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SCHOOL OF MEDICINE AND HEALTH SCIENCES
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DISCIPLINE OF BIOCHEMISTRY & MOLECULAR BIOLOGY
MASTER OF MEDICINE PART 1 – COMMON CORE

STEROIDOGENESIS – An Overview

What are Steroid Hormones?

- ❑ Steroid hormones: group of hormones synthesized from Cholesterol.
- ❑ Important steroid hormones in humans: Estradiol, Testosterone, Progesterone, Aldosterone, and Cortisol (**Fig. 1**).

What are the Pathways for Biosynthesis of Steroid Hormones?

- ❑ Pathways for biosynthesis of steroid hormones are shown in **Fig. 2**.
- ❑ Steroid hormone class synthesized by a given tissue depends upon:
 - Its complement of Peptide Hormone Receptors,
 - Its response to Peptide Hormone Stimulation
 - Its Genetically Expressed Complement of Enzymes

How do steroid hormones exist in plasma?

- ❑ Steroid hormones are hydrophobic, thus they bind to Specific Hormone Binding Glycoproteins in plasma (bound fractions of steroid hormones).
- ❑ Small amount of Steroid hormone usually remains Unbound or Free in plasma.
- ❑ Unbound or “Free” fraction of steroid hormone in plasma is biologically active.
- ❑ Measurement of Free (unbound) steroid hormone status or binding protein levels is important in the diagnosis of patients with certain 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.
- ❑ Specific Nucleotide Sequences in DNA are called Hormone Responsive Elements (HREs).
- ❑ Interaction of steroid hormone-receptor complexes with DNA leads to altered rates of Transcription of associated Genes in Target cells (**Fig 3**)

How does SHBG affect plasma levels of Sex Steroid Hormones?

- ❑ Testosterone and Estradiol circulate in plasma mostly bound Sex Hormone Binding Globulin (SHBG).
- ❑ SHBG has a higher affinity for Testosterone than for Estradiol
- ❑ Testosterone decreases SHBG synthesis by the liver

- ❑ Estradiol stimulates SHBG synthesis by the liver, thus SHBG levels in females is about twice that in males
- ❑ Factors that 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 itself increases SHBG concentration and Testosterone decreases it, this system functions as a Biological Servomechanism.
- ❑ Testosterone and SHGB 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 secretion of Sex Steroids (Fig. 4)?

- ❑ Secretion is regulated by Hypothalamic-Pituitary-Gonadal Axis (HPG-axis)
- ❑ Hypothalamus releases Gonadotrophin-Releasing Hormone (GnRH)
- ❑ GnRH acts on Anterior Pituitary, which then produces the Gonadotrophins:
 - Luteinizing Hormone (LH) and
 - Follicle-Stimulating Hormone (FSH).
- ❑ Gonadotrophins (LH and FSH) act cooperatively on the Ovaries in female and Testes in male to stimulate Sex Hormone secretion and reproductive processes
- ❑ Regulation of secretion of sex steroids is by Negative Feedback on HPG –axis.
- ❑ Inhibin produced by the Gonads feed back inhibits production of FSH.

TAKE NOTE:

- ❑ Concentrations of Estradiol are low before Puberty, but then rise rapidly and fluctuate cyclically during reproductive life.
- ❑ After menopause, plasma Estradiol concentrations fall despite high circulating concentrations of Gonadotrophins.
- ❑ Normal hormonal control of Menstrual Cycle depends on the interaction of hormones secreted from the Hypothalamus, Anterior Pituitary and Ovary.

What are some of the disorders of Female Sex Hormones?

- ❑ Some disorders of female sex hormones include:
 - Sub-fertility, Amenorrhoea, Oligomenorrhoea
- ❑ **Hirsutism:** (increase in body hair with male pattern distribution).
 - In most cases it is genetic in origin and benign
 - Commonest Pathological cause: Polycystic Ovarian Syndrome (PCOS)
 - Serious diseases must be excluded when investigating Hirsute women
- ❑ **Virilism:** Although uncommon it is a sign of serious disease.
 - Testosterone levels are usually elevated
 - There may be evidence of excessive Androgen action such as:
 - Clitoral enlargement, Hair growth in a male pattern, Deepening of the voice and Breast atrophy.
 - Tumors of the ovary or of the adrenal are the likely cause.

Why carry out the Androgen Screen in female?

- ❑ Observation of an elevated Testosterone in a female should always be investigated further
- ❑ A decrease in plasma SHBG concentration is evidence of elevated Androgen, because synthesis of SHBG is depressed by Testosterone.
- ❑ It may not be immediately apparent whether the source of the Testosterone is the Ovary or the Adrenal Cortex.
- ❑ “Androgen Screen” may be used to establish the source of the Testosterone
 - Androgen Screen is carried out by measuring the concentration of other Androgens in plasma, such as:
 - Dehydroepiandrosterone Sulfate (DHAS) and Androstenedione

Schematic representation of the Androgen Screen is shown in (**Fig. 5**):

- ❑ If both DHA Sulfate and Androstenedione are elevated, it suggests that the Adrenal gland is overproducing Androgens.
- ❑ If DHS Sulfate in normal but Androstenedione is elevated, it suggests that the Ovary is the source of the Androgen overproduction.

Endocrine Investigation in the Sub-fertile Female:

- ❑ Investigation of Infertile Female depends on Phase of the Menstrual Cycle.
 - If there is a Regular Menstrual Cycle:
 - Progesterone should be measured in the middle of the Luteal Phase (day 21).
 - If Progesterone is high (> 30 nmol/L):
 - ❑ The patient has ovulated 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.
- ❑ In female who present with Irregular or Absent Menstruation (Oligomenorrhoea or Amenorrhoea) or who are not ovulating, hormone measurements may be diagnostic.
- ❑ A protocol for Investigation is shown in **Fig. 6**.
- ❑ Measurement of Estradiol and Gonadotrophin concentrations may detect:
 - Primary Ovarian Failure or
 - Polycystic Ovarian Syndrome.
- ❑ Measurement of Prolactin, and Androgens may also assist.

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

- ❑ **Primary Ovarian Failure:**
 - ❑ Indicated by elevated Gonadotrophins and Low Estradiol concentration (Post-Menopausal Pattern).
 - ❑ Hormone replacement therapy assists libido and prevents Osteoporosis, but does not restore fertility.
- ❑ **Hypogonadotropic – Hypogonadism:**
 - ❑ Subnormal Gonadotrophins and Estradiol concentrations suggests the presence of a Hypothalamic-Pituitary lesion.
 - ❑ Mechanisms responsible for the Amenorrhoea or Oligomenorrhoea in female with normal Gonadotrophin and Estradiol concentrations remain to be elucidated.

- ❑ **Hyperprolactinaemia:**
 - ❑ Prolactin acts directly on the Mammary Glands to control Lactation.
 - ❑ Gonadal function is impaired by elevated circulation of Prolactin concentrations.
 - ❑ Hyperprolactinaemia is common and can cause Infertility in both sexes.
 - ❑ An early indication in women is Amenorrhoea and Galactorrhoea.

- ❑ Some causes of Hyperprolactinaemia include:
 - ❑ Stress;
 - ❑ Drugs (e.g., Estrogens, Phenothiazines, Alpha-Methyl Dopa, Metoclopramide)
 - ❑ Primary Hypothyroidism (Prolactin is stimulated by the raised TRH);
 - ❑ Pituitary diseases.

- ❑ **Polycystic Ovarian Syndrome:**
 - ❑ Indicated by elevated plasma 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

LET US TAKE A BRIEF LOOK AT PCOS

- ❑ Before considering PCOS, one needs to relate the condition known as Insulin Resistance to Hyperinsulinemia in female.

What is Insulin Resistance?

- ❑ Insulin Resistance is a poorly understood phenomenon in which tissues fail to respond to Insulin.
- ❑ Some of the reasons may include:
 - The number or affinity of Insulin Receptors is reduced in some patients;
 - Others may have normal Insulin binding, but abnormal Post-Receptor responses, such as, problems with activation of Glucose Transport
 - High expression of Tumor Necrosis Factor- α (TNF- α) in the fat cells of Obese Individuals.
 - ❑ The greater the quantity of body fat in a susceptible individual, the greater the resistance of normally Insulin-Sensitive cells to the action of Insulin.

- Insulin Resistance simply means:
 - That the ability of Insulin to dispose of glucose in the Liver, Skeletal Muscle, Adipose tissue and other peripheral tissues is compromised
- When Insulin Resistance, or Reduced Insulin Sensitivity exists, the body attempts to overcome this resistance by secreting more Insulin from the Pancreas.
- This compensatory state of **Hyperinsulinemia** (high insulin levels in the blood) is used as a marker for the Insulin Resistance Syndrome.

What causes Insulin Resistance?

- Exact mechanism for Insulin Resistance is not known.
- Hypothesis proposed include the following:
 - Post-Receptor Defect in Adipose Tissue.
 - Abnormalities in the Regulation of Expression of the Insulin Gene have been shown to be associated with Hyperinsulinemia.
- Despite Insulin Resistance in Adipose Tissue and Skeletal Muscle, the Ovary remains relatively sensitive to Insulin, and
- Both Insulin and Insulin-like Growth Factor-1 have stimulatory effects on the production of **Androgen** by the Ovary.

How does Hyperinsulinemia relate to infertility in female?

- The central, probably heritable, biochemical abnormality of PCOS is Hyperinsulinemia.
- Hyperinsulinemia leads to the condition called **Hyper-Androgenism**:
 - Ovarian Overproduction of Testosterone
 - Adrenal Overproduction of Androgens (DHA Sulfate and Androstenedione)
- These changes, particularly the increased Testosterone in turn affects the Hypothalamus-Pituitary-Ovarian axis (HPA axis in female), leading to abnormal production of LH and FSH.
- Consequences of abnormal LH and FSH production include:
 - Ovarian underproduction of Estrogen, along with
 - Abnormal production of Progesterone,
 - Overproduction of Testosterone, which consequently results in Amenorrhea and Infertility.

What is the biochemical basis for Polycystic Ovarian Syndrome (PCOS)?

- Biochemical basis of PCOS is not clearly understood.
- A number of theories have been suggested, these include the following:
 - Evidence of Autosomal Transmission related to strong Genetic Clustering.
 - A Gene or Series of Genes causes the Ovaries to become Sensitive to Insulin stimulation, causing the Ovary to Overproduce Androgen, while blocking the Maturation of Follicles.
 - The major underlying disorder in PCOS is Insulin Resistance, with the resultant Hyperinsulinemia stimulating excess Ovarian Androgen production.

How does defect in Insulin metabolism promote Hyper-Androgenism in PCOS?

- Exact mechanism whereby defects in Insulin metabolism promote increased Androgen activity in PCOS is not fully understood.
 - A number of Hypotheses have been suggested:
 - Insulin inhibits the biosynthesis of SHBG in the liver, which leads to an increase in plasma level of Free Testosterone.
 - Insulin also inhibits the biosynthesis of Insulin-like Growth Factor-1 (IGF-1) Binding Protein in the liver.
 - Reduction in plasma levels of IGF-1 Binding Protein causes an increase in plasma concentration of circulating Free Insulin-like Growth Factor –1 (IGF-1), which further enhances Ovarian Androgen production.
- In most cases of PCOS the Ovary is said to be the major site of excess Androgen production, but some women with PCOS may have an Adrenal contribution to the increased Androgen production.

Other Steroid Hormones

Adrenal cortex is the source of two important steroid hormones:

- **Cortisol:**
 - Main **Glucocorticoid** in humans.
 - Glucocorticoids are 21-Carbon steroids, promotes Gluconeogenesis
 - Natural or Synthetic steroids with Cortisol-like effects are called Glucocorticoids.
- **Aldosterone:**
 - Main **Mineralocorticoid in humans.**
 - Mineralocorticoids are 21-Carbon steroids, promotes retention of Na⁺ ions and excretion of K⁺ and H⁺ ions, particularly in the kidneys.
 - Natural or Synthetic steroids with Aldosterone-like effects are called Mineralocorticoids.

CORTISOL (Glucocorticoid):

How is Cortisol produced (Fig 2)?

- Cortisol is synthesized from Cholesterol delivered to the Adrenal Gland mainly by Low-Density Lipoprotein (LDL-Cholesterol).
- Number of LDL receptors is increased when the Adrenal Glands are stimulated by **AdrenoCorticoTrophic Hormone (ACTH, also called Corticotrophin)**.
- Adrenal glands are also capable of synthesizing Cortisol from acetate, though this appears to be of minor importance.

How is the secretion of Cortisol regulated? (See Fig. 7)

- Secretion of Cortisol is regulated via the Hypothalamic-Pituitary-Adrenocortical axis (HPA-axis) with classic negative feedback control.
 - Corticotrophin-Releasing Hormone (CRH) is secreted by the Hypothalamus under the influence of Cerebral Factors.
 - Binding of CRH to the Anterior Pituitary induces production of a large molecular weight protein known as **Pro-opiomelanocortin (POMC)**.
 - POMC is then cleaved into various fragments, including **ACTH, Melanocyte-Stimulating Hormones (MSH), beta-Lipotrophins, and beta-Endorphins**.
 - ACTH (Corticotrophin), a 39-Amino-Acid hormone, acts on the Adrenal Cortex stimulating synthesis and secretion of Cortisol.
 - Hypothalamic secretion of CRH and Pituitary secretion of ACTH are modulated by Cortisol in Negative Feedback Loops.
 - In humans only Cortisol exerts the Negative Feedback for ACTH release. None of the other Glucocorticoids block secretion of ACTH.
- In certain cases of enzyme deficiencies (e.g., 21-Hydroxylase):
 - Cortisol is not produced and ACTH secretion is unchecked and Adrenal Hyperplasia occurs leading to the Clinical condition called Congenital Adrenal Hyperplasia (CAH).

Cortisol Binding in Plasma:

- Cortisol secreted by the Adrenal Cortex is transported in plasma mainly bound to **Corticosteroid-Binding Globulin (CBG, also called Transcortin)**.
- It is the unbound fraction (Free fraction) of Cortisol in plasma that is Biologically active
- Plasma level of CBG is affected by several factors:
 - Pregnancy and Estrogen treatment (e.g., oral contraceptives) increases Plasma CBG.
 - Hypo-proteinaemic state (e.g., nephrotic syndrome) causes decrease in plasma CBG
 - Parallel changes occur in plasma concentration of total Cortisol.
 - Cortisol metabolism occurs mainly in the Liver, and products of Cortisol metabolism can be detected in the Urine as **17-Hydroxycorticosteroids (17-OHCS)**.

ALDOSTERONE (Mineralocorticoid):**Primary Regulators of Aldosterone secretion is:**

- Renin-Angiotensin system – it stimulates Aldosterone production,
- Increased plasma K^+ ions stimulates Aldosterone production,
- Decreased plasma K^+ ions inhibit Aldosterone production.

Renin – Angiotensin – Aldosterone system: (Fig. 8)**Some effects of Aldosterone:**

- Primary role is in Na^+ metabolism; Primary target is Distal Tubules.
- Actions of Aldosterone cause the Kidneys, Gut, and Salivary/Sweat Glands to affect Electrolyte Balance.
- Aldosterone stimulates re-absorption of Na^+ ions and secretion of K^+ and H^+ ions.
- Excess Aldosterone causes Hyponatremia, Hypokalemia, Alkalosis
- Aldosterone deficiency causes Hyponatremia, Hyperkalemia, Acidosis
- Effect on Na^+ and K^+ ions depends on intake of these Cations:
 - Increased Na^+ intake = Increased K^+ secretion)