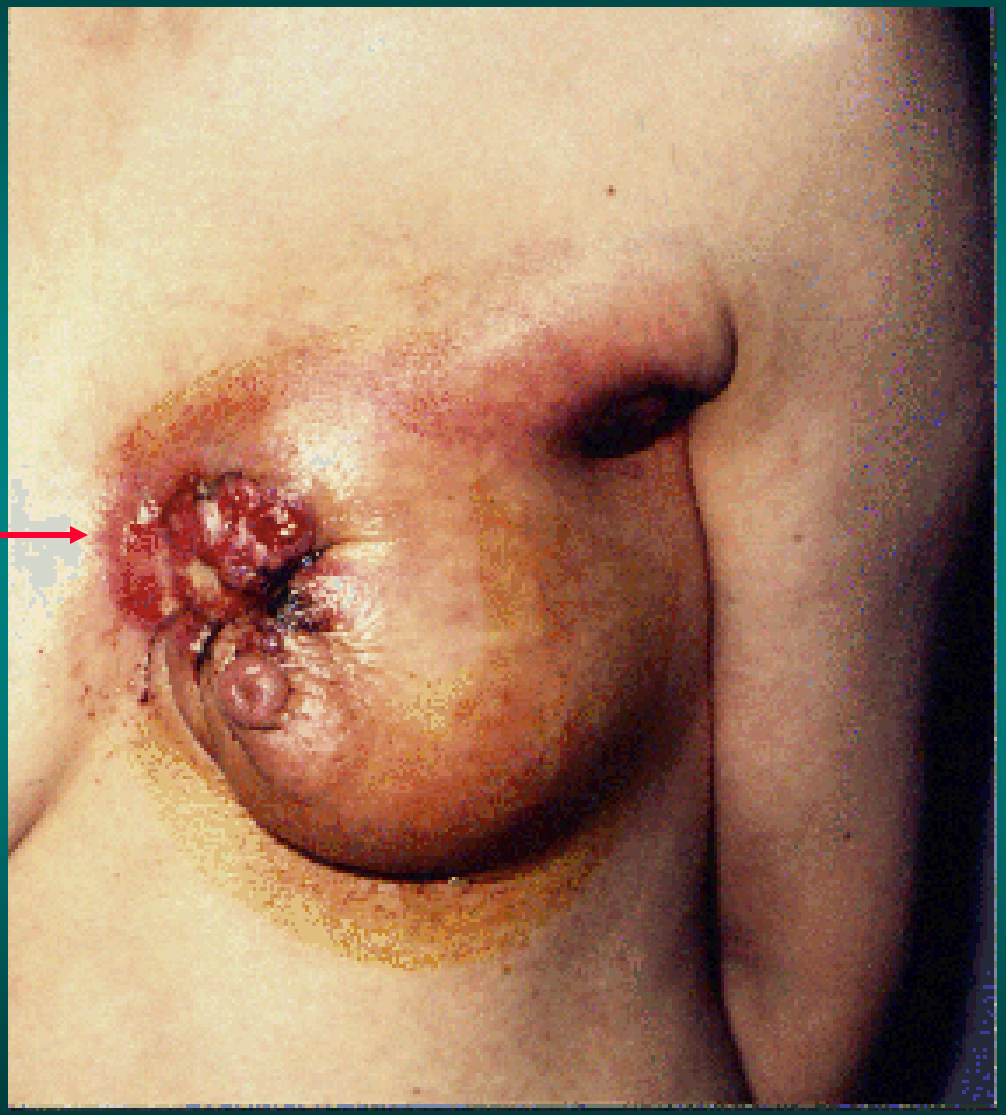


Typical breast cancer present as a painless bump

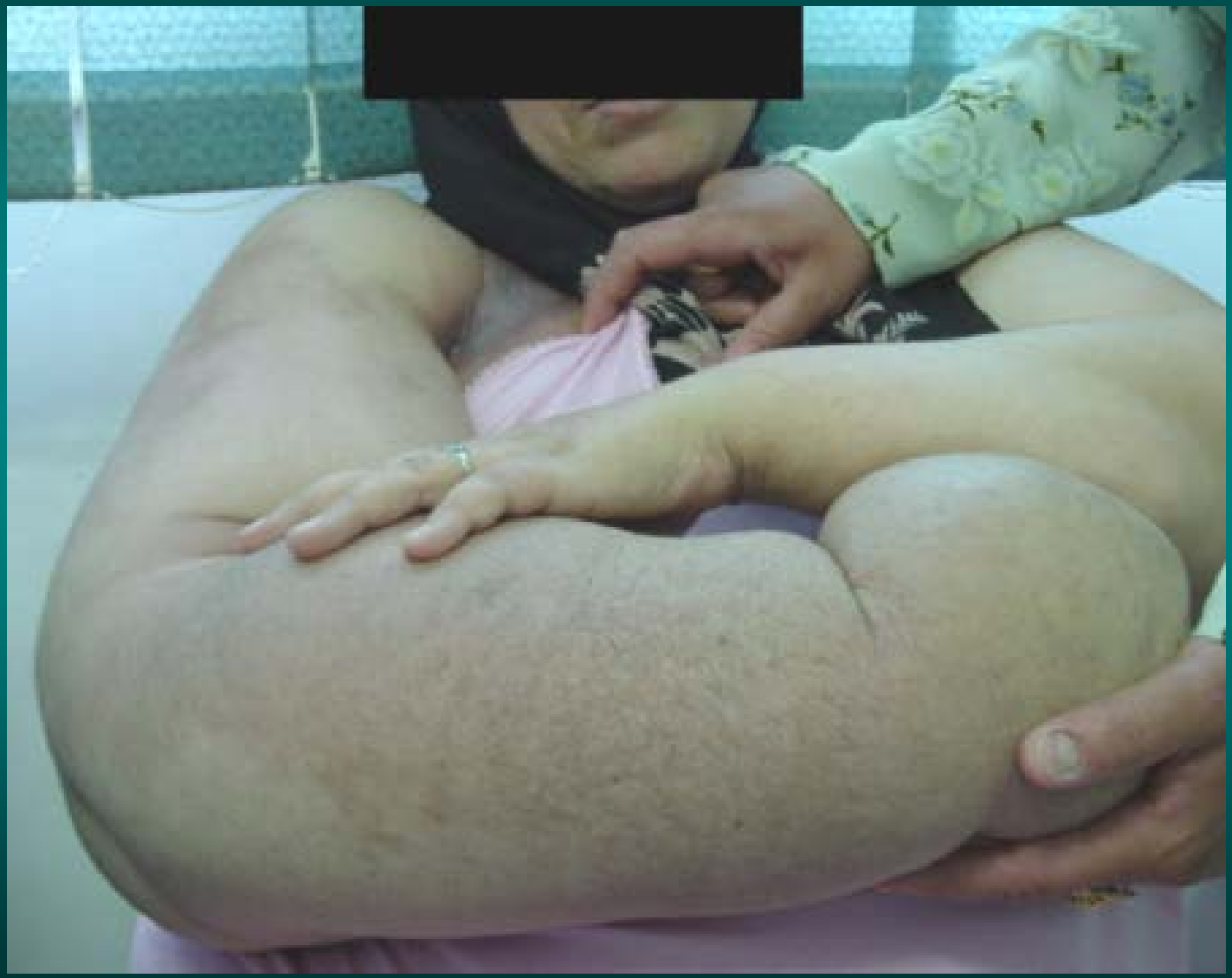
Locally advanced breast carcinoma



A tumor accompanied by ulceration



Lymphoedema



Estrogen synthesis in breast cancer

- Estrogens are produced in many types of cancers, regardless of the presence or absence of ER and PR, both in pre- and postmenopausal women.

- Estrogen level in the breast tumors in postmenopausal women can be ~20-fold higher than in serum.

- About 70% of breast cancers have receptors for estrogens and progesterone. Estrogen is a major mitogen for this type of cancer.

- There is a strong correlation between the level of estrogenes in post-menopausal women and the risk of breast cancer.

Estrogen in breast cancer:

↑ Proliferation ↓ Apoptosis

Breast cancer – antiestrogenic therapy

- It was notified in 1896 that ovarectomy leads to significant decrease in breast carcinoma (G. Beadson, Lancet).

- However, already in 1900 assessed that ovarectomy helped only in 30% of cases, and improvements lasted only for ~2 years. Despite these limitations endocrynic therapy became a standard in treatment of breast cancer. It was surgical antiestrogenic therapy:

* ovarectomy (in premenopausal women)
* adrenalectomy (in post-menopausal women)

- Surgical ovarectomy has been replaced by ovary irradiation

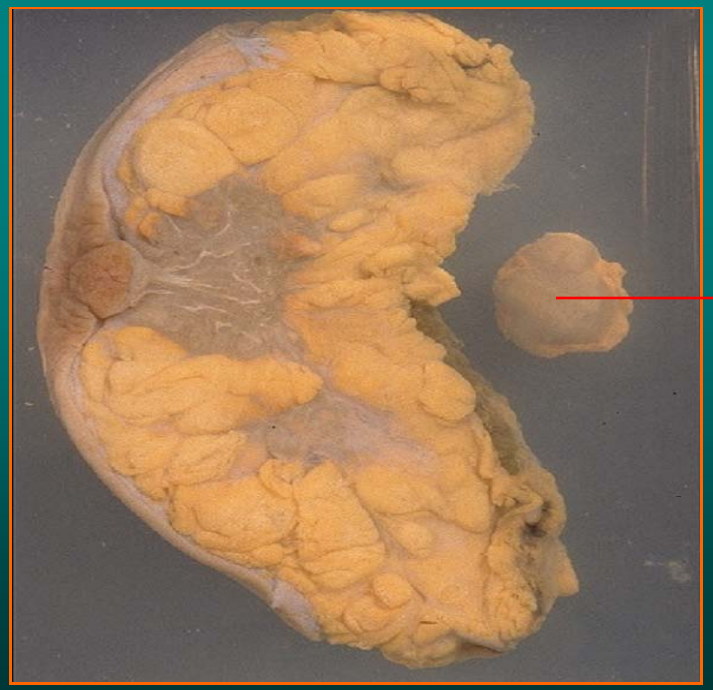
- For ~20 years pharmacological antiestrogenic therapy is applied using:

• ER antagonists
• aromatase inhibitors

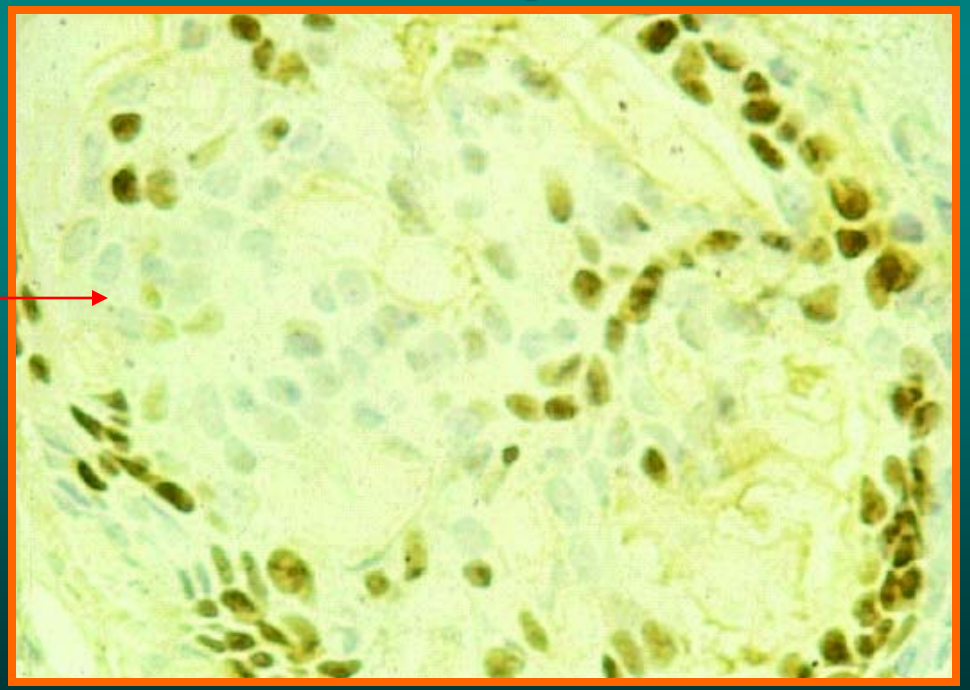
Antiestrogenic therapy – selection of patients

- It was found in 1971 that antiestrogenic therapy is effective in treatment of cancers with a high level of ER expression (improvement was observed in 60% of patients)

- Immunohistochemistry evidenced that cell in the same tumor may have differen level of ER expression. Therefore, the antiestrogenic therapy is less effective in the late stages of cancer (higher diversity of cells).



Cancer with different expression of ER



SERM (selective estrogen-receptor modulators)
Tamoxifen

- Tamoxifen is an antiestrogen, which blocks binding of estrogen to ER.

- It was invented during study on contraceptive pills in 1960's.

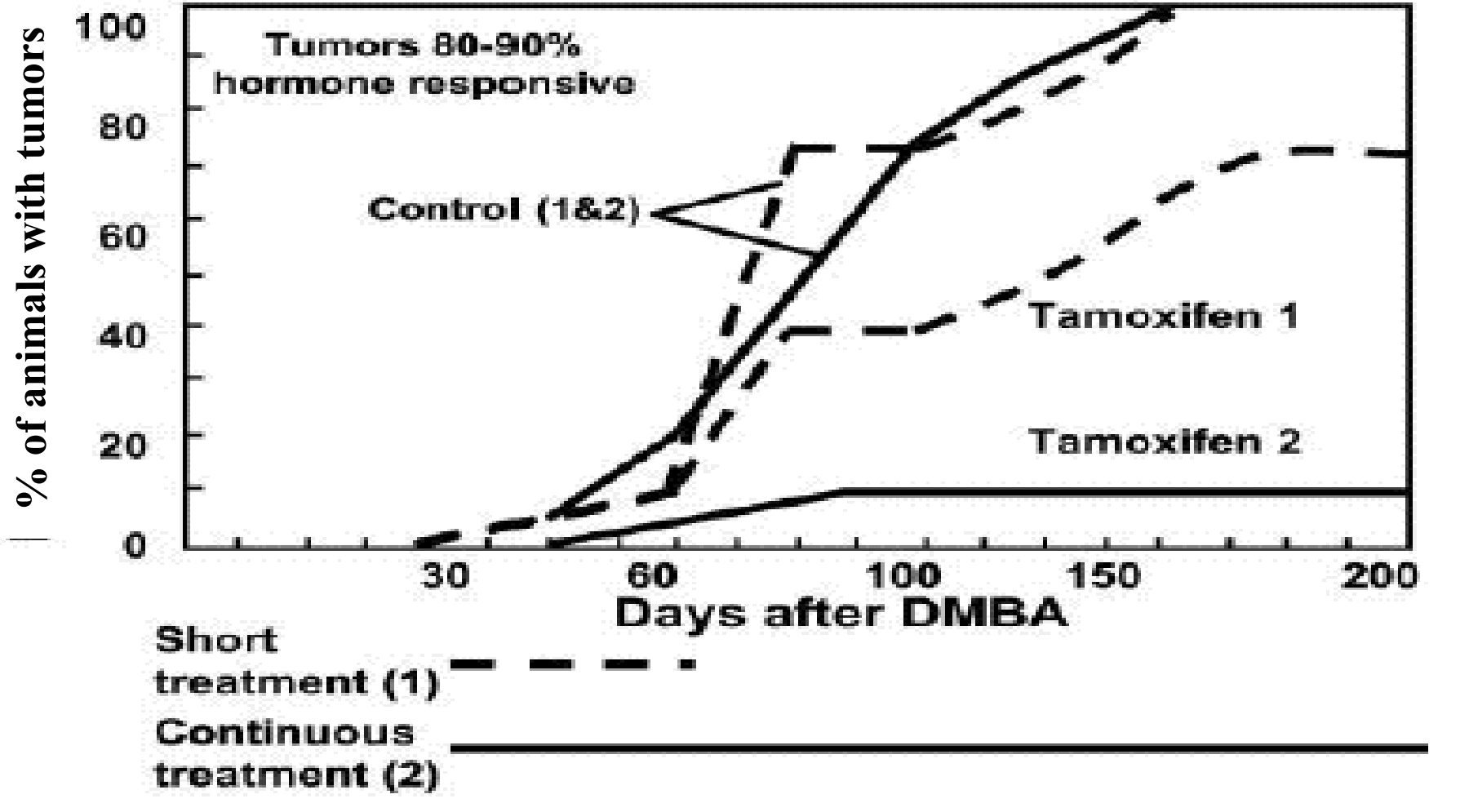
- In 1970's laboratory strategies of breast cancer prevention using tamoxifen was elaborated. However, it did not stir up the interest of clinicians, who believed in chemotherapy.

- 25 years later tamoxifen was found to be effective also in clinical practice – under condition of selection of appropriate group of patiens (cancers with high level of ER) and long-term application (more than 5 years)

- Tamoxifen could be useful in women at high risk of breast cancer.

Comparison of short-term and long-term treatment with tamoxifen on the growth of breast cancer in rats

Pharmacologically induced cancer.
Treatment with tamoxifen began 30 days after induction



Tamoxifen – clinical trials

- More than 13 000 women at high risk of breast cancer (before and after menopause) were treated for 5 years with tamoxifen or placebo.

- Tamoxifen decreased the risk of breast cancer by 50%.

There were, however, serious side-effects:

- Tendency to increased bone fracture rate

- Increase (2.6 - 4 fold) frequency of uterus cancer in postmenopausal women.

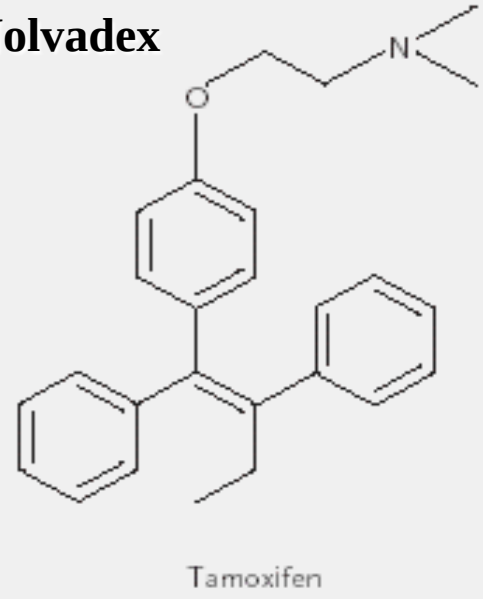
In the late 1980s it was recommended as a drug protecting the women with a high risk of breast cancer, but not in general population because of the risk of cervical cancer.

Tamoxifen – clinical trials

- Tamoxifen therapy when started directly after surgical removal of early stage of estrogene-sensitive cancer (with ER expression) decreased mortality after 5 years by **28%.**

- After employment of tamoxifen in UK, number of deaths caused by breast cancer decreased from 16 000 to 12 800.

Nolvadex



Tamoxifen

Thank you and see you next week...

What would be profitable to remember in June:

- Structure and isoforms of ER and PR

- Role of ER in osteoporosis

- ER inhibitor and aromatase inhibitors in treatment of breast cancer

Slides can be found in the library and at the Heme Oxygenase Fan Club page:

https://biotka.mol.uj.edu.pl/~hemeoxygenase

Venus from Schelklingen
(35,000-40,000 years ago)