

**SCHOOL OF MEDICINE AND HEALTH SCIENCES
DIVISION OF BASIC MEDICAL SCIENCES
DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY
BMLS 3; BDS IV
CLINICAL BIOCHEMISTRY: GONADAL FUNCTION**

What are steroid hormones?

- Steroid hormones are the group of hormones that are synthesized from Cholesterol.

Briefly outline the pathways for biosynthesis of steroid hormones:

- Pathways for the biosynthesis of steroid hormones are shown in **Fig. 1**.
- Particular steroid hormone class synthesized by a given cell type depends upon:
 - Its complement of peptide hormone receptors,
 - Its response to peptide hormone stimulation and
 - Its genetically expressed complement of enzymes.

How do steroid hormones exist in plasma?

- Steroid hormones are not soluble in aqueous medium, thus they bind to specific hormone binding Glycoproteins in plasma (bound fraction of hormone).
- A small amount of the steroid hormone usually remains unbound or free in plasma.
- Unbound or “Free” fraction of the hormone in plasma is biologically active.
- Measurement of Free hormone status or binding protein levels is important in the diagnosis of patients with steroid hormone disorders.

What is the general mode or mechanism of action of steroid hormones?

- Steroid hormones exert their action by passing through the cell membrane and binding to intracellular receptors (formation of steroid hormone-receptor complex)
- Steroid Hormone-Receptor Complex exerts its action in the Nucleus of Target cells.
- Steroid Hormone-Receptor Complex binds to Specific Nucleotide Sequences in the DNA of Responsive Genes.
- These DNA sequences are identified as Hormone Responsive Elements (HREs).
- Interaction of steroid hormone-receptor complexes with DNA leads to altered rates of Transcription of the associated Genes in Target cells.

What are the Sex Steroid Hormones?

- Both Testosterone and Estradiol are known as Sex Steroid Hormones.
- Testosterone: Testes synthesize the Principal Androgen in male.
- Estradiol (Oestradiol): Principal female hormone is secreted by the Ovaries, varies widely in concentration in plasma throughout the female menstrual cycle.
- Steroids with Estradiol-like action are called Estrogens.
- **Progesterone** is a product of the Ovary and is secreted when a Corpus Luteum forms after Ovulation.
- Normal female plasma contains Testosterone:

- Half comes from the Ovary and
- Half from peripheral conversion of **Androstenedione** and **Dehydroepiandrosterone (DHEA) Sulphate**, secreted by the Adrenal Cortex.
- Estradiol is present in low concentration in normal male plasma.

What is the function of SHBG and how does it affect plasma levels of the Sex Steroids?

- Testosterone and Estradiol circulate in plasma mostly bound to plasma proteins, particularly Sex Hormone Binding Globulin (SHBG).
- SHBG has a higher affinity for Testosterone than for Estradiol.
- Estradiol stimulates SHBG synthesis by the liver,
- Testosterone decreases SHBG synthesis by the liver
- Plasma concentration of SHBG in females is twice that in males.
- Factors, which alter the concentrations of SHBG in plasma, alter the Ratio of Unbound Testosterone to Unbound Estradiol.

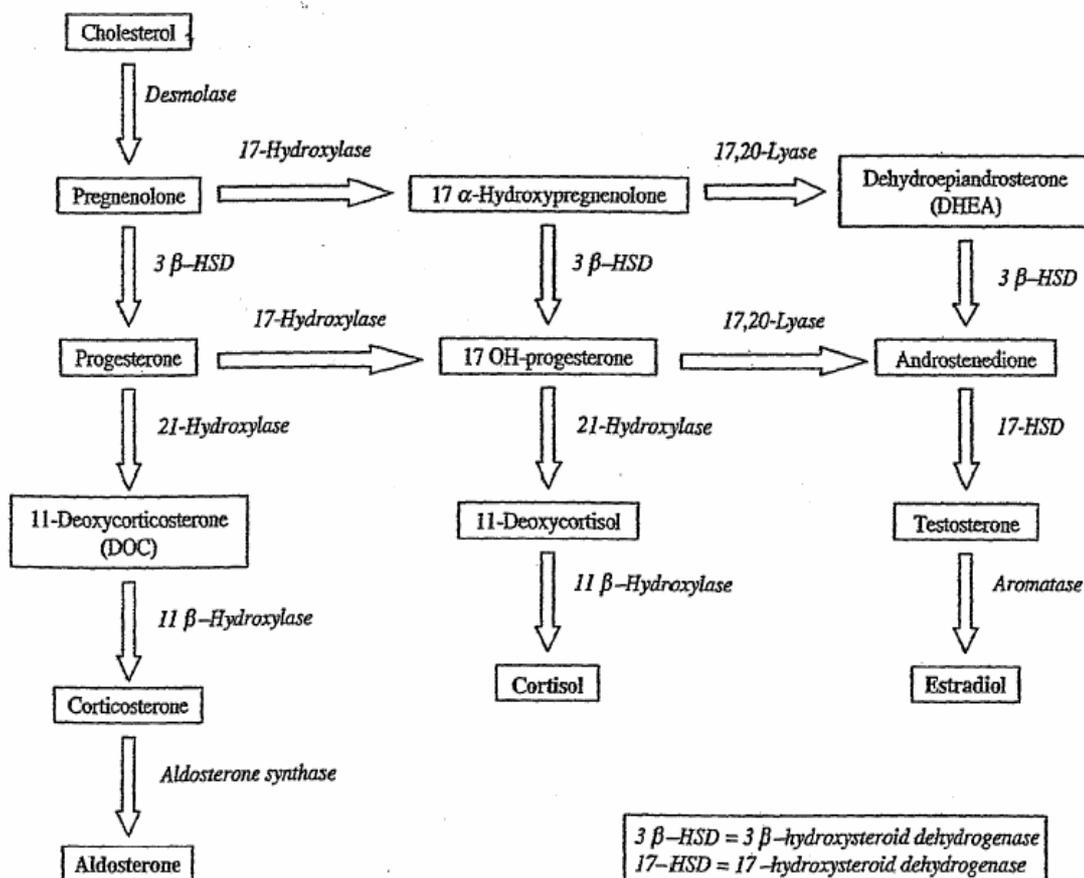
- In both sexes the effect of:
 - An increase in SHBG is to increase Estradiol-like effects, (**Why?**)
 - A decrease in SHBG is to increase Androgen effects (**Why?**)

- As Estradiol it increases SHBG concentration and Testosterone decreases it, this system functions as a Biological Servomechanism.
- Testosterone and SHBG concentrations are sometimes reported by the laboratory as a Ratio (the Free Androgen Index), which gives a clearer indication of Androgen status than does serum Testosterone alone.

What axis regulates the secretion of the sex steroids?

- Secretion is regulated by the Hypothalamic-Pituitary-Gonadal Axis (HPG-axis)
- Hypothalamus releases the Hormone called Gonadotropin-Releasing Hormone (GnRH)
- GnRH acts on the Anterior Pituitary to stimulate synthesis and release of the Gonadotropins:
 - Luteinizing Hormone (LH) and
 - Follicle-Stimulating Hormone (FSH).
- Gonadotropins (LH and FSH) act cooperatively on the Ovaries in the female and the Testes in the male to stimulate Sex Hormone secretion and reproductive processes
- Regulation of the secretion of sex steroid hormones is by Negative Feed-back on HPG –axis.
- Inhibin produced by the Gonads also feed back inhibits the production of FSH.

Figure 1:
Diagram the steroid hormone pathway.



Steroid Pathway.

What are the functions of male gonads?

- Testes secrete Testosterone and produces Spermatozoa
- LH influences development of Leydig cells and their secretion of Testosterone
- FSH influences Sertoli cell function
- FSH and LH are required for stimulation and maintenance of Spermatogenesis (Fig. 2)
- Testosterone is responsible for:
 - Growth and function of Prostate and Epididymis,
 - Development of male secondary sex characteristics (hair growth, deep voice and characteristic musculature)

- ❑ Biological activity of Testosterone is due to its conversion to Dihydrotestosterone by 5-alpha-Reductase in some tissues.

What are some of the disorders of male sex hormones?

- ❑ Hypogonadism may result in deficient sperm production and decreased testosterone secretion
- ❑ Hypogonadism may be caused by:
 - ❑ Testicular deficiency (Primary disorders or Hypergonadotrophic Hypogonadism)
 - ❑ Defect in Hypothalamus or Pituitary (Secondary disorders or Hypogonadotrophic Hypogonadism)
- ❑ FSH and LH or only LH may be absent in Hypogonadotrophic Hypogonadism
- ❑ Generalized failure of Pituitary function may occur

What are some of the causes of Primary Hypogonadism?

- ❑ Congenital defects, such as, Klinefelter's syndrome or Testicular Agenesis
- ❑ Acquired defects due to Testicular Infections (e.g., Mumps), Trauma, Irradiation, or Cytotoxic drugs

What are some of the causes of Secondary Hypogonadism?

- ❑ Pituitary tumours
- ❑ Hypothalamic disorders such as Kallmann's syndrome

What is the test to establish Hypogonadism?

- ❑ Dynamic tests, such as, stimulation with GnRH may help to establish the cause of Hypogonadism in some patients

What are some of the disorders of male sexual differentiation?

- ❑ Disorders of male sexual differentiation are rare
- ❑ Testosterone production may be impaired
- ❑ In Testicular Feminization syndrome, Androgen receptors are inactive and target tissues cannot respond to stimulation by circulating Testosterone

What are the functions of female sex hormone (Estradiol)?

- ❑ Estradiol:
 - ❑ Responsible for Female secondary sex characteristics
 - ❑ Stimulation of follicular growth
 - ❑ Development of the Endometrium

What Axis is involved in regulation the female sex hormone?

- ❑ Hypothalamus-Anterior Pituitary Ovarian Axis (HPO-Axis) See **Fig. 3A**

TAKE NOTE:

- ❑ Estradiol secretion is low before puberty,
- ❑ At puberty Estradiol secretion rise rapidly and fluctuate cyclically throughout reproductive life
- ❑ After Menopause, plasma Estradiol concentrations fall despite high circulating concentrations of Gonadotropins

What hormones influence the menstrual cycle?

- Menstrual cycle is influenced by cyclic variations of hormones produced by Hypothalamus (Gn-RH), Anterior Pituitary (FSH & LH) and Ovary (Estradiol and Progesterone)
- **Figs 3B & 3C** shows changes in plasma concentrations of FSH, LH, Estradiol and Progesterone in normal menstrual cycle
- Developing Graafian follicles in Ovaries respond to cyclical stimulus of Gonadotrophins by secreting Estradiol-17 β and Oestrone;
 - Both are metabolized to a third Estrogen called Estriol
- After Ovulation, Corpus Luteum secretes Progesterone as well as Estrogens
- Changes in Uterus are determined by Ovarian Steroid output
 - Changes are modified if pregnancy occurs
- Progesterone acts on Uterus and is essential for maintenance of early pregnancy
- Estradiol-17 β may stimulate or inhibit secretion of Gonadotrophins, depending on its concentration in plasma;
 - Stimulating effect of Estradiol-17 β can be prevented by high plasma concentration of Progesterone

What are some of the disorders related to female sex hormones?

- **Subfertility, Amenorrhoea, Oligomenorrhoea**
- **Hirsutism:**
 - Increase in body hair with male pattern distribution
 - Majority of cases (termed idiopathic) it is genetic in origin and benign
 - Commonest pathological cause is Polycystic Ovarian Disease
 - Essential to exclude serious disease when investigating Hirsute women
- **Virilism:**
 - Testosterone concentrations are usually markedly elevated
 - Evidence of excessive Androgen action such as:
 - Clitoral Enlargement, Hair Growth in a male pattern, Deepening of Voice Breast Atrophy
 - Tumours of Ovary or Adrenal are the likely cause

Oligomenorrhoea and Amenorrhoea:

- Women with Oligomenorrhoea or Amenorrhoea may present because of concerns they have regarding their:
 - Bleeding pattern, Infertility, Hirsutism, Virilism, or a combination of these
- Need to exclude the following as possible cause:
 - Physiological causes of Amenorrhoea (Pregnancy, Lactation)
 - Anatomical abnormalities
- Amenorrhoea may be:
 - Primary (i.e., patient has never menstruated), thus abnormal development may be the likely cause, or
 - Secondary to various causes as listed in Table below

- ❑ Summary of some endocrine causes of infertility that may have to be considered, especially if there are menstrual abnormalities also

Site of Lesion	Examples:
Hypothalamus	<ul style="list-style-type: none"> ❑ Anorexia Nervosa, ❑ Severe weight loss, ❑ Stress (Psychological and/or Physical), ❑ Gn-RH deficiency (Kallmann's syndrome), ❑ Tumours (e.g., acromegaly)
Anterior Pituitary	<ul style="list-style-type: none"> ❑ Hyper-prolactinaemia, ❑ Hypopituitarism, ❑ Functional tumours (e.g., Cushing's disease), ❑ Isolated deficiency of FSH or of LH
Ovaries	<ul style="list-style-type: none"> ❑ Polycystic ovary syndrome, ❑ Ovarian failure (may be auto-immune, chromosomal, iatrogenic i.e., after cancer therapy, or idiopathic), ❑ Ovarian tumours
Receptor defect	<ul style="list-style-type: none"> ❑ Testicular feminization syndrome
Other endocrine diseases	<ul style="list-style-type: none"> ❑ Diabetes Mellitus, ❑ Thyrotoxicosis, ❑ Adrenal dysfunction (e.g., late-onset CAH)

What suggested laboratory tests can assist in diagnosis?

- ❑ Measurement of plasma concentrations of hormones that affect HPO-axis
 - ❑ FSH, LH, Estradiol-17 β , Prolactin, TSH, and FT4
- ❑ If Hirsutism or Virilization is present, measure:
 - ❑ Plasma Testosterone, Androstenedione and Dehydroepiandrosterone Sulphate (DHAS)

What is the Androgen Screen in Female and how is it interpreted?

(Fig. 4)

- ❑ Observation of Elevated Testosterone in a female must be Investigated further
- ❑ Androgen screen test is to identify the source of the Testosterone
- ❑ A decrease SHBG concentration is evidence of Elevated Androgen (**Why?**)
 - Because Testosterone inhibits synthesis of SHBG in the Liver
- ❑ Source of Testosterone may be either the Ovary or Adrenal Cortex
- ❑ By measuring concentration of other Androgens such as Androstenedione and DHA Sulphate (an "Androgen screen"), the source of Testosterone can be identified (**Fig. 4**)
- ❑ An elevated DHA sulphate suggests that Adrenal or an Adrenal tumor is overproducing Androgens
- ❑ If the Ovary is the source then only Androstenedione will be raised

Endocrine Investigation in the Subfertile Female: (Fig. 5)

- ❑ Investigation of Infertile female depends on Phase of Menstrual Cycle
- ❑ Female presenting with Regular Menstrual Cycle:
 - Measure Serum Progesterone in the Middle of Luteal Phase (day 21)
 - If Progesterone is High (> 30 nmol/L), Ovulated has occurred and there is no need for further Endocrine Investigation
 - Other causes of Subfertility should be sought
 - If Progesterone is Low (< 10 nmol/L), Ovulation has not occurred
- ❑ Female presenting with Irregular or Absent Menstruation (Oligomenorrhoea or Amenorrhoea) or who are not Ovulating,
 - ❑ Hormone measurements may be Diagnostic
 - ❑ Protocols for investigation are shown in **Figs. 5, 6, 7, and 8**
- ❑ Measurement of Estradiol and Gonadotrophin concentrations may detect:
 - Primary Ovarian Failure or Polycystic Ovarian Disease
- ❑ Measurement of Prolactin, and Androgens may also assist

What are some of the Endocrine causes of Subfertility in females?

- ❑ **Primary Ovarian Failure:**
 - ❑ Indicated by elevated Gonadotropins and Low Estradiol concentration (a post-menopausal pattern)
 - ❑ Hormone replacement therapy assists libido and prevents osteoporosis, but does not restore fertility
- ❑ **Polycystic Ovarian Disease:**
 - ❑ Indicated by Elevated LH and normal FSH
 - ❑ Estradiol measurements are often unhelpful
 - ❑ Hirsutism, a feature of this condition, is associated with raised Testosterone and subnormal sex hormone binding protein concentrations
- ❑ **Hypogonadotropic Hypogonadism:**
 - ❑ Subnormal Gonadotrophin and Estradiol concentrations suggests the presence of a Hypothalamic-Pituitary Lesion such as interferences from a Pituitary Tumour
 - ❑ Mechanism responsible for Amenorrhoea or Oligomenorrhoea in female with Normal Gonadotrophin and Estradiol concentrations is not clear
- ❑ **Hyperprolactinaemia:**
 - ❑ Prolactin acts directly on Mammary Glands to control lactation
 - ❑ Gonadal function is impaired by elevated circulation of Prolactin
 - ❑ Hyperprolactinaemia is common and can cause infertility in both sexes
 - ❑ Early indication in women is Amenorrhoea and Galactorrhoea
- ❑ **Some causes of Hyperprolactinaemia:**
 - ❑ Stress;
 - ❑ Drugs (e.g., estrogens, phenothiazines, alpha-methyl dopa, metoclopramide);

- Primary Hypothyroidism (Prolactin is stimulated by the raised TRH);
- Pituitary diseases

Interpretation of Fig. 6 (Investigation of Oligomenorrhoea and Amenorrhoea)

- Fig. 6 summarizes the interpretation of investigations commonly performed in patients with menstrual abnormalities
- **Plasma level of Prolactin High:**
 - Result needs to be confirmed by repeating the investigation
 - Even then, it must be interpreted with caution, since stress, certain drugs, Hypothyroidism and Chronic Renal Failure can all lead to elevation in plasma concentration of Prolactin
- **Plasma level of Prolactin Normal:**
- Three possible scenarios:
 - **High plasma levels of FSH and LH; Low plasma level of Estradiol-17 β**
 - Indicates Primary Ovarian Failure,
 - May be due to Chromosomal Abnormality, Chemotherapy or Auto-immune disease, or it may be Idiopathic due to Premature Menopause
 - **High Plasma level of LH, Plasma levels of FSH and Estradiol-17 β Low or at Lower limit of Normal**
 - May indicate Polycystic Ovarian Syndrome
 - **Plasma level of FSH, LH and Oestradiol-17 β are all low, or at lower limits of reference ranges**
 - May indicate Hypothalamic, Pituitary or other endocrine disease
 - Before this possibility is investigated, a Progesterone challenge test should be performed

How is the Progesterone challenge carried out and interpreted?

- Progesterone challenge test is carried out by given the patient a certain amount of Progesterone (Medroxy-progesterone) daily for 5 days
- Menstrual bleeding in the week following Progesterone withdrawal indicates that there has been adequate Priming of the Endometrium by Estrogens;
- Polycystic Ovary Syndrome may be the diagnosis in these patients

Interpretation of Fig. 7 (Investigation of Female Infertility with Normal Menstruation)

- In patients who menstruate normally it is important to establish whether the cycles are Ovulatory or Anovulatory
- Measure Serum level of Progesterone on one occasion between days 19 and 23 of the cycle,
- Monitor response in three separate cycles
- Ovulatory cycle is indicated if serum level of Progesterone is greater than 30nmol/L

- ❑ Anovulatory cycles is strongly indicated if Progesterone level is less than 10 nmol/L
- ❑ In patients with serum concentration of Progesterone between 10 and 30 nmol/L, the cycles may be Ovulatory, but there may be a defect in the Luteal Phase leading to decreased fertility

Interpretation of Fig. 8 (Investigation of Hirsutism in females):

- ❑ Scheme for investigation of female Hirsutism (Fig. 8)
- ❑ Measure serum level of Testosterone and Dehydroepiandrosterone sulphate (DHAS)
- ❑ DHAS is a specific Adrenal product in females
- ❑ Hirsutism is usually idiopathic in women with normal levels of DHAS
- ❑ Detailed investigation, may reveal evidence of Adrenal excess due:
 - ❑ To low plasma concentration of SHBG accompanied by increased serum concentration of Free Androgens, or
 - ❑ To increased conversion of Testosterone to 5 α -Dihydrotestosterone (5 α -DHT) in the skin
- ❑ **A second group** of Hirsute women have moderate increased serum concentration of Testosterone, secondary to increased production by the Ovaries or Adrenals, and often associated with Menstrual Irregularity
- ❑ If the underlying cause is Late-Onset Congenital Adrenal Hyperplasia due to Partial deficiency of 21-hydroxylase this can be confirmed by injecting 250 mg Synacthen, Intramuscularly and then measure the serum concentration of 17 α -Hydroxyprogesterone one hour later
- ❑ In a patient with CAH, there will be an increase in serum concentration of this compound to more than twice the upper reference value
- ❑ Polycystic Ovarian Syndrome (Stein-Leventhal syndrome) is a more common cause of Hirsutism, with patients often having Irregular Menses, moderately increased serum Testosterone levels and serum DHAS with increased plasma concentration of LH
- ❑ **Third group** of Hirsute women have considerably increased serum concentrations of Testosterone and DHAS, and may be excluded as should rarer causes of abnormalities, e.g., ovarian or adrenal tumours

Fig. 2: HYPOTHALAMUS-ANTERIOR PITUITARY-TESTICULAR AXIS (HPT-AXIS) IN MALES

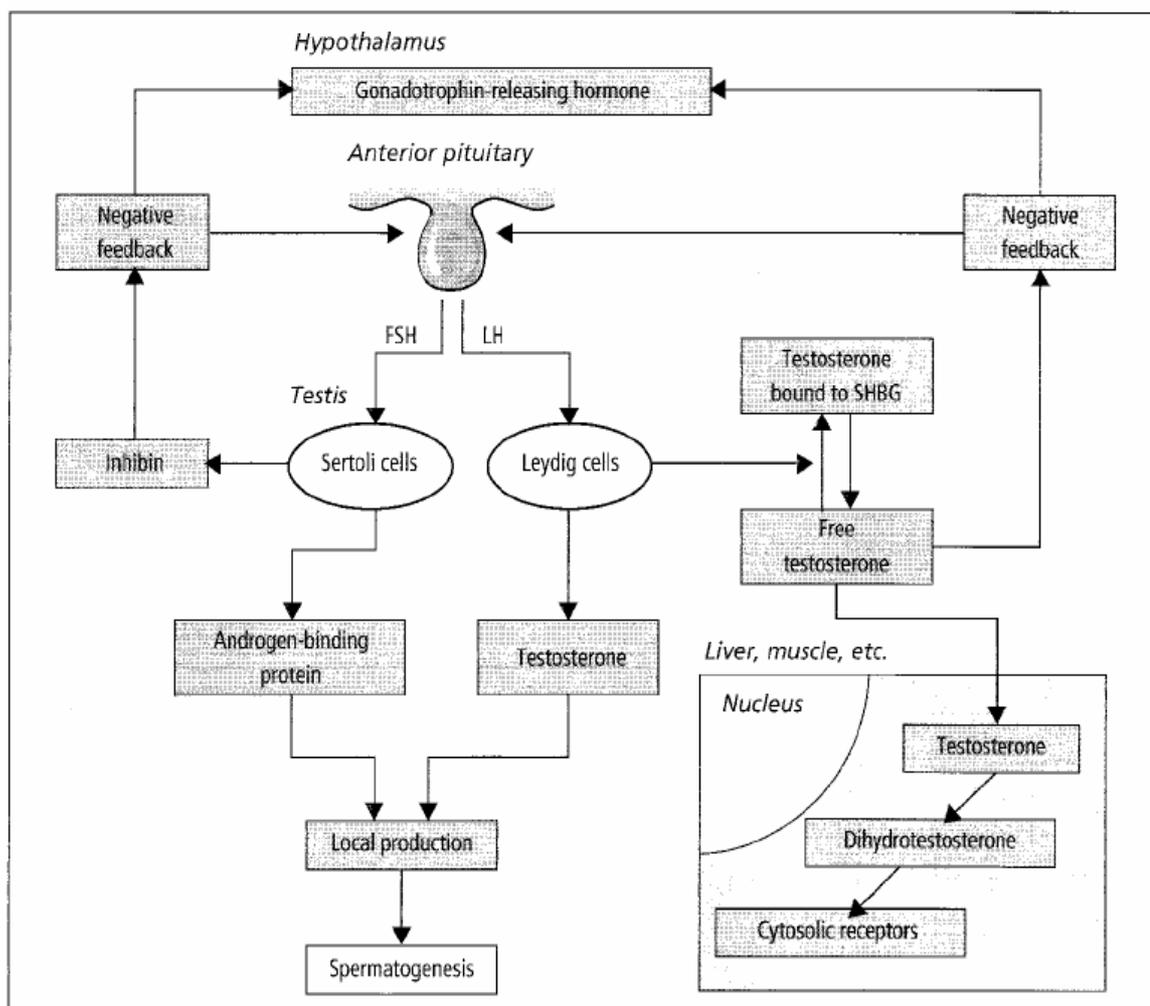


Figure 17.1 The hypothalamic–pituitary–testicular axis. SHBG: sex hormone-binding globulin. Activin from Sertoli cells stimulates FSH release.

NOTE: 5- α -Reductase catalyzes the conversion of Testosterone to Dihydrotestosterone

Fig. 3A: HYPOTHALAMUS-ANTERIOR PITUITARY OVARIAN AXIS (HPO-AXIS) IN FEMALES

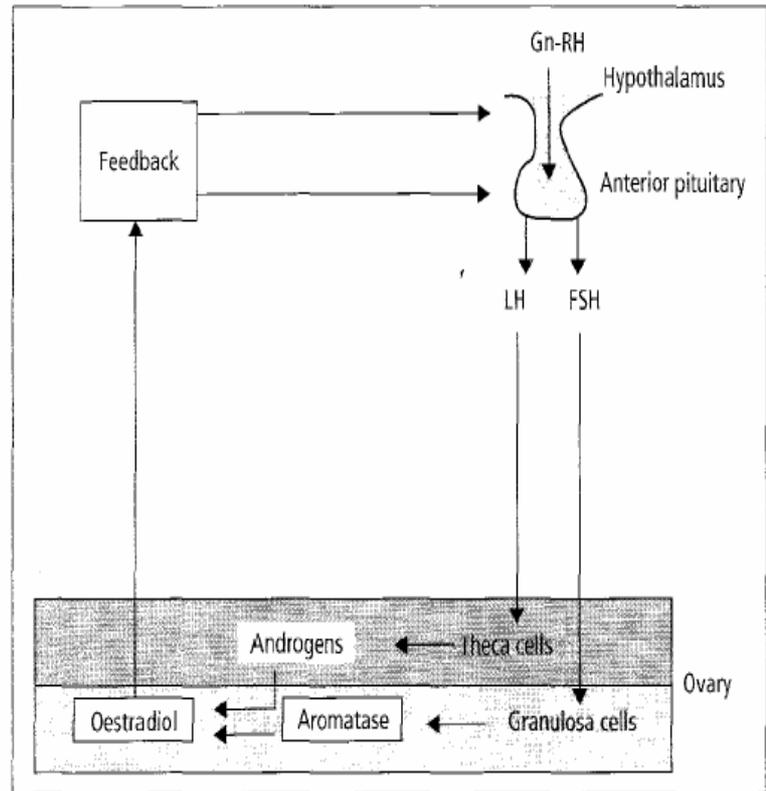


Figure 17.3 The hypothalamic-pituitary-ovarian axis. Activins, inhibins and progesterone also have a role in regulating the cycle.

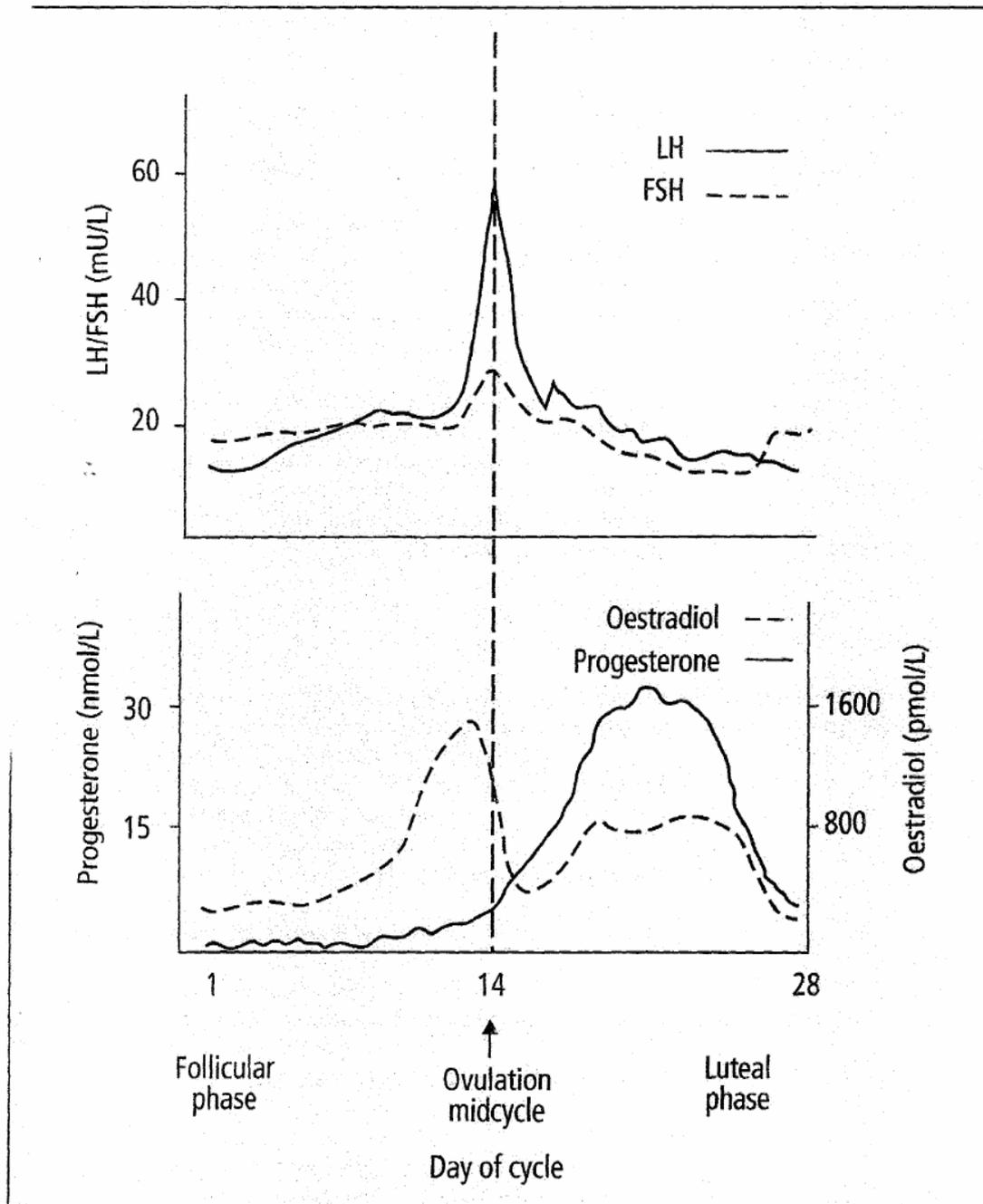
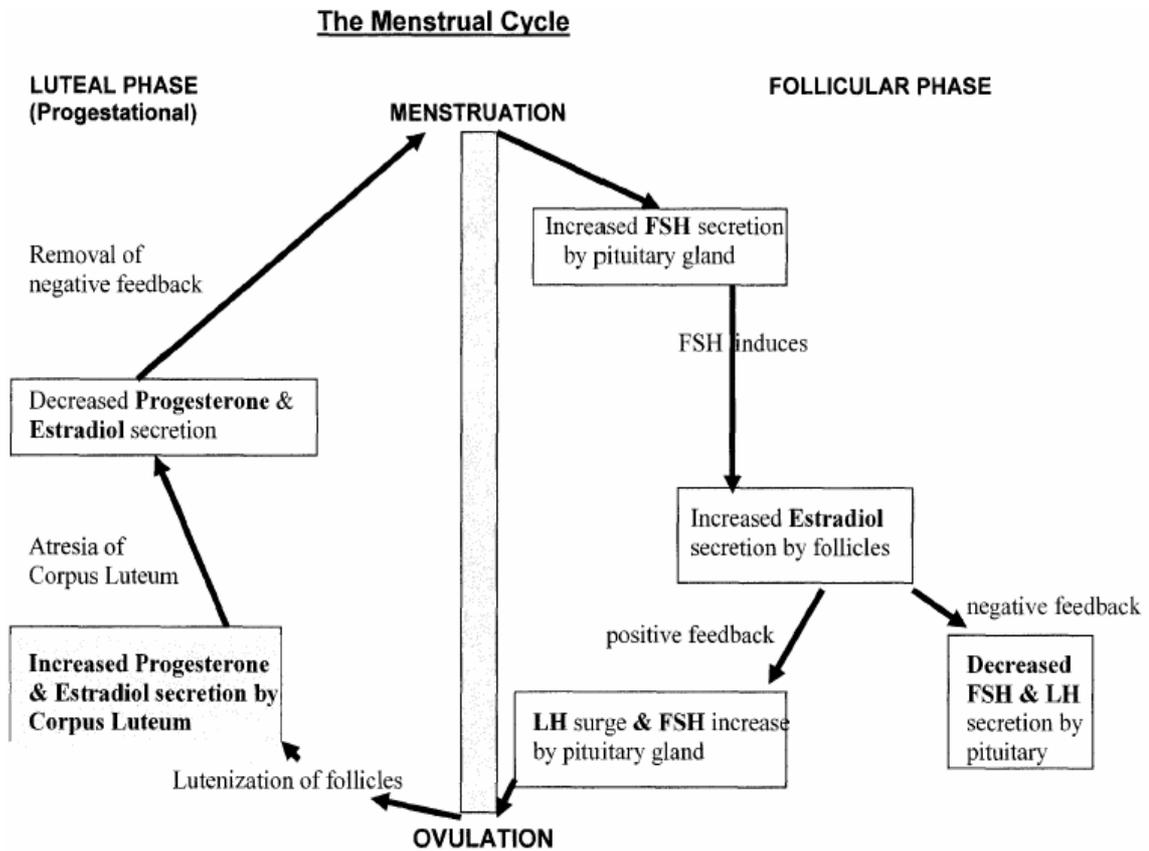
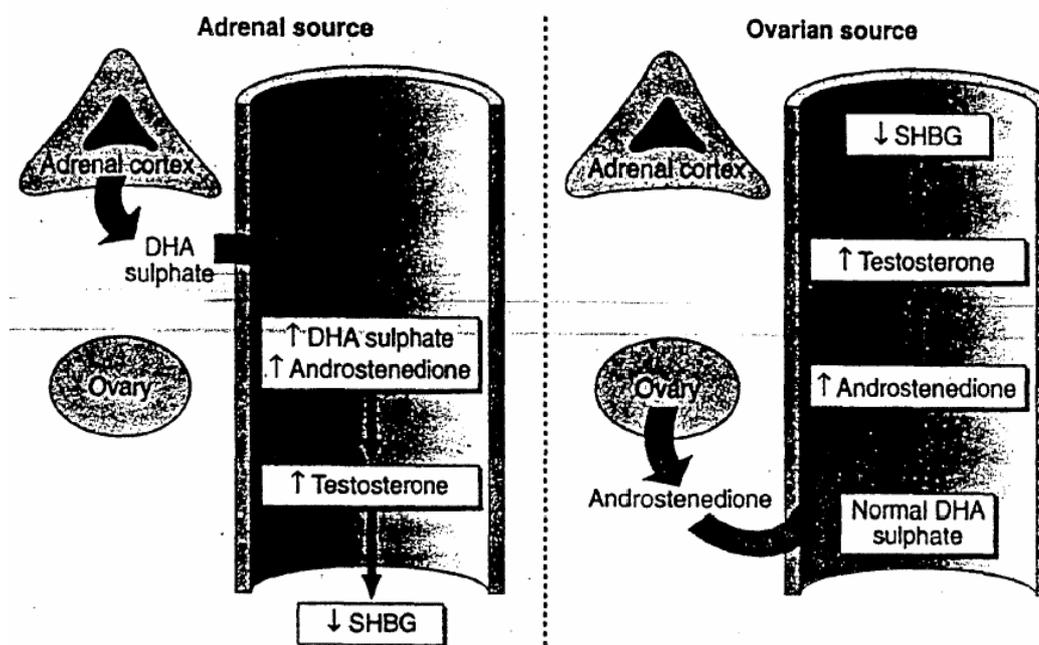
Fig. 3B: Hormonal changes in the menstrual cycle

Fig. 3C: Hormonal changes in Menstrual cycle showing positive and negative feedback effects of Estradiol



CHECK THE HANDOUTS GIVEN TO YOU IN CLASS FOR ALL THE OTHER DIAGRAMS (FIGURES: 5 to 8).

Fig. 4: Adrenal Screen in female.



Investigation of an elevated testosterone concentration in a woman.

CLINICAL BIOCHEMISTRY STUDY QUESTIONS IN GONADAL FUNCTION

1. What are steroid hormones?
2. How do steroid hormones exist in plasma?
3. What is the general mode or mechanism of action of steroid hormones?
4. What are the Sex Steroid Hormones?
5. What is the function of SHBG?
6. How does SHBG affect plasma levels of the Sex Steroids?
7. Why does increase in SHBG cause increase in Estradiol-like effects?
8. Why does decrease in SHBG cause increase in Androgen effects?
9. What Axis Regulates Secretion of the Sex Steroid Hormones?
10. What are the functions of the Female Sex Hormone?
11. What are some of the disorders of the Female Sex Hormone?
12. Describe how the Androgen Screen in Female is carried out?
13. How are Endocrine Investigations in the Sub fertile Female carried out?
14. What are some of the endocrine causes of sub-fertility in female?