SN EDUCATION PRESENTS

Male Hormone Bloodwork Panel

From Fertility to Masculinity

Dr Dean St. Mart

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Introduction

- Dr Dean St. Mart PhD.

- Formulator for Supplement Needs (www.supplementneeds.co.uk)
- Pharmacologist for Atlas Laboratories (AlphaGenix)
- Background in Drug Design and Pharmacology
- 1st Class Honours Degree in Chemistry and Pharmaceutical Chemistry
- PhD in Synthetic Organic Chemistry.

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Overview

- Introduction to Male Hypothalamic Pituitary (Gonadal) Axis (HPTA).
- Introduction to Male Hormone panel of bloodwork.
- **Basic understanding of:**
  - Testosterone
  - Luteinizing Hormone (LH)
  - Follicle Stimulating Hormone (FSH)
  - SHBG
  - Estradiol
  - Prolactin

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Why Investigate the Male HPTA

- What investigate the Male HPTA?
  - Male Hypogonadism – Late Onset, Anabolic-Androgenic Steroid Induced.
  - Fertility issues – Low sperm production.

- **<u>Symptoms of Hypogonadism:</u>**
  - Sexual dysfunction (reduced libido, erectile dysfunction, diminished penile sensation, difficulty attaining orgasm, reduced ejaculate with orgasm.
  - Reduced energy, vitality, or stamina; poor sleep.
  - Depressed mood or diminished sense of well being.
  - Increased irritability.
  - Difficuly concentrating and other cognitive problems.
  - Hot flushes (with acute onset in some cases.

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Hypogonadism

- **Signs of Hypogonadism:**
  - Anemia.
  - Muscle wasting (sarcopenia).
  - Reduced Bone Mass or Bone Mineral Density (BMD).
  - Absence or Regression of Secondary Sex Characteristics.
  - Abdominal Visceral Adiposity (i.e. belly fat).
  - Oligospermia or Azoospermia (Low or Zero Sperm).

- Hypogonadism is characterized by **low serum total testosterone levels** (<300 ng/dL or 10.5 nmol) **alongside 2 or more symptoms or signs as described previously.**

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Primary Hypogonadism

- Primary (Hypergonadotropic) Hypogonadism refers to Testicular disorders and is characterised by Low Serum Total Testosterone despite high levels of Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH).
- Pituitary is functioning normally – but Leydig cells are unresponsive.

- **<u>Causes of Primary Hypogonadism:</u>**
  - Genetic conditions (Klinefelter syndrome, gonadal dysgenesis).
  - Anatomic defects.
  - Infection.
  - Tumour or Varicocele.
  - Injury.
  - Iatrogenic causes (caused by surgery or certain medications like AAS).
  - Alcohol abuse.

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Secondary Hypogonadism

- Secondary (Hypogonadotropic) Hypogonadism is used to refer to a deficient release of Gonadotropin-Releasing Hormone (GnRH) and is characterised by low-normal or low levels of FSH, LH and Total Testosterone.
- Testicles are functioning normally – but there is a signalling issue from Hypothalamus to the Pituitary.

- **<u>Causes of Secondary Hypogonadism:</u>**
  - Hyperprolactinemia (often secondary to pituitary adenoma or tumour).
  - GnRH deficiency with anosmia (Kallmann syndrome; delayed puberty with loss of smell).
  - Hypothalamic lesions or disorders (Head injury).
  - Pituitary lesions or disorders.
  - AAS usage.
  - Malnutrition.

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Late Onset Hypogonadism

- Late-onset hypogonadism (LOH) is characterized by low testosterone (T) levels and clinical symptoms.
- Sexual symptoms and fatigue are the earliest and most common presentations.
- Other symptoms include depression, sleep alterations, poor concentration, and metabolic disorders.
- Total testosterone (TT) and free testosterone (FT) concentrations decrease with increasing age in men.
- LOH is the result of a gradual drop in T; a steady decline in T levels of **about 1% per year**.
- However, decreases in T concentrations with age are gradual and vary between individuals,
  - Higher rates of decline in men with adiposity and comorbid diseases.
- LOH has the combined features of both primary and secondary hypogonadism.

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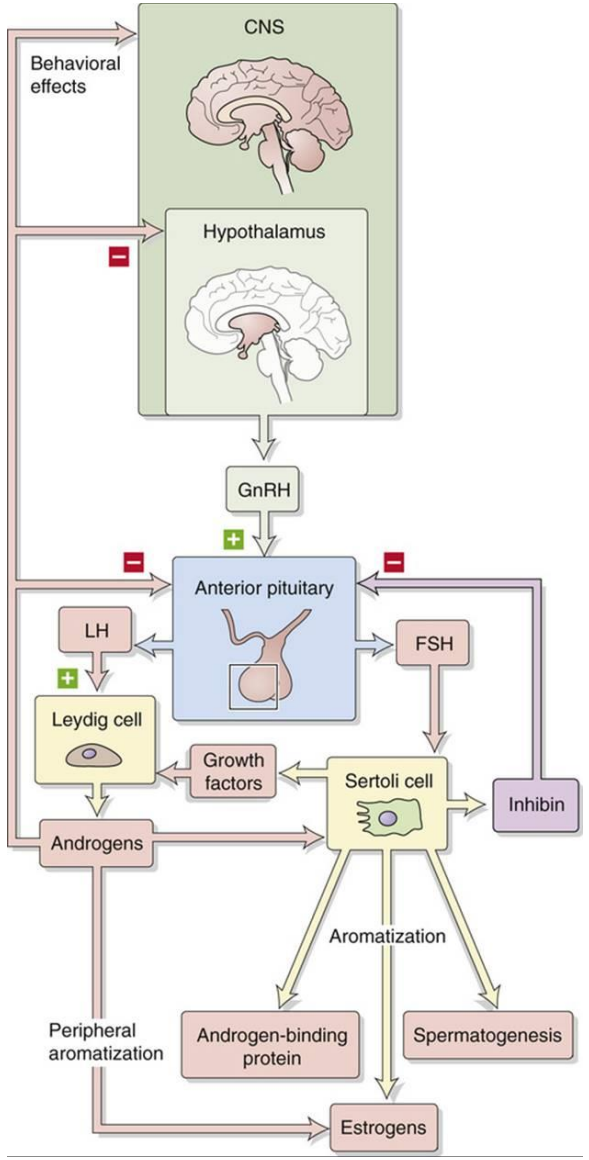
Male HPTA

- Hypothalamic Pituitary (Gonadal) Axis.
  - Hypothalamus, Pituitary gland (both anterior and posterior) and the Testes.
- The Hypothalamus secretes gonadotrophin releasing hormone (GnRH).
- GnRH stimulates Anterior pituitary gland to secrete two hormones
  - Follicle-stimulating hormone (FSH) and Luteinizing hormone (LH).
  - As well as adrenocorticotropin, growth hormone (GH), prolactin, and thyroid-stimulating hormone (TSH).
- LH acts on Leydig cells to produce Testosterone.
- FSH stimulates the Sertoli and Sertoli-Germ cells to produce sperm.
- **Negative feedback system**
  - Testosterone produced by Leydig cells / Estradiol can also negatively feedback.
  - Inhibin B produced by Sertoli cells.



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Male Testosterone Synthesis

- **GnRH** made in the **Hypothalamus** binds to a membrane receptor on **pituitary** gonadotrophs.
- Stimulates the biosynthesis and **secretion of LH** from Pituitary.
- LH then travels and binds **LH Receptors (LHR)** on the surface of **Leydig cells**.

- cAMP is then produced by the Leydig cells; which, through **protein kinase A (PKA),** is of critical importance in steroid formation.
- Acute stimulation of PKA allows the **translocation of cholesterol** to the **outer mitochondrial membrane**.

- **Cholesterol** is transferred from the **outer to the inner mitochondrial membrane** where it is converted to **pregnenolone** by **CYP11A1**.
- **Chronic stimulation of Leydig cells by LH** and cAMP is essential for regulating the expression levels of the proteins and enzymes involved in steroidogenesis.

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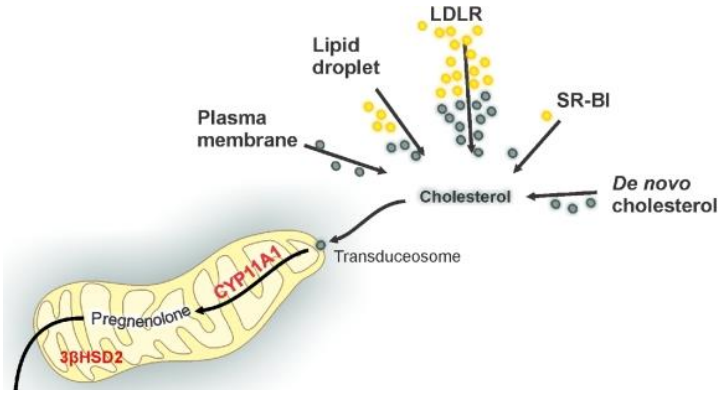
Testosterone Synthesis

- 2 classes of enzymes involved in testosterone biosynthesis
  - Cytochrome P450 (CYPs) proteins of the **mitochondria**.
  - Hydroxysteroid dehydrogenases (HSDs) of the **smooth endoplasmic reticulum.**

- Cholesterol is imported into mitochondria through a large protein complex, the **transduceosome**.
- Cholesterol reaches **CYP11A1 (cytochrome P450 cholesterol side chain cleavage) in the inner mitochondrial membrane** where it is metabolized to **pregnenolone**.



- **Pregnenolone** is converted by **3β-HSD** located at the mitochondria and endoplasmic reticulum to Progesterone and Androstenedione.

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Enzymes - Testosterone Synthesis

- **<u>Four steroidogenic enzymes are involved in testosterone biosynthesis from cholesterol:</u>**

1. Cytochrome P450 Family 11 Subfamily A1 (**CYP11A1**),
2. 3β-hydroxysteroid dehydrogenase/Δ5 → Δ4 isomerase (**3β-HSD**)
3. **CYP17A1**
4. **17β-HSD3**

- **CYP11A1** is located in **mitochondria** at the **inner mitochondrial membrane**.

- **3β-HSD** is present in mitochondria but predominantly in **smooth endoplasmic reticulum**.

- **CYP17A1 and 17β-HSD3** are found only in **smooth endoplasmic reticulum**.

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Testosterone Synthesis

- **CYP11A1 (aka P450scc) (inside your mitochondria) catalyzes** the **conversion of cholesterol to pregnenolone**.

- **CYP11A1 determines the synthetic capacity of the Leydig cells.**

- The **proximity of 3β-HSD to CYP17A1 and 17β-HSD3** in the **smooth endoplasmic reticulum** makes it possible for each intermediate involved in testosterone synthesis to move easily and directly to the next enzyme.

- Adrenal glands produce testosterone also in males – **less than 5% total production.**

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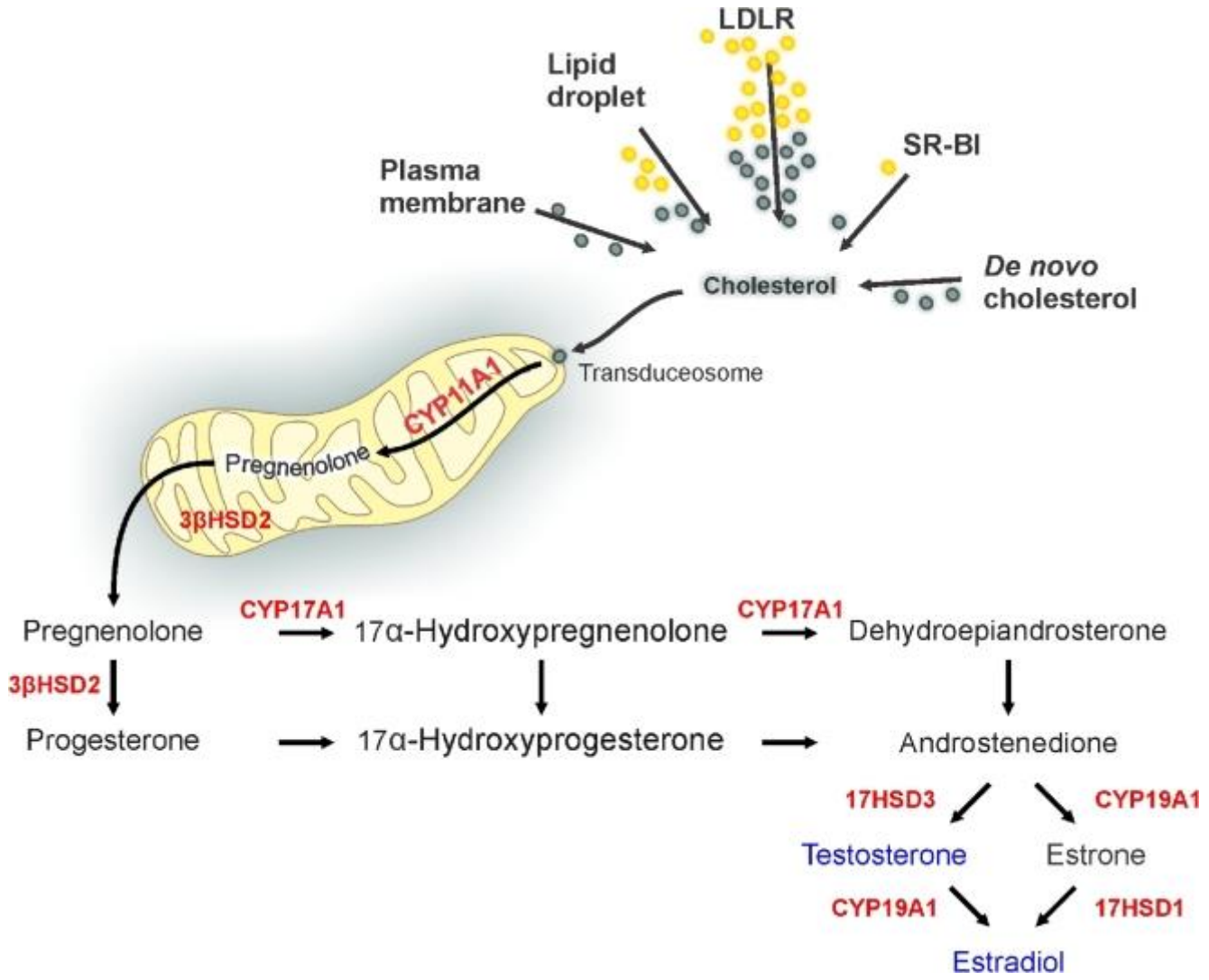
Cholesterol Synthesis of Testosterone



Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biol Reprod. 2018 Jul 1;99(1):101-111. doi: 10.1093/biolre/ioy059. PMID: 29566165; PMCID: PMC6044347.

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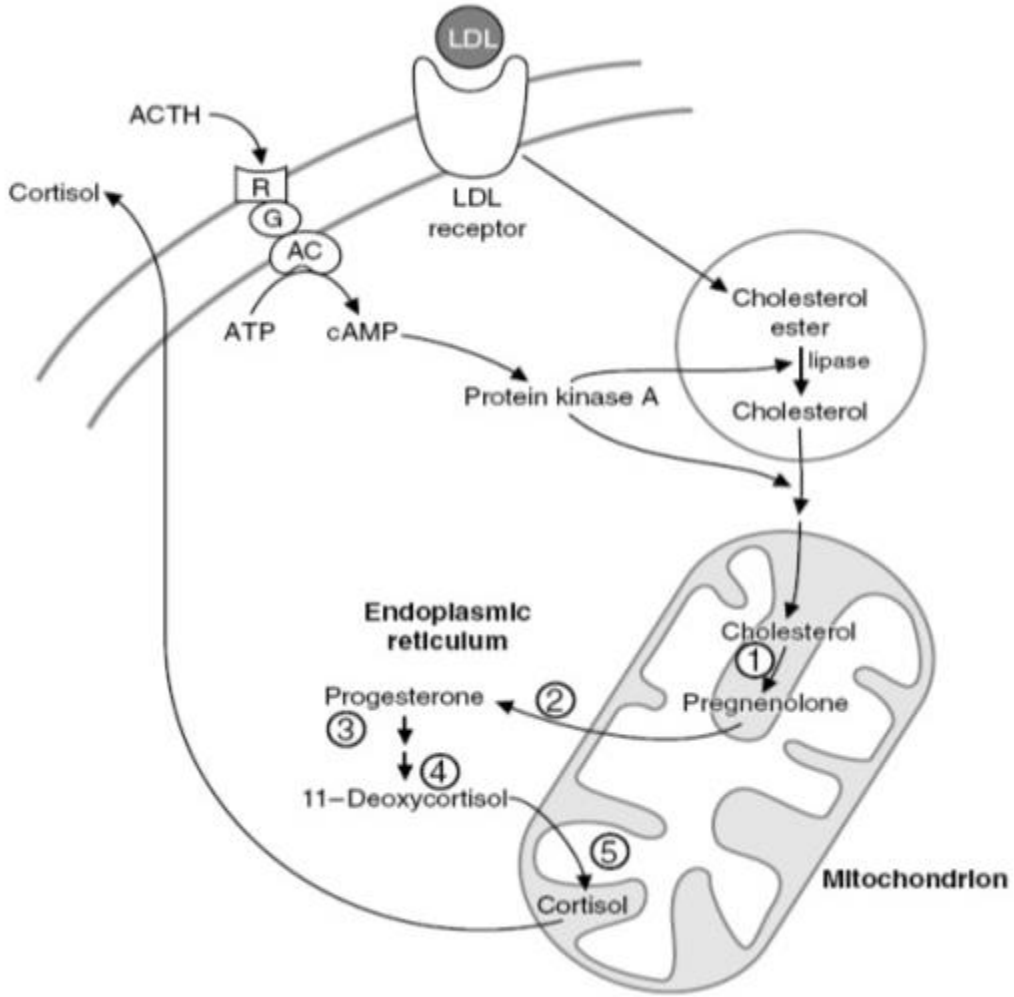
Adrenal Formation of Testosterone



Less than 5% total production

Held PK, Bird IM, Heather NL. Newborn Screening for Congenital Adrenal Hyperplasia: Review of Factors Affecting Screening Accuracy. Int J Neonatal Screen. 2020 Aug 23;6(3):67. doi: 10.3390/ijns6030067. PMID: 33117906; PMCID: PMC7569755.

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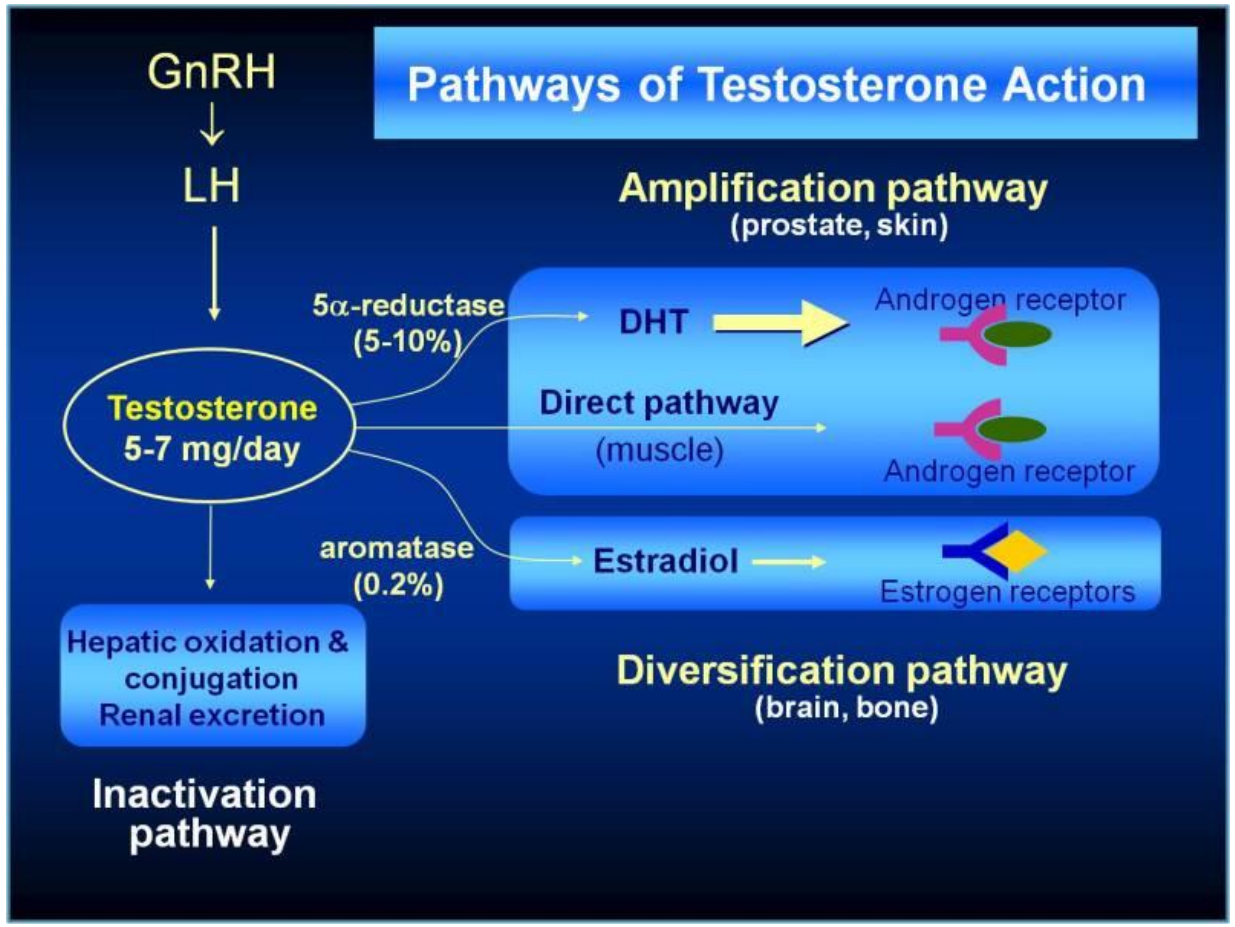
Testosterone Production



Handelsman DJ. Androgen Physiology, Pharmacology, Use and Misuse. [Updated 2020 Oct 5]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279000/

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Factors of Low Testosterone Production

- Negative feedback of Estradiol on Hypothalamic production of GnRH.
  - Androgen Therapy / AAS use.
  - Endogenous CYP19A aromatase.
  - Xenoestrogens from environment.
- Brain injury / Impact.
- Physical blockage / Damage to Testicle -> Varicocele, Injury, Fibrosis.
- Lack of nutrition -> cholesterol precursor, mitochondria nutrients.
- Mitochondrial oxidative stress -> lack of Magnesium, Copper, Glutathione etc.
  - CYP11A1 located in mitochondria could be being downregulated.
  - Pathogens, oxidative stress.

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Testosterone

- **Serum Total Testosterone** measurement of **both bound and un-bound** (free) testosterone.
- Testosterone and dihydrotestosterone (DHT) circulate in plasma unbound (free approximately 2 - 3%) bound to specific plasma proteins (sex hormone-binding globulin SHBG) and weakly bound to nonspecific proteins such as albumin.

- The SHBG-bound fraction is **biologically inactive** because of the high binding affinity of SHBG.
- **Free testosterone** measures the free fraction ready to bind to the Androgen Receptor (AR).
- **Bioavailable testosterone** includes free plus weakly bound to albumin.

- **Reference Range:**
  - Total Testosterone 280 to 800 ng/dL **or** 9.7 to 27.7 nmol/L
  - "Optimal" 600 to 800 ng/dL **or** 20.8 to 27.7 nmol/L
  - Free Testosterone 0.22 to 0.62 nmol/L

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Sex Hormone Binding Globulin

- Nearly **60% of testosterone is bound** to high-affinity protein called sex hormone-binding globulin (SHBG).
- **Albumin** accounts for binding to the **remaining nearly 38%** of the hormone.
- A **small fraction (nearly 2% males**, 1% in females) represents the **physiologically active free form** that mediates the biological action of the hormone at the target tissues in both sexes.

- SHBG is produced by the **liver** and its production is controlled by certain hormonal as well as physiological and pathological factors.
- SHBG **binds both** testosterone and estrogens with **higher affinity of binding to testosterone** than to estrogens (**affinity to testosterone is 5 times greater** than to estrogens).
- In addition to binding steroids, SHBG binds to receptor sites on plasma membranes.

- An **increase in SHBG** concentration **decreases the bioavailability of testosterone** and thus decreases the free hormone levels **without noticeable change in total hormone levels**.
- Therefore, an inverse relationship between SHBG concentration and free testosterone status.

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Sex Hormone Binding Globulin

Decrease
Androgens
Obesity
Insulin resistance
Metabolic syndrome
Type 2 diabetes mellitus
Gestational diabetes mellitus
Polycystic ovary syndrome
Non-alcoholic fatty liver disease
Acromegaly
Cushing's syndrome
Congenital adrenal hyperplasia
Hyperprolactinemia
Tumor necrosis factor alpha
Interleukin-1 beta

Increase
Estrogens
Pregnancy (Estrogens)
Weight loss
Alcoholic cirrhosis
Hepatitis-B and hepatitis-C infection
Hemochromatosis
Hyperthyroidism
Growth hormone deficiency
Acute intermittent porphyria
First generation anticonvulsants

Aydın B, Winters SJ. Sex Hormone-Binding Globulin in Children and Adolescents. Journal of Clinical Research in Pediatric Endocrinology. 2016 Mar;8(1):1-12..

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LH and FSH

- The pituitary cells that synthesize LH are called **gonadotropes.**
  - The majority of the gonadotropes synthesize both LH and FSH, whereas the two other categories are composed of cells that exclusively synthesize either FSH or LH.
- The gonadotropes are stimulated by the **hypothalamic polypeptide, GnRH,** whose secretion is **pulsatile**. **GnRH is released by neurons** that terminate on the blood vessels running along the pituitary stalk.
- GnRH release occurs under the stimulation of a peptide product named **kisspeptin.**
- Kisspeptin is produced mainly in the hypothalamus and regulated upon a feedback mechanism played by steroid hormones and acts through a **G protein-coupled receptor (GPCR).**
- Kisspeptin supports the pulses of GnRH to the gonadotropes in the pituitary gland.
- The frequency of the GnRH pulses results in concurrent, selective pulsatile secretion of LH or FSH.
- GnRH binds to a GPCR -> activating the Gq/11 proteins -> stimulating phospholipase C.

- Phospholipase C generates 2 intracellular messenger **inositol 1,4,5-triphosphate** and **diacylglycerol.** These intracellular messengers activate **protein kinase C (PKC)** and increase intracellular calcium.
- PKC and calcium regulate the ***expression of the LHB gene.***
  Secretion of LH is regulated by the **increase in intracellular calcium**.

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LH and FSH

- LH and FSH are made from similar genes and thus have similar properties.
- They are both glycoproteins made up of an alpha and beta subunit.

- The **alpha subunit** is the **same** between the two hormones.
- The **beta subunit of each is different** and gives each hormone its **biological specificity.**

- Patients with hypogonadotropic hypogonadism usually have an issue with GnRH signaling.
  - Decrease in FSH and LH secretion.

- Decrease in FSH and LH contributes to both decreased androgen levels as well as reduced spermatogenesis.
- Giving these patients pulsatile GnRH or LH (or hCG) and FSH can help increase spermatogenesis and thus increase the sperm concentration in the ejaculate.

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LH and FSH

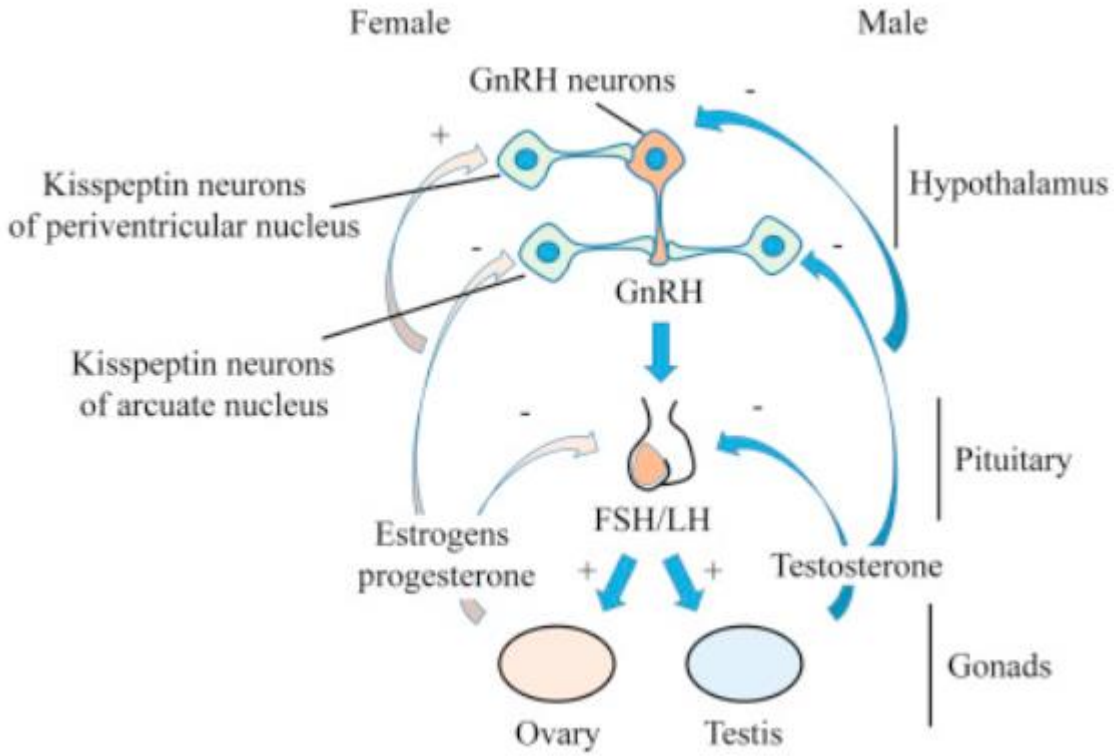

Fig. 1. Feedback controls of gonadotropins' production. In the hypothalamus, kisspeptin neurons stimulate GnRH secretion under the control of steroid feedback. GnRH release is pulsatile and induces the release of LH and FSH by the anterior pituitary. In the gonads, gonadotropins promote gamete formation and the production of steroid hormones, which, in turn, regulate GnRH release by a feedback mechanism. In females, steroid hormones estrogens and progesterone exert a positive feedback on kisspeptin neurons of the periventricular nucleus, inducing the preovulatory surge of GnRH and LH. On the contrary, estrogens and progesterone inhibit kisspeptin production in the arcuate nucleus. In the male, testosterone exerts a negative feedback on kisspeptin neurons of the arcuate nucleus, as well as on GnRH neurons and pituitary, inhibiting GnRH and gonadotropins release.

Livio Casarini, Daniele Santi, Gary R. Marshall, Manuela Simoni,
Luteinizing Hormone (LH); Encyclopedia of Endocrine Diseases (Second Edition), Academic Press, 2018, Pages 142-148

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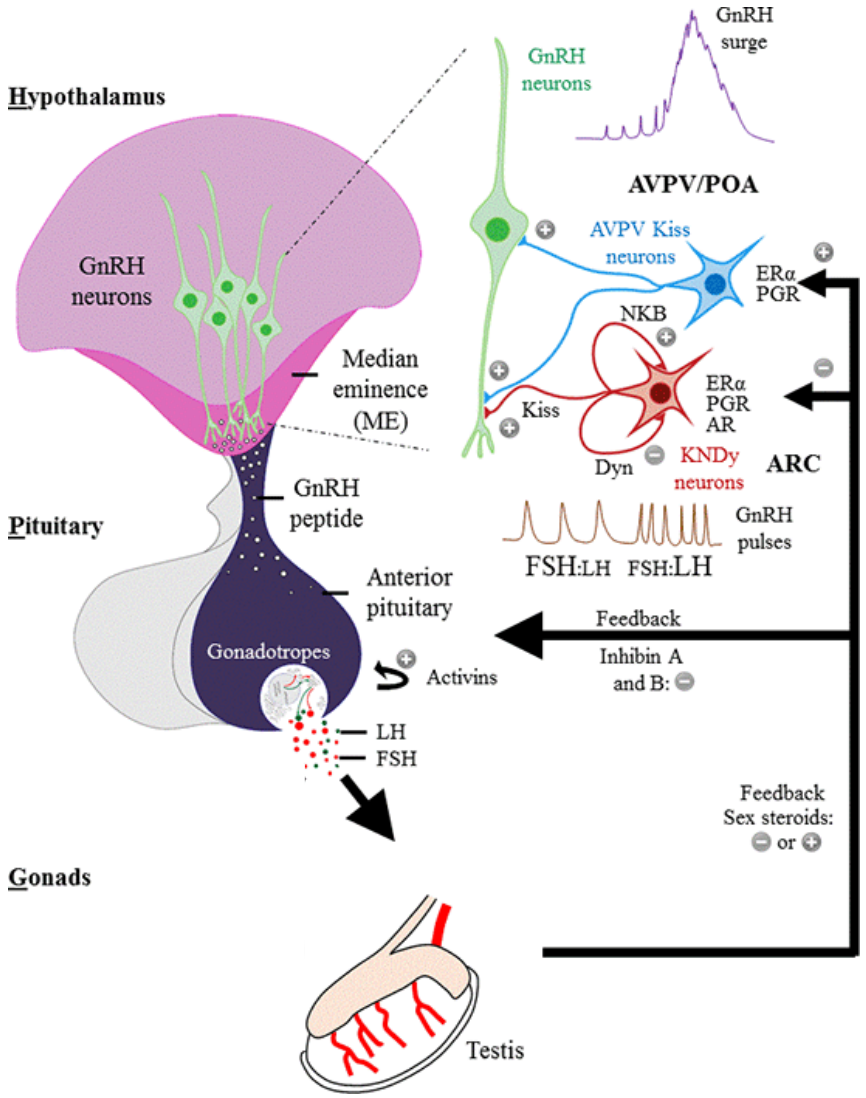
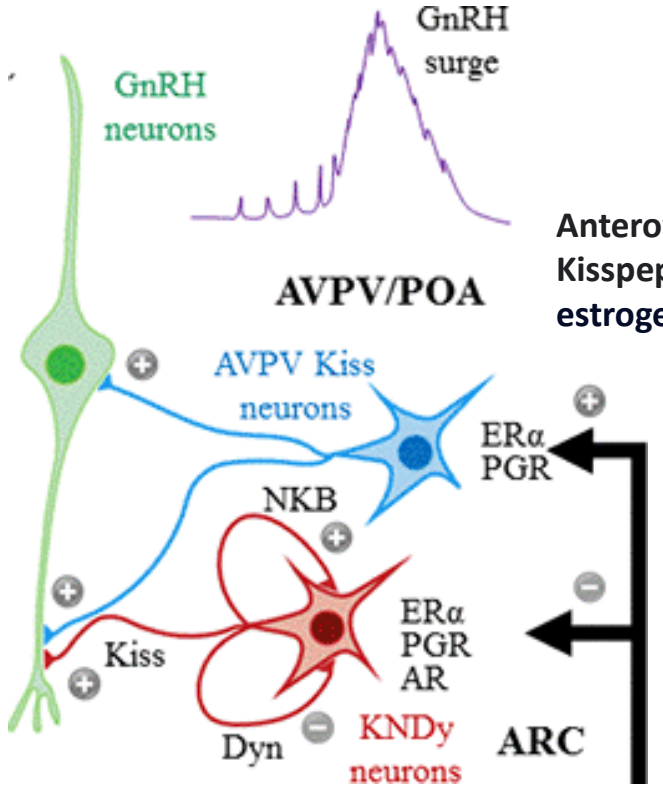
GnRH Regulation





Anteroventral Periventricular Nucleus Kisspeptin neurons express estrogen receptor α,

Bernard, D., Li, Y., Toufaily, C., & Schang, G. (2019, May 23). Regulation of Gonadotropins. *Oxford Research Encyclopedia of Neuroscience*.https://doi.org/10.1093/acrefore/9780190264086.013.58

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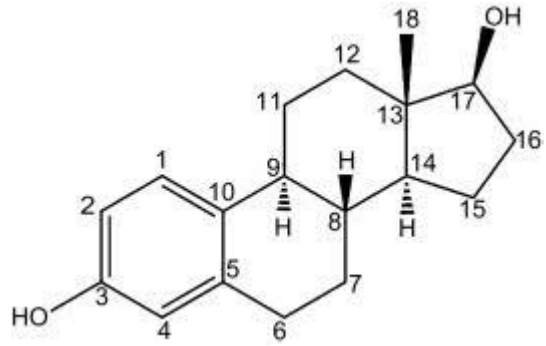
Estradiol



- There are two main types of estrogen in men:
  - Estrone (E1) and Estradiol (E2) – small conversion to Estriol (E3).

- Estradiol in men is essential for modulating libido, erectile function, and spermatogenesis.
- Estrogen receptors, as well as **Aromatase**, the enzyme that converts testosterone to estrogen, are abundant in brain, penis, and testis, organs important for sexual function.
- In the brain, estradiol synthesis is increased in areas related to sexual arousal.
  - In the penis, estrogen receptors are found throughout the corpus cavernosum with high concentration around neurovascular bundles.
- Low testosterone and elevated estrogen increase the incidence of erectile dysfunction independently of one another.
- In the testes, spermatogenesis is modulated at every level by estrogen.
- Regulation of testicular cells by estradiol shows both an inhibitory and a stimulatory influence, indicating an intricate symphony of dose-dependent and temporally sensitive modulation.

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Estradiol

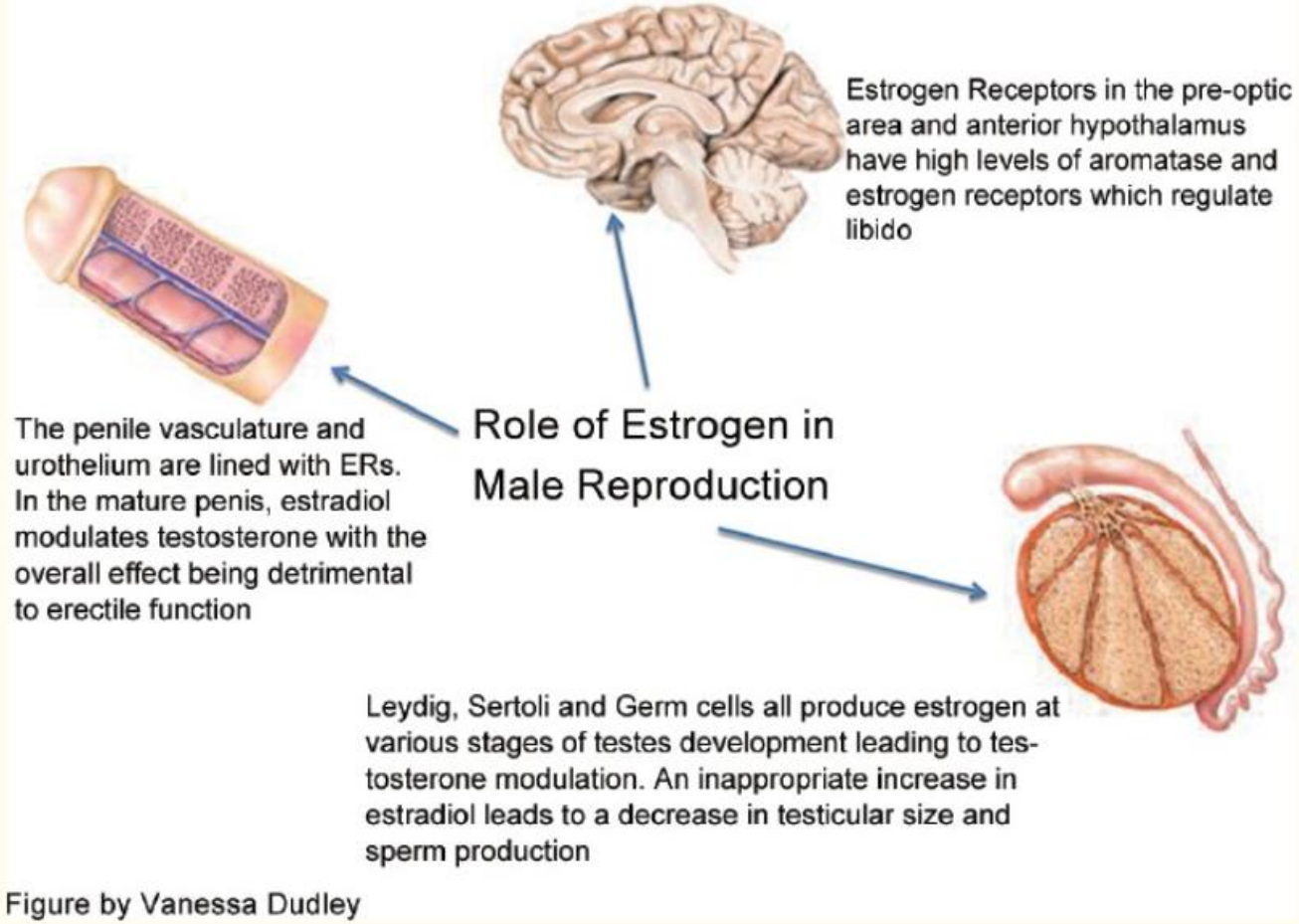

Figure by Vanessa Dudley

Figure 1

The role of estrogen in male reproduction.

Schulster, Michael[1]; Bernie, Aaron M[1]; Ramasamy, Ranjith[2,].
The role of estradiol in male reproductive function. Asian Journal of Andrology 18(3):p 435-440, May–Jun 2016. | DOI: 10.4103/1008-682X.173932

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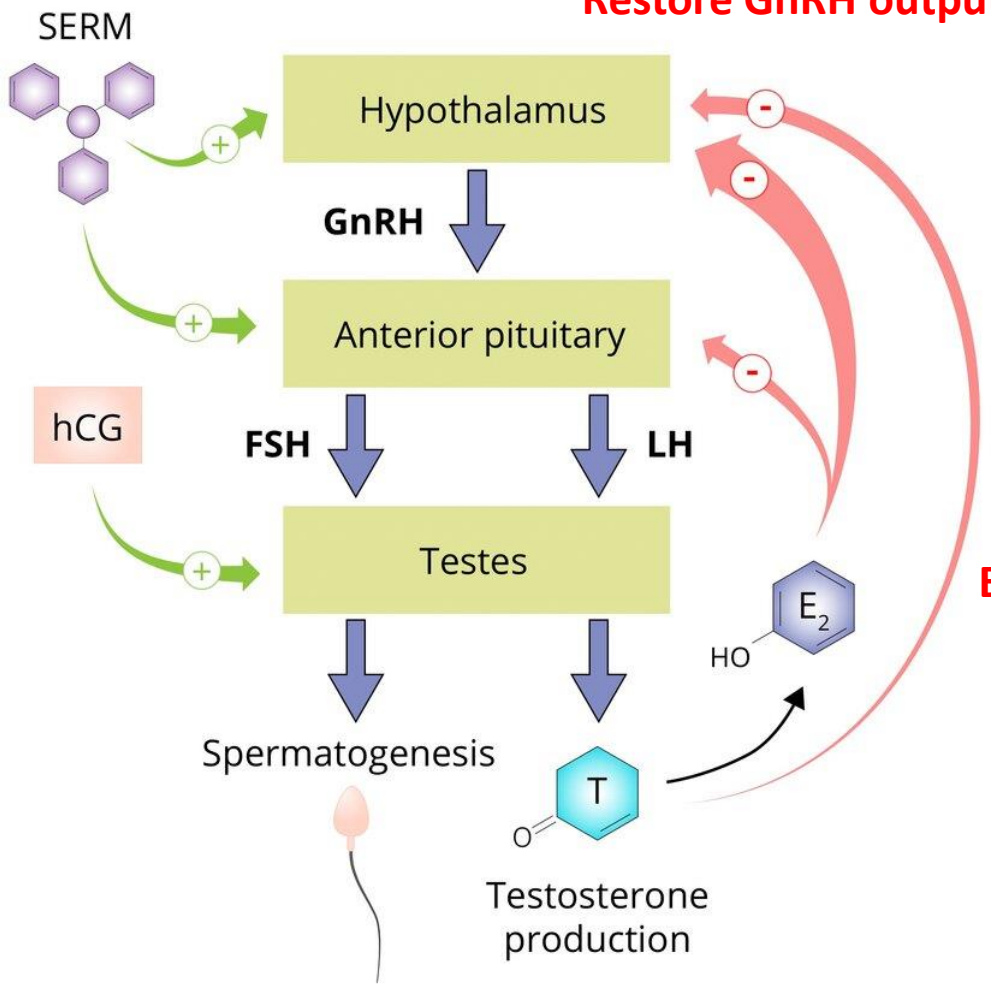
Low Testosterone Production

Restore GnRH output from Hypothalamus.



Estradiol main negative feedback……

Bond P, Smit DL, de Ronde W. Anabolic-androgenic steroids: How do they work and what are the risks? Front Endocrinol (Lausanne). 2022 Dec 19;13:1059473. doi: 10.3389/fendo.2022.1059473. PMID: 36644692; PMCID: PMC9837614.

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Prolactin

- Prolactin synthesized in the adenohypophyseal (pituitary) lactotrophs, has **no known target organ or defined role** in male reproduction.

- Although the functional significance of prolactin to male reproduction has not been unequivocally established, the hormone has been associated primarily with male infertility.

- Acute hyperprolactinemia is known to suppress testosterone synthesis and male fertility through **prolactin-induced hypersecretion of adrenal corticoids** or by inhibiting the secretion of GnRH through prolactin receptors on hypothalamic dopaminergic neurons

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Prolactin

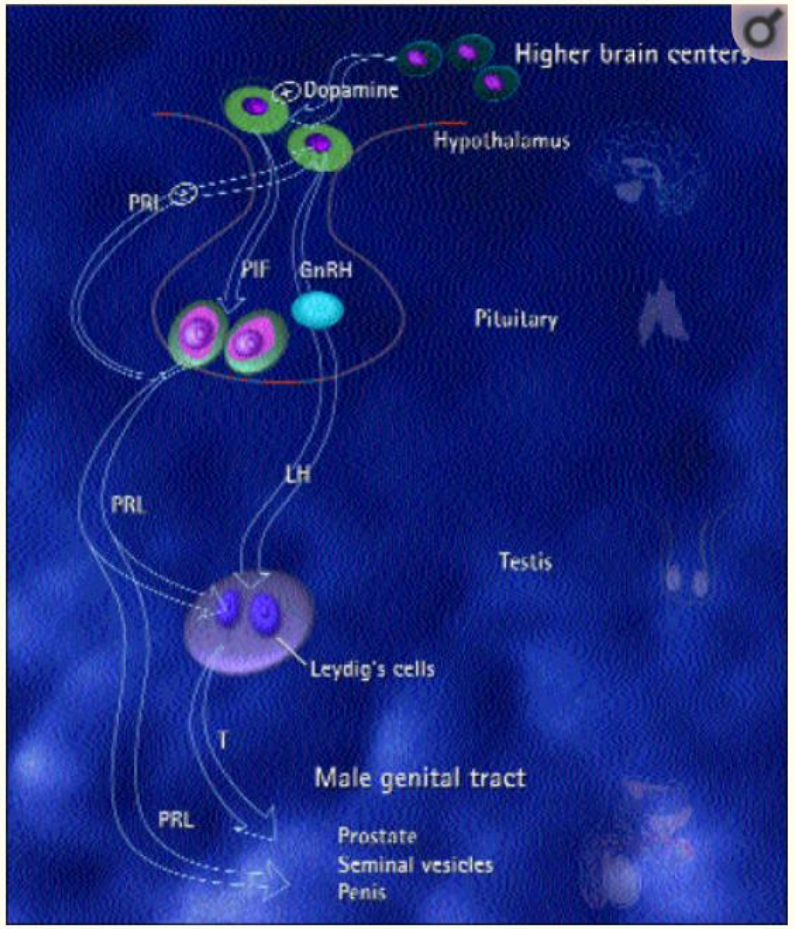

Figure 2

Dopamine from higher brain centers stimulates release of prolactin inhibitory factor (PIF), and prolactin (PRL) from the pituitary inhibits gonadotropin-releasing hormone (GnRH) secretion. Bromocriptine, a dopamine agonist, works to further increase production of PIF to decrease PRL production. LH, Luteinizing hormone.

Zeitlin SI, Rajfer J. Hyperprolactinemia and erectile dysfunction. *Rev Urol*. 2000;2(1):39-42.

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Prolactin

- **What is hyperprolactinemia?**
  - Higher-than-normal levels of the hormone **prolactin** in the blood.
- **What causes hyperprolactinemia?**
  - Growth or tumour on the pituitary gland called a prolactinoma.
  - Certain prescription medicines can also increase prolactin levels.
    - High blood pressure (such as calcium-channel blockers and methyldopa)
    - Tricyclic and SSRI antidepressants
    - Estradiol (Elevated Estradiol)
  - **Hypothyroidism** or underactive thyroid— not produce enough thyroid hormone
  - Low brain dopamine levels
- **Signs and symptoms of hyperprolactinemia?**
  - **Erectile dysfunction** —trouble getting or keeping an erection.
  - Breast enlargement (**Gynecomastia)** and Breast milk production (very rare).
  - Decreased muscle mass and body hair.
  - Headaches

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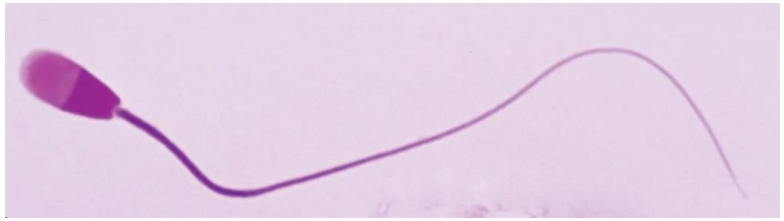
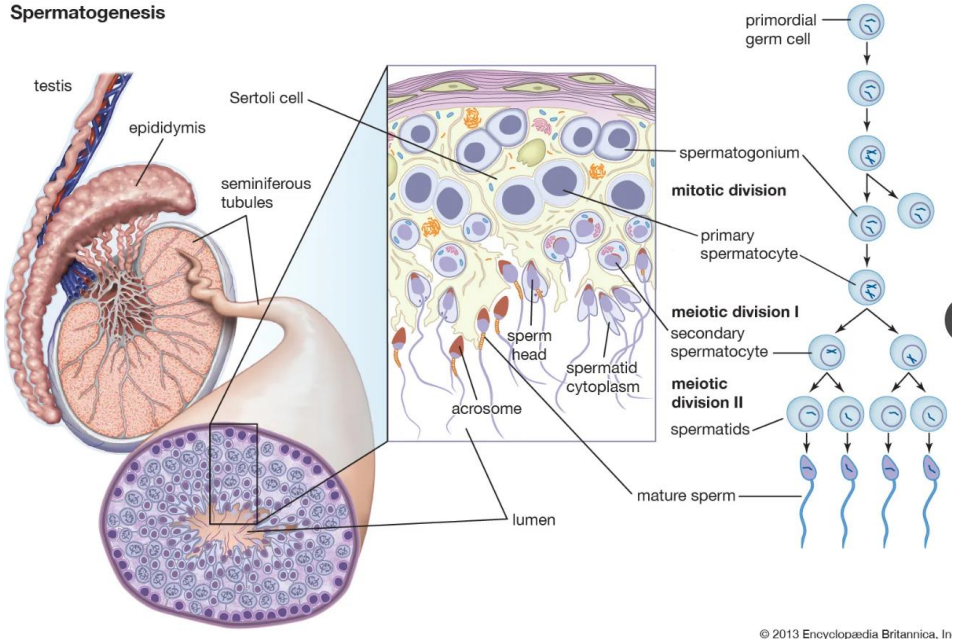
Spermatogenesis

- **Spermatogenesis** is the process by which sperm cell production occurs.
- Sperm production takes place inside the **seminiferous tubules**, which is a convoluted cluster of tubes located inside the testes.
- **Testosterone production** occurs in cells surrounding the seminiferous tubules, called **Leydig cells**.
- After being formed, sperm cells travel outside of the tubules into the **epididymis**, where they **mature and prepare for ejaculation**.





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Spermatogenesis

- For humans, the entire process of spermatogenesis is estimated as taking 74 days but can be as high as approximately 120 days (Between 10-20 weeks approx.).
- Each day, some 100 million sperm are made in each human testicle.
- Each ejaculation releases approx. 200 million sperm.
- Unused sperm are either resorbed or passed out of the body in urine.
- During his lifetime, a human male can produce 10^{12} to 10^{13} sperm.

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Medical Treatment for Infertility

Substance	Administration	Dosage and frequency	Current availability
GnRH	Subcutaneous infusion pump	25-200 ng/kg per pulse every 2 hours	Only in specialty centers or part of clinical trials
Human chorionic-gonadotropin (hCG)	Subcutaneous/intramuscular	1,500-3,000 IU 2 times/week	Available, FDA approved for treatment of infertility due to gonadotropin deficiency
Human menopausal gonadotropin (hMG)	Subcutaneous/intramuscular	75 IU 2-3 times/week	Available, FDA approved for treatment of infertility due to gonadotropin deficiency
Highly purified or recombinant human follicle-stimulating hormone (rhFSH)	Subcutaneous/intramuscular	100-150 IU 2-3 times/week	Available, FDA approved for treatment of infertility due to gonadotropin deficiency
Dopamine agonist	Oral	Cabergoline (0.5-1 mg twice weekly), bromocriptine (2.5-5.0 mg twice weekly)	FDA approval for treatment of hyperprolactinaemia
Aromatase inhibitors	Oral	Anastrozole 1 mg/day	Off label use
		Letrozole 2.5 mg/day	Off label use
		Testolactone	Not available in the USA
Selective estrogen receptor modulators (SERMs)	Oral	Clomiphene citrate titrate to 50 mg/day	Off label use
		Tamoxifen 20 mg/day, toremifene 60 mg/day, raloxifene 60 mg/day	Off label use

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Thank you for listening