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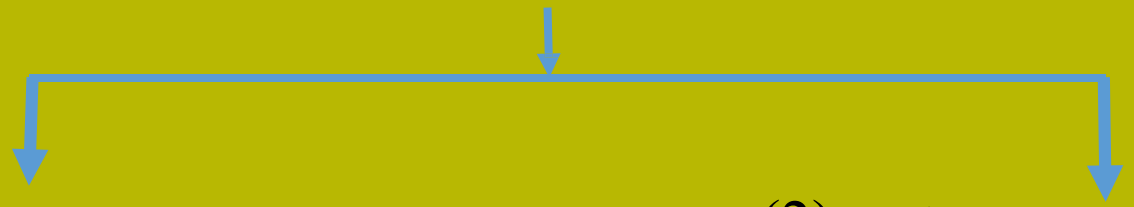
- Introduction .
- Physiology of Reproductive endocrinology .
- Current Anti fertility drugs available.
- Pre Clinical screening of Anti fertility Drugs :

➢ In vitro

➢ In vivo

Anti Fertility drugs :

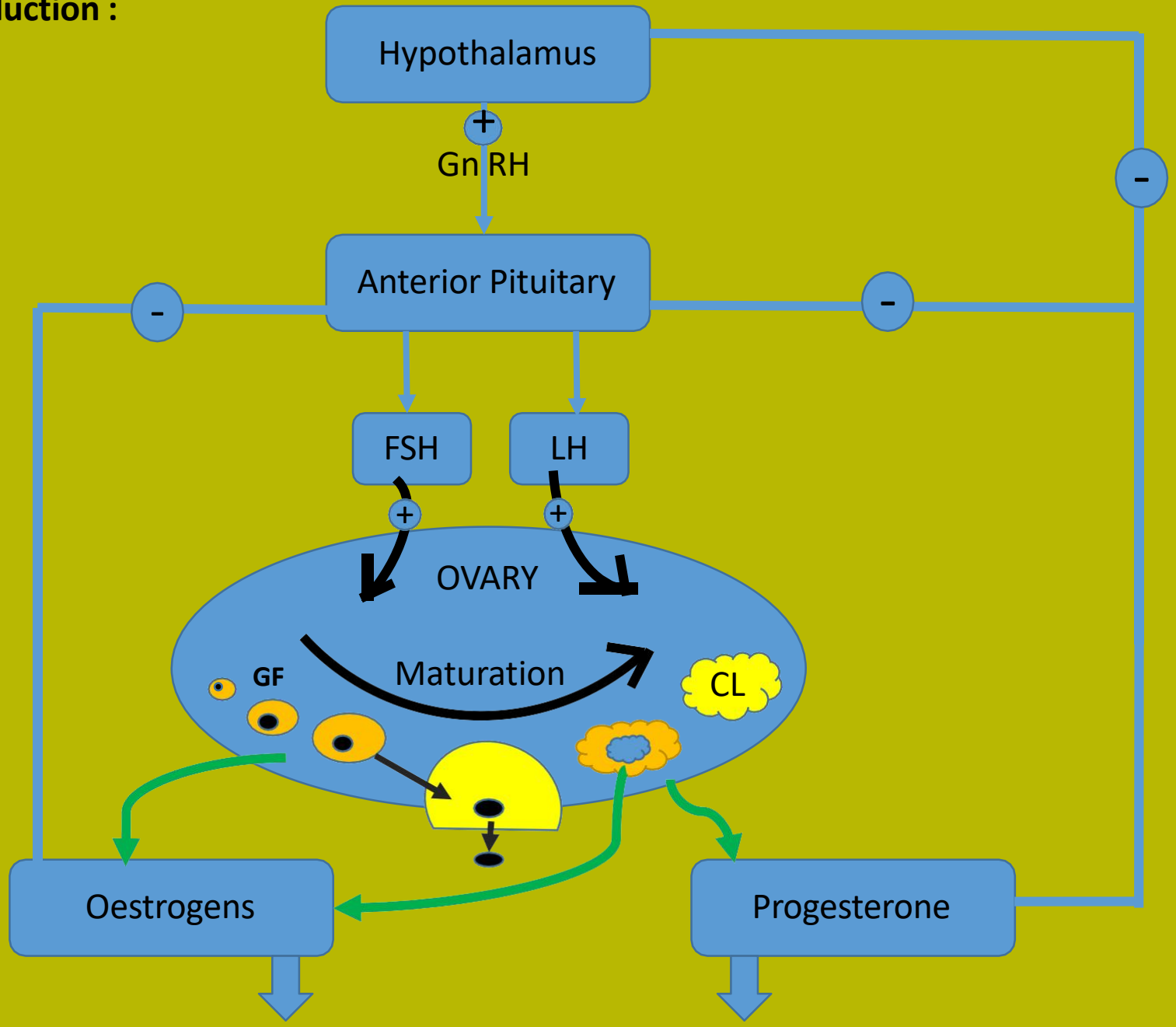
- Antifertility drugs are chemical substances which suppress the action of hormones that promote pregnancy. These drugs actually reduce the chances of pregnancy and act as a protection. Antifertility drugs are made up of derivatives of synthetic progesterone or a combination of derivatives of estrogen and progesterone .
- These are also known as **contraceptive agents**.
- **Contraception** is the method of preventing normal process of ovulation, fertilization and ovum implantation , nothing but pregnancy.
- These are classified into two types.



(1) Female contraceptive agents.

(2) Male contraceptive agents .

Endocrine control of Reproduction :



Act on Reproductive tract & other tissues

Oestrogens :

- Only three estrogens are present in significant quantities in the plasma of the human female: β-*estradiol, estrone,* and *estriol.*
- The principal estrogen secreted by the ovaries is β-estradiol.
- They play an essential role in the growth and development of female secondary sexual characteristics.
- Major site of Oestrogen production is Ovary in premenopausal female.
- Oestrogen stimulates synthesis of progesterone receptors but progesterone inhibits the synthesis of Oestrogen receptors .

- *Synthetic oestrogens available :*
- ***Steroidal :*** Ethinylestradiol, Mestranol, Tibolone.
- ***Nonsteroidal :*** Diethylstilbestrol (stilbestrol) Hexestrol, Dienestrol

Effects of Oestrogens :

- Growth and development of female reproductive system.
- Feedback inhibition of Gonadotrophin (LH/FSH) secretion.
- Maintain bone mass by decreasing the bone sorption.
- Increased risk of breast , endometrial & cervical carcinoma .
- **Mechanism of action:**
- Estrogens bind to specific nuclear receptors in target cells and produce effects by regulating protein synthesis. Estrogen receptors (ERs) have been demonstrated in female sex organs, breast, pituitary, liver, bone, blood vessels, heart, CNS and in certain hormone responsive breast carcinoma cells.
- Two ERs designated ERα and ERβ have been identified, cloned and structurally characterized.

• Major Uses :

- Hormone replacement therapy in post menopausal women . (progesterone added)
- Is a component **of oral contraceptives**.
- Oestrogens reduce testosterone production due to feedback inhibition of LH secretion.(Testesterone dependent prostatic carcinoma).

• Progestins :

- Most important of the progestins is progesterone , another progestin, 17-α-hydroxyprogesterone.
- It is mainly secreated by corpus luteum.
- Large amounts of progesterone are also secreted by the placenta during pregnancy, especially after the fourth month of gestation.
- Unlike other steroid receptors, the progesterone receptor (PR) has a limited distribution in the body ,confined mostly to the female genital tract, breast, CNS and pituitary.
- The progesterone receptor is normally present in the nucleus of target cells (Nuclear receptor) .
- **Synthetic progestins available** :
- Medroxyprogesterone acetate , Megestrol acetate , Dydrogesterone , Hydroxyprogesterone caproate
- ***Newer compound :*** Nomegestrol acetate , Levonorgestrel , Desogestrel.
- **Major Uses :**
- **Oral contraception** & Hormone replacement therapy (in comination with oestrogens) .
- For Amenorrhea , Abnormal uterine bleeding ,premature labour , Luteal phase support for infertility.

Luteinizing hormone

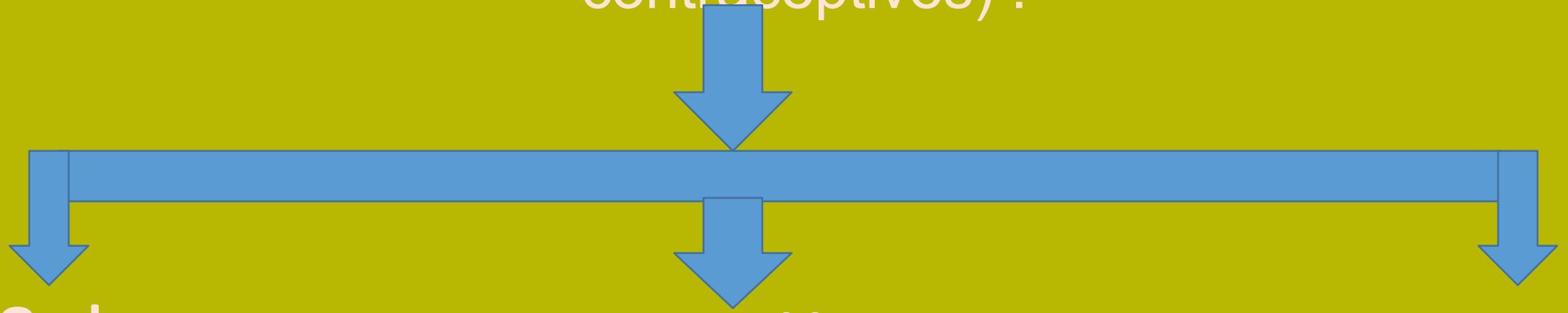
- In females, an acute rise of LH ("**LH surge**") **triggers ovulation** and development of the **corpus luteum**.
- In males, where LH had also been called **interstitial cell–stimulating hormone** (**ICSH**), it stimulates leydig cell production of **Testesterone**.

Follicle stimulating hormone

- In both *males* and *females*, FSH stimulates the maturation of primordial germ cells.
- FSH sustains **spermatogenesis** in Males.
- FSH stimulates the growth and development of immature **ovarian follicles** in the ovary.

LH & FSH **act synergistically** with each other .

Types of contraceptive preparations (female contraceptives) :



Oral

1. Combined pill
2. Phased regimens
3. Mini pill (progestin only)
4. Postcoital (Emergency)

Injectable

1. Long acting progestin only
2. Long acting progestin + Long acting Oestrogen

Implants

1. Biodegradable implants
2. Non biodegradable implants

Mechanism of Action :

- Hormonal contraceptives interfere with fertility in many ways; the relative importance depends on the type of method as:

1. **Inhibition of Gn release** from pituitary by reinforcement of **normal feedback inhibition by** progestin,reducing frequency of LH secretory pulses (an optimum pulse frequency is required for triggering ovulation) while **the estrogen primarily reduces FSH secretion**. (minipill and progestin-only)

2. Thick ***cervical mucus*** secretion *hostile to sperm penetration* is evoked by progestin action.

3. Even if ovulation and fertilization occur, the blastocyst may **fail to implant** because endometrium is either hyperproliferative or hypersecretory .(minipill & post coital)

4. ***Uterine and tubal contractions*** may be modified to disfavour fertilization. This action contributes to the efficacy of minipills and postcoital pill.

1. Combined pill :

- Oestrogen + progesterone containing preparation.
- 2nd gen contain less Oestrogen & progestin to reduce side effects
- 3rd gen contain new progestin like Desogestrel along with anovulatory progestin like 19 nor-Testesterone.
- Taken daily for 21 days, 5th menstrual day ----

7 day gap after 2nd menstruation ----

2. **Phased regimen** :

- These have been introduced to permit reduction in total steroid dose without compromising efficacy. These are biphasic or triphasic.
- Oestrogen level constant with low progestin ------ ist regimen
- Oestrogen level constant with ⇧ progestin ------ 2nd regimen
- Oestrogen level constant with ⇧ ⇧ progestin ------- 3rd regimen

3) Minipill (progestin only pill) :

- A low-dose progestin only pill is taken daily continuously without any gap. The menstrual cycle tends to become irregular
- The efficacy is lower (96–98%) compared to 98–99.9% with combined pill.

4) Postcoital (emergency) contraception :

- Currently 3 regimens are available :
- a) Levonorgestrel 0.5 mg + ethinylestradiol 0.1 mg taken as early as possible but within 72 hours of unprotected intercourse and repeated after 12 hours.
- (b) Levonorgestrel alone 0.75 mg taken twice with 12 hour gap within 72 hours of intercourse.
- (c) Mifepristone 600 mg single dose taken within 72 hours of intercourse

Injectables :

- These have been developed to obviate need for daily ingestion of pills. They are given i.m. as oily solution; are highly effective.
- Menstrual irregularities, excessive bleeding or amenorrhoea are very common.
- Two types of preparations have been tested:
- **(i) *Long acting progestin alone :*** injected once in 2–3 months depending on the steroid. Two compounds have been marketed:
- (a) Depot medroxyprogesterone acetate.
- (b) Norethindrone (Norethisterone) enanthate.
- **ii) *Long acting progestin + long acting estrogen :*** —
- Once a month. These have been tested to a more limited extent, but a combination of Medroxyprogesterone + estradiol cypionate has been approved by US-FDA for i.m. injection every month. Main advantage is that they allow a reasonable menstrual bleeding pattern in most cases.

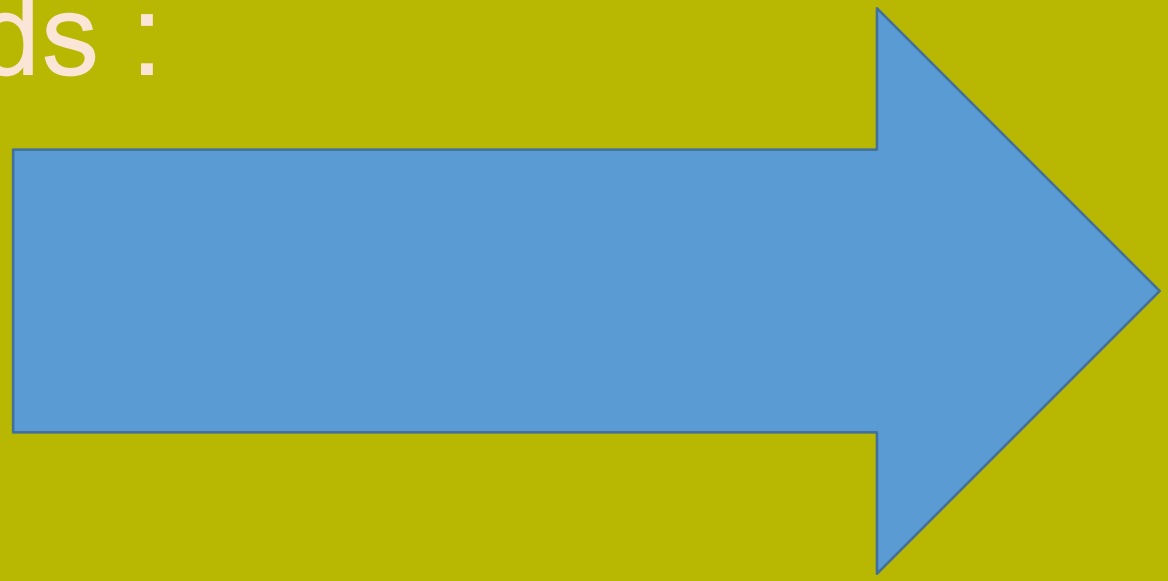
Implants (Novel formulations):

- **Implants** These are drug delivery systems implanted under the skin, from which the steroid is released slowly over a period of 1–5 years. They consist of either—
- **(a) Biodegradable polymeric matrices**—do not need to be removed on expiry.
- **(b) Non-biodegradable rubber membranes—**have to be removed on expiry.

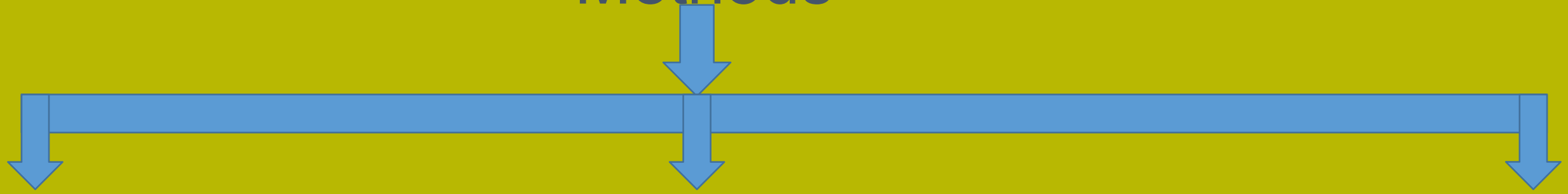
MALE CONTRACEPTIVE :

- The only way to suppress male fertility by drugs is to inhibit spermatogenesis. Though considerable effort has been made in this direction and effective drugs have been found, no satisfactory/ acceptable solution is yet tangible.
- Drugs and approaches tried are—
- 1. Antiandrogens : Act by direct action on testes; cause unacceptable loss of libido.
- 2. Estrogens and progestins : Act by suppressing Gns— cause unacceptable feminization.
- 3. Androgens : They inhibit Gns but have poor efficacy; combination with progestin is more efficacious, preserves libido, but is still not reliable.
- 4. Superactive Gn RH analogues : They inhibit Gn release after continued action; also inhibit testosterone secretion— produce impotence, loss of libido.
- 5. Cytotoxic drugs : Cadmium, nitrofurans and indoles suppress spermatogenesis, but are toxic, often produce irreversible action.

Screening methods :



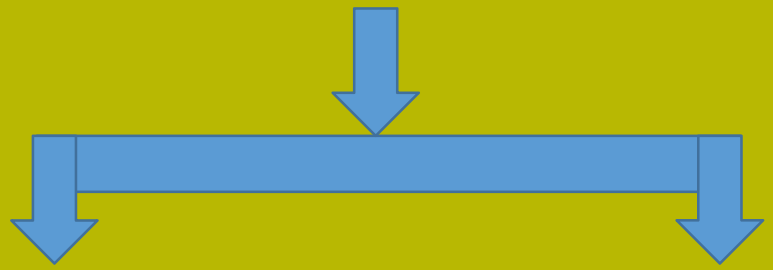
Methods



Antiovulatory Activity

1. HCG induced ovulation in rats.
2. Cupric acetate induced ovulation in rabbits.

Progestational activity



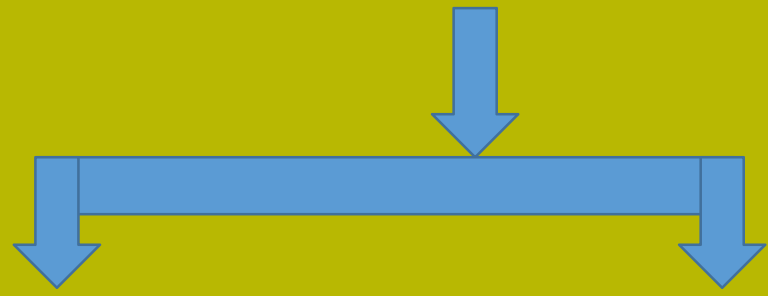
<u>In vivo methods</u> :

1. Pregnancy maintainence test.
2. Proliferation of uterine endometrium in oestrogen primed rabbits (Clauberg Mcphail test)

In vitro methods :

1. Progesterone receptor binding assay.

Oestrogenic activity



<u>In vivo methods</u> :

1. Vaginal opening
2. Assay for water uptake
3. 4-day uterine weight assay
4. Vaginal cornification
5. Chick oviduct method

<u>In vitro methods</u> :

1. Oestrogenic receptor binding assay
2. Potency assay

Tests used for screening :

Anti ovulatory activity :

1) HCG – Induced ovulation in rats :

Rationale :

- Immature female albino rats do not ovulate sponataneously and do not show cyclic changes of the vaginal epithelium .
- Priming with HCG induces follicular maturation , followed by sponataneous ovulation 2 days later .
- Injection of anti ovulatory drugs, prior to induction procedure will prevent ovulation .
- This principle is used for the screening of anti-ovulatory agents

Procedure :

- Immature female albino rats 24-26 days of age are used for the experiment .
- The animals are treated with various test drugs in a different dose levels .
- After the administration of the test drug ,HCG is given exogenously for ovulation.
- After 2 days ,animals are sacrificed ,ovaries are dissected out ,preserved in 10% buffered *formalin* and subjected to histopathological evaluation .
- The results are compared with the control group.

2) Cupric acetate-induced ovulation in rabbits :

- Rationale :
- The rabbits are reflex ovulators .They ovulate within a few hours after mating or after mechanical stimulation of vagina or sometimes even prescence of males ,or administration of certain chemicals like cupric acetate .

- The rabbit ovulates within a few hours after injection of cupric acetate (0.3mg/kg i.v of 1 %cupric acetate in 0.9 saline) .
- Injection of anti ovulatory drugs ,24 hours before the induction procedure prevents ovulation.

Procedure:

- Sexually mature female rabbits,weighing 3-4 kg are used for the study.Animals are kept in isolation for atleast 21 days to ensure ,they are not pregnant and to prevent the induction of ovulation by mating .
- They are treated with test drug and 24 hours later cupric acetate is given .The rabbits are scarificed and ovaries are examined 18-24 hrs later .
- The total number of ovulation points on both ovaries are recorded for each animal .
- Then the ovaries and uterus are excised out and preserved in 10% buffered formalin and subjected to histopathological evaluation .

Oestrogenic activity :

- A primary therapeutic use of Oestrogen is in contraception .The rationale for these preperations is that excess exogenous Oestrogen inhibits FSH & LH , thus prevents ovulation.

In vivo Methods :

1) vaginal opening :

- This assay is based on the principal that vaginal opening occurs in immature female albino mice & rats by treating with Oestrogenic compounds . The sign of complete vaginal opening is observed as a sign of Oestrogenic activity.

Procedure :

- Immature female animals (18 day old mice , 21 day old rats) are used for the study.
- The test & standard drugs are administered to the animals intramuscularly in cotton seed oil . The time of complete vaginal opening can be observed as a sign of Oestrogenic activity

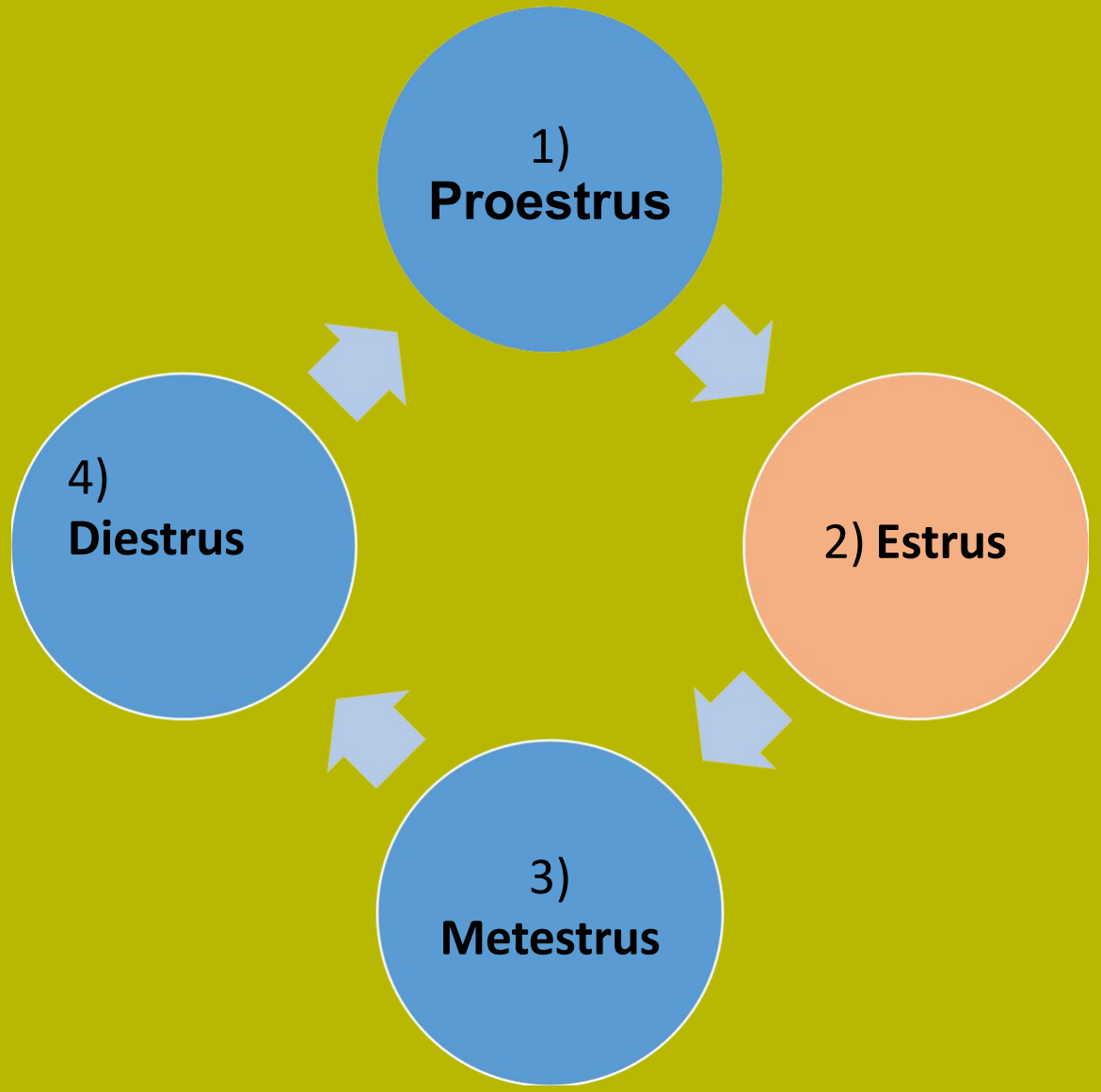
2) Vaginal cornification :

- Rationale :

- This assay is based on the fact that rats and mice exhibit a *cyclical ovulation* with associated changes in the secretion of hormones ,this lead to the changes in the vaginal epithelial cells.The oestrus cycle is classified into

1. Proestrus.
2. Estrus.
3. Metestrus.
4. Diestrus .

- Drugs with Oestrogenic activity change the animal into *estrus stage*.



Stage of estrus cycle in rats

Stage of estrus cycle in rats :

- The estrus cycle is a cascade of hormonal and behavioral events which are highly synchronized and repetitive. The short and precise estrus cycle of the laboratory rats has been a useful model for reproductive studies .

- The estrous cycle of rat is usually completed in 4-5 days as :

1. Proestrus : is beginning of new cycle ,follicles start maturing under the influence of GH , smear is characterized by by nucleated epithelial cells ,stage lasts for about 12 hrs.
2. Estrus : uterus is enlarged and extended due to fluid accumulation , oestrogen secreation is at its peak , smear shows squamous cornified cells. Estrus means period of heat or sexual receptivity. (12 hrs).
3. Metestrus : ovary contains corpora lutea , secreating progesterone , indicated by mixture of leukocytes & cornified epithelial cells indicating post ovulatory stage.(21 hrs)
4. Diestrus : corpora lutea regress & declining secretion of oestrogen & progesterone causes regression of the uterus, smear shows only leukocytes . (57 hrs)

3) Chick oviduct method :

- Rationale :
- The weight of oviduct of young chicken is increased, dose – dependently by natural and synthetic Oestrogen .This principle is used for the screening Oestrogenic compounds.

- Procedure :
- 7 days old pullet chicks are injected subcutaneously , twice daily with solutions of the test compound in various doses for 6 days .
- Doses between 0.020 & 0.5 µg ,17 β oestradiol per animal serve as standard .
- 6-10 chicks are used for each dosage group.
- On the day after the last injection , the animals are sacrificed and weight of the body and oviduct is determined .

Progestational Activity :

In vivo methods :

Proliferation of uterine endometrium in oestrogen primed rabbits (Clauberg Mcphail test)

Principal :

- Female rabbits weighing between 800-1000g are primed with estradiol and followed by the administration of progestational compound leading to the proliferation of the endometrium and converted into secretory phase .

Procedure :

- Female rabbits weighing 800- 1000g are primed with injection of estradiol 0.5mcg/ml in aqueous solution daily
  .on day 7 treatment is begun .
- The total dose is given in 5 equally divided fraction daily over 5 days .
- 24hrs after the last injection , animals are killed and uteri are dissected out and frozen sections of segment of middle portion of one horn is prepared and examined for histological interpretation .
- For interpretation of progestational proliferation of endometrium , beginning of glandular development may be
  graded 1 & endometrium consisting only of glandular tissue may be graded 4