EFFECT OF *JATROPHA TANJORENSIS* LEAF EXTRACT ON SOME FERTILITY HORMONES AND TESTICULAR FUNCTION IN ADULT MALE WISTAR RATS

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CERTIFICATION

This is to certify this project on "**THE EFFECT OF JATROPHA TANJORENSIS LEAVES ON FERTILITY HORMONES AND TESTICULAR FUNCTION IN ADULT MALE WISTAR RATS**" was carried out by **ASEMOTA EMILY**, with the matriculation number **BMS2101609** in partial fulfillment of the requirement of the award of Bachelor of Science Degree (B.Sc) in the Department of Physiology, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

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DEDICATION

First and foremost, I dedicate this work to God Almighty, my Creator, my unwavering pillar of strength, and the ultimate source of my inspiration, wisdom, knowledge, and understanding. It is by His grace and through His power alone that I have been able to navigate this journey. On His wings, I have soared.

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ABSTRACT

Jatropha tanjorensis (JT), commonly known as "hospital too far," is widely consumed as a vegetable and used in traditional medicine to treat ailments like anemia and diabetes. However, scientific evidence regarding its impact on male reproductive health is limited and conflicting, with some reports suggesting potential antifertility effects. This study aimed to investigate the effects of *Jatropha tanjorensis* methanol leaf extract on fertility hormones and the testes in male Wistar rats. Fourteen (14) male Wistar rats were divided into a control group (n=7), which received distilled water, and a test group (n=7), which was administered 500mg/kg of the methanol leaf extract of *J. tanjorensis* orally for four (4) weeks. At the end of the experiment, serum levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), testosterone, and prolactin were analyzed. Testicular weights were also measured. Data were analyzed using ANOVA, with $p < 0.05$ considered significant. Compared to the control group, administration of the *J. tanjorensis* extract resulted in a significant decrease in serum testosterone levels (2.350 ± 0.132 U/L vs. 3.225 ± 0.312 U/L, p=0.0416). Concurrently, the extract caused a significant increase in both serum LH (9.450 ± 0.595 µg/l vs. 5.975 ± 1.217 µg/l, p=0.0426) and FSH (15.65 ± 0.307 µg/l vs. 9.075 ± 2.04 µg/l, p=0.0190). There were no significant differences in prolactin levels or mean testicular weight between the groups. The findings demonstrate that the methanol extract of *Jatropha tanjorensis* disrupts the reproductive hormonal profile in male Wistar rats. The combination of low testosterone with high levels of LH and FSH is indicative of hypergonadotropic hypogonadism, suggesting a direct inhibitory effect on testicular steroidogenesis (primary hypogonadism). These results indicate that the consumption of *J. tanjorensis* leaves may pose a potential risk to male reproductive health by impairing testicular function.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

Jatropha tanjorensis plant belongs to the Euphorbiaceae family, commonly known as "hospital too far," catholic vegetable, "Iyana Ipaja." It is widely consumed in the southwestern region of Nigeria as soup. It is a perennial herb, which shows intermediacy in phenotypic characters between Jatropha curcas and Jatropha gossypifolia (Oyewole, 2011). The plant leaves are widely used in the southwestern region of Nigeria for making vegetable soup. The leaves are also used traditionally in the treatment of anemia (as a hematinic agent), diabetes and cardiovascular diseases, which are all diseases caused by production of free radicals (Oseghale *et al*., 2025). The leaf extract has hypoglycaemic properties and is taken as medicine for the treatment of diabetic symptoms (Anhwange *et al*., 2019). It has been reported that Jatropha leaves are rich in beta blockers, anti-cancer agents, anti-anaemic, anti-microbial activities, anti-plasmodial and anti-oxidant effects against oxidative stress induced by malaria parasite (Omoregie, and Sisodia, 2011; Ahaotu *et al*., 2013). Fertility hormones and testes in male Wistar rats include several key components related to reproductive function, hormone regulation, and testicular structure and activity. Testosterone is the primary male hormone regulating sex differentiation, producing male sex characteristics, spermatogenesis, and fertility (Basaria, 2013). Testosterone is responsible for the development of primary sexual development, which includes testicular descent, spermatogenesis, enlargement of the penis and testes, and increasing libido (Plant and Marshall, 2001). Testosterone levels rise significantly during puberty (around 40-50 days of age) and peak around 76 days in Wistar rats before stabilizing to adult levels (Singh *et al*., 2015). Luteinizing hormone (LH) is secreted by the anterior pituitary and plays a role in reproductive functions such as synthesis of androgens in males. It is a glycoprotein hormone that is co-secreted along with follicle-stimulating hormone by the gonadotrophin cells in the adenohypophysis (anterior pituitary). Luteinizing hormone is a part of a neurological pathway comprised of the hypothalamus, the pituitary gland, and the gonads. In this pathway, LH release is stimulated by gonadotropin-releasing hormone (GnRH) and inhibited by estrogen in females and testosterone in males. LH has various functions, which differ between women

and men. In both sexes, LH contributes to the maturation of primordial germ cells. In male, LH causes the testes' Leydig cells to produce testosterone. In female, LH triggers the creation of steroid hormones from the ovaries (Ilahi and Ilahi, 2022). The pituitary gland regulates human reproduction through the refined, combined action of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH) on the gonads. Upon transport in the bloodstream, FSH reaches the gonads, stimulating follicle development in females and spermatogenesis in males (Rastrelli *et al*., 2014).

1.2 STATEMENT OF PROBLEM

Despite the traditional use of *Jatropha tanjorensis* leaves in herbal medicine and its reported medicinal properties, there is limited and conflicting scientific evidence regarding its impact on male reproductive health, particularly on fertility hormones and testicular function. Some studies suggest that bioactive compounds in *Jatropha tanjorensis* such as phytol and lupeol may exert antifertility effects by significantly reducing serum levels of key gonadal hormones, FSH, LH, and testosterone and impairing sperm parameters in male. Conversely, other reports indicate potential fertility-enhancing effects at certain doses. Additionally, the mechanisms by which *Jatropha tanjorensis* influences testicular histology and hormonal balance remain inadequately understood. This uncertainty poses a challenge in validating the safety and efficacy of *Jatropha tanjorensis* leaves for therapeutic use related to male fertility. Therefore, there is a need to systematically investigate and clarify the effects of *Jatropha tanjorensis* leaf extract on fertility hormones and testicular structure and function in male to provide scientific evidence that can guide its medicinal application and address potential reproductive risks.

1.3 JUSTIFICATION OF STUDY

Given the widespread reliance on herbal remedies in many developing countries, including Nigeria, where a high percentage of the population depends on plant-based medicine, it is crucial to clarify the dose-dependent effects and safety profile of *Jatropha tanjorensis* on male fertility. This study will provide valuable insights into the hormonal and histological

impacts of *Jatropha tanjorensis* leaves on the testes of male Wistar rats, thereby informing its potential therapeutic application or risks in male reproductive health.

1.4 AIM OF STUDY

The aim of this study is to evaluate the effect of *Jatropha tanjorensis* leaf extract on fertility hormones and testes in male wistar rats.

1.5 RESEARCH QUESTION

1. What is the effect of leaf extract of Jatropha tanjorensis on serum levels of Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH), Testosterone, and Prolactin, as well as on testicular weight and histology in adult male Wistar rats?

1.6 SPECIFIC OBJECTIVES

1. To evaluate the fertility hormones and testes in male wistar rats following the consumption of *Jatropha tanjorensis* leaves.
2. To compare the changes of the group of animals that consume *Jatropha tanjorensis* leaves to the control group.

CHAPTER TWO

2.0 LITERATURE REVIEW

1.1 JATROPHA TANJORENSIS

Jatropha tanjorensis plant belongs to the Euphorbiaceae family, commonly known as "hospital too far," catholic vegetable, "Iyana Ipaja." It is widely consumed in the southwestern region of Nigeria as soup. It is a perennial herb, which shows intermediacy in phenotypic characters between Jatropha curcas and Jatropha gossypifolia (Omoregie and Osagie 2007). The plant leaves are widely used in the southwestern region of Nigeria for making vegetable soup. The leaves are also used traditionally in the treatment of anemia (as a hematinic agent), diabetes and cardiovascular diseases, which are all diseases caused by production of free radicals (Iwalewa *et al*. 2005). The leaf extract has hypoglycaemic properties and is taken as medicine for the treatment of diabetic symptoms (Adeleke *et al*., 2004). It has been reported that Jatropha leaves are rich in beta blockers, anti-cancer agents, anti-anaemic, anti-microbial activities, anti-plasmodial and anti-oxidant effects against oxidative stress induced by malaria parasite (Omoregie, and Sisodia, 2011; Ahaotu *et al*., 2013).

1.2 FERTILITY HORMONES

1.2.1 TESTOSTERONE

Testosterone is the primary male sex hormone and androgen in males. In humans, testosterone plays a key role in the development of male reproductive tissues such as testicles and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair (Singh *et al*., 2015). It is associated with increased aggression, sex drive, dominance, courtship display, and a wide range of behavioral characteristics (Silva *et al*., 2013). In addition, testosterone in both sexes is involved in health and well-being, where it has a significant effect on overall mood, cognition, social and sexual behavior, metabolism and energy output, the cardiovascular system, and in the prevention of osteoporosis (Smith and Batur, 2020; Durdiakova *et al*., 2011). Insufficient levels of testosterone in men may lead to abnormalities including frailty, accumulation of adipose fat tissue within the body, anxiety and depression, sexual performance issues, and bone loss. Testosterone is a steroid hormone from the androstane class containing a ketone and a hydroxyl group at positions three and seventeen respectively. It is biosynthesized in several steps from cholesterol and is converted in the liver to inactive metabolites (Luetjens and Weinbauer, 2012). It exerts its action through binding to and activation of the androgen receptor (Luetjens and Weinbauer, 2012). In humans and most other vertebrates, testosterone is secreted primarily by the testicles of males and, to a lesser extent, the ovaries of females. On average, in adult males, levels of testosterone are about seven to eight times as great as in adult females (Durdiakova *et al*., 2011). As the metabolism of testosterone in males is more pronounced, the daily production is about 20 times greater in men. Females are also more sensitive to the hormone. In addition to its role as a natural hormone, testosterone is used as a medication to treat hypogonadism and breast cancer (Tyagi *et al*., 2017). Since testosterone levels decrease as men age, testosterone is sometimes used in older men to counteract this deficiency. It is also used illicitly to enhance physique and performance, for instance in athletes. In general, androgens such as testosterone promote protein synthesis and thus growth of tissues with androgen receptors (Xu *et al*., 2019). Testosterone can be described as having anabolic and androgenic (virilising) effects, though these categorical descriptions are somewhat arbitrary, as there is a great deal of mutual overlap between them. The relative potency of these effects can depend on various factors and is a topic of ongoing research (Čeponis *et al*., 2017). Testosterone can either directly exert effects on target tissues or be

metabolized by 5α-reductase into dihydrotestosterone (DHT) or aromatized to estradiol (E2). Both testosterone and DHT bind to an androgen receptor; however, DHT has a stronger binding affinity than testosterone and may have more androgenic effect in certain tissues at lower levels (Čeponis *et al*., 2017).

- *Anabolic effects* include growth of muscle mass and strength, increased bone density and strength, and stimulation of linear growth and bone maturation.

- *Androgenic effects* include maturation of the sex organs, particularly the penis, and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of facial hair (such as the beard) and axillary (underarm) hair. Many of these fall into the category of male secondary sex characteristics.

Testosterone effects can also be classified by the age of usual occurrence. For postnatal effects in both males and females, these are mostly dependent on the levels and duration of circulating free testosterone (Sfetcu, 2014). Factors affecting testosterone levels may include:

- Age: Testosterone levels gradually reduce as men age (Huhtaniemi, 2014). This effect is sometimes referred to as andropause or late-onset hypogonadism (Huhtaniemi, 2014).

- Exercise: Resistance training increases testosterone levels acutely (Vingren *et al*., 2010), however, in older men, that increase can be avoided by protein ingestion (Tyagi *et al*., 2017). Endurance training in men may lead to lower testosterone levels (Tyagi *et al*., 2017).

- Nutrients: Vitamin A deficiency may lead to sub-optimal plasma testosterone levels (Monson *et al*., 2023). The secosteroid vitamin D in levels of 400–1000 IU/d (10–25 μg/d) raises testosterone levels (Pilz *et al*., 2011). Zinc deficiency lowers testosterone levels but over-supplementation has no effect on serum testosterone (Rotter *et al*., 2015)

- Weight loss: Reduction in weight may result in an increase in testosterone levels. Fat cells synthesize the enzyme aromatase, which converts testosterone, the male sex hormone, into estradiol, the female sex hormone (Håkonsen *et al*., 2011). However,

no clear association between body mass index and testosterone levels has been found (MacDonald *et al*., 2010).

- Miscellaneous: Sleep: (REM sleep) increases nocturnal testosterone levels (Ukraintseva *et al*., 2018).
- Behavior: Dominance challenges can, in some cases, stimulate increased testosterone release in men (Ukraintseva *et al*., 2018).
- Foods: Natural or man-made antiandrogens including spearmint tea reduce testosterone levels (Ukraintseva *et al*., 2018). Licorice can decrease the production of testosterone and this effect is greater in females (Minnetti *et al*., 2022).
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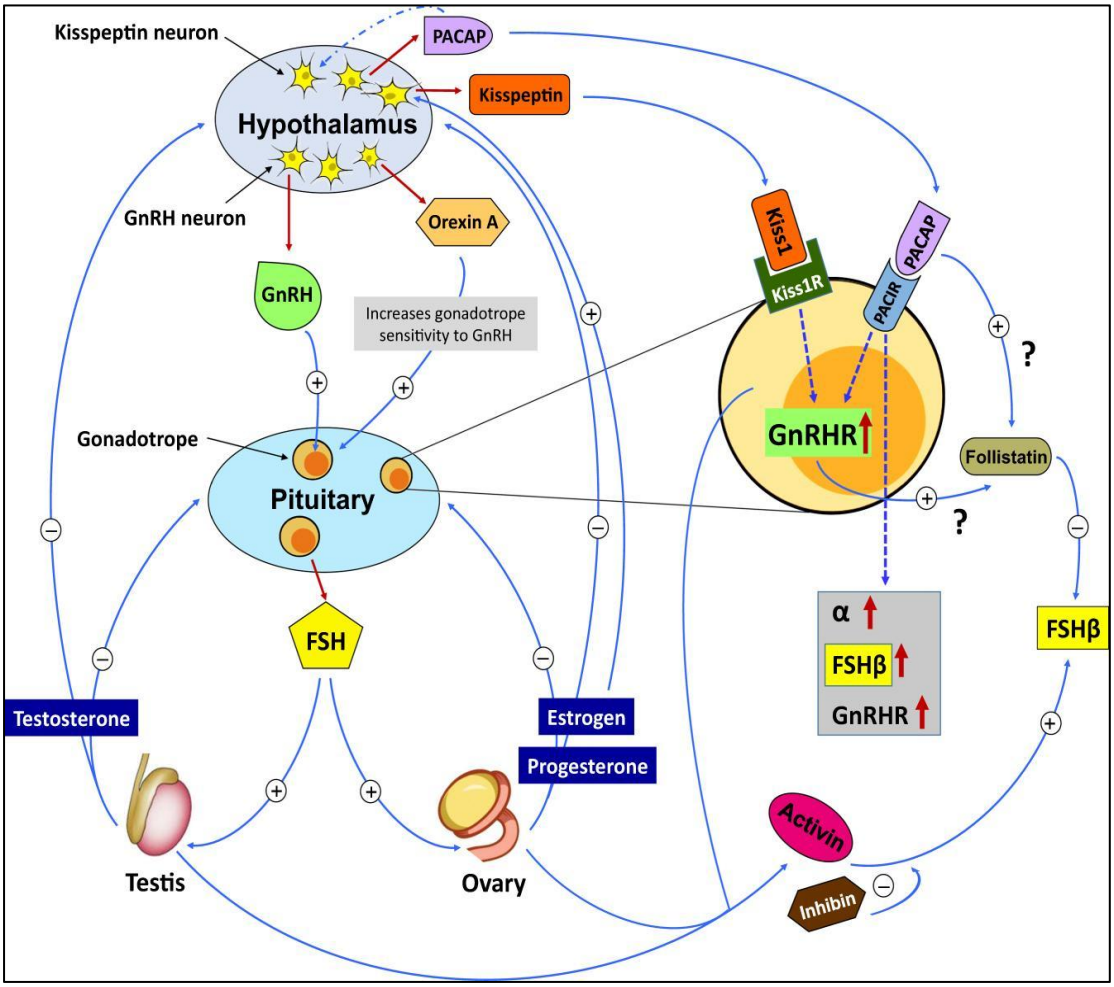

Figure 2. 1 A diagrammatic illustration of the synthesis, secretion and regulation of testosterone, LH and FSH (Das and Kumar, 2018).

1.2.2 LUTEINIZING HORMONE (LH)

Luteinizing hormone (LH, also known as luteinising hormone, lutropin and sometimes lutrophin) is a hormone produced by gonadotropic cells in the anterior pituitary gland. The production of LH is regulated by gonadotropin-releasing hormone (GnRH) from the hypothalamus (Stamatiades and Kaiser, 2018). In females, an acute rise of LH known as an 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 testosterone. It acts synergistically with follicle-stimulating hormone (FSH). LH is a heterodimeric glycoprotein. Each monomeric unit is a glycoprotein molecule; one alpha and one beta subunit make the full, functional protein. Its structure is similar to that of the other glycoprotein hormones, follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The protein dimer contains 2 glycopeptidic subunits (labeled alpha- and beta- subunits) that are non-covalently associated (Jiang *et al*., 2014):

- The *alpha subunits* of LH, FSH, TSH, and hCG are identical, and contain 92 amino acids in human but 96 amino acids in almost all other vertebrate species (glycoprotein hormones do not exist in invertebrates) (Atre *et al*., 2024).

- The *beta subunits* vary. LH has a beta subunit of 120 amino acids (LHB) that confers its specific biologic action and is responsible for the specificity of the interaction with the LH receptor (Ando, 2021). This beta subunit contains an amino acid sequence that exhibits large homologies with that of the beta subunit of hCG and both stimulate the same receptor. However, the hCG beta subunit contains an additional 24 amino acids, and the two hormones differ in the composition of their sugar moieties (Strickland *et al*., 2019).

The different composition of these oligosaccharides affects bioactivity and speed of degradation. The biologic half-life of LH is 20 minutes, shorter than that of FSH (3–4 hours) and hCG (24 hours). The biological half-life of LH is 23 hours subcutaneous (Ezcurra and Humaidan, 2014) or terminal half-life of 10-12 hours (le Cotonnec *et al*., 1998). LH acts upon the Leydig cells of the testes and is regulated by gonadotropin-releasing hormone (GnRH). The Leydig cells produce testosterone under the control of LH. LH binds to LH receptors on the membrane surface of Leydig cells. Binding to this receptor causes an increase in cyclic adenosine monophosphate (cAMP), a secondary messenger, which allows

cholesterol to translocate into the mitochondria. Within the mitochondria, cholesterol is converted to pregnenolone by CYP11A1 (Zirkin and Papadopoulos, 2018). Pregnenolone is then converted to dehydroepiandrosterone (DHEA) (Koskenniemi *et al*., 2017). DHEA is then converted to androstenedione by 3β-hydroxysteroid dehydrogenase (3β-HSD) (Liu *et al*., 2015) and then finally converted to testosterone by 17β-hydroxysteroid dehydrogenase (HSD17B). The onset of puberty is controlled by two major hormones: FSH initiates spermatogenesis and LH signals the release of testosterone, an androgen that exerts both endocrine activity and intratesticular activity on spermatogenesis (Bernard et al., 2019). LH is released from the pituitary gland, and is controlled by pulses of gonadotropin-releasing hormone. When bloodstream testosterone levels are low, the pituitary gland is stimulated to release LH. As the levels of testosterone increase, it will act on the pituitary through a negative feedback loop and inhibit the release of GnRH and LH consequently (Lei *et al*., 2025). Androgens (including testosterone and dihydrotestosterone) inhibit monoamine oxidase (MAO) in the pineal gland, leading to increased melatonin and reduced LH and FSH by melatonin-induced increase of Gonadotropin-Inhibitory Hormone (GnIH) (Ubuka *et al*., 2014) synthesis and secretion. Testosterone can also be aromatized into estradiol (E2) to inhibit LH. E2 decreases pulse amplitude and responsiveness to GnRH from the hypothalamus onto the pituitary (Bernard *et al*., 2019). Changes in LH and testosterone blood levels and pulse secretions are induced by changes in sexual arousal in human males (Hohl *et al*., 2018).

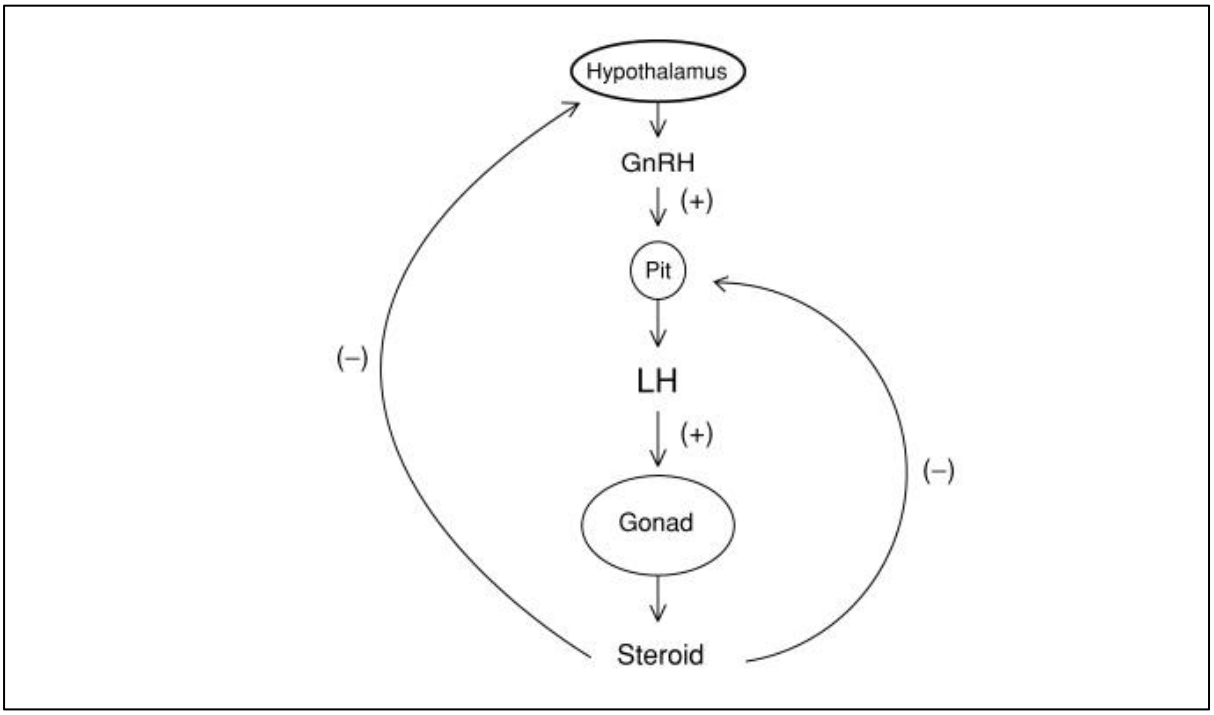

Figure 2. 2 Schematic illustration of the synthesis, secretion and regulation of LH

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1.2.3 FOLICLE STIMULATING HORMONE

Follicle-stimulating hormone (FSH) is a gonadotropin, a glycoprotein polypeptide hormone (Cahoreau *et al*., 2015). FSH is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) work together in the reproductive system. FSH is a 35.5 kDa glycoprotein heterodimer, consisting of two polypeptide units, alpha and beta. Its structure is similar to those of luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The alpha subunits of the glycoproteins LH, FSH, TSH, and hCG are identical and consist of 96 amino acids, while the beta subunits vary. Both subunits are required for biological activity. FSH has a beta subunit of 111 amino acids (FSH β), which confers its specific biologic action, and is responsible for interaction with the follicle-stimulating hormone receptor (Jiang *et al*., 2012). FSH regulates the development, growth, pubertal maturation and reproductive processes of the human body (Ulloa-Aguirre *et al*., 2018).

- In both *males* and *females*, FSH stimulates the maturation of primordial germ cells.
- In *males*, FSH induces Sertoli cells to secrete androgen-binding proteins (ABPs), regulated by inhibin's negative feedback mechanism on the anterior pituitary. Specifically, activation of Sertoli cells by FSH sustains spermatogenesis and stimulates inhibin B secretion.
- In *females*, FSH initiates follicular growth, specifically affecting granulosa cells. With the concomitant rise in inhibin B, FSH levels then decline in the late follicular phase. This seems to be critical in selecting only the most advanced follicle to proceed to ovulation. At the end of the luteal phase, there is a slight rise in FSH that seems to be of importance to start the next ovulatory cycle.

Control of FSH release from the pituitary gland is unknown. Low frequency gonadotropin-releasing hormone (GnRH) pulses increase FSH mRNA levels in the rat, but is not directly correlated with an increase in circulating FSH (Sharma *et al*., 2012). FSH stimulates primary spermatocytes to undergo the first division of meiosis, to form secondary spermatocytes. FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes (Das and Kumar, 2018), and is critical for the initiation of spermatogenesis.

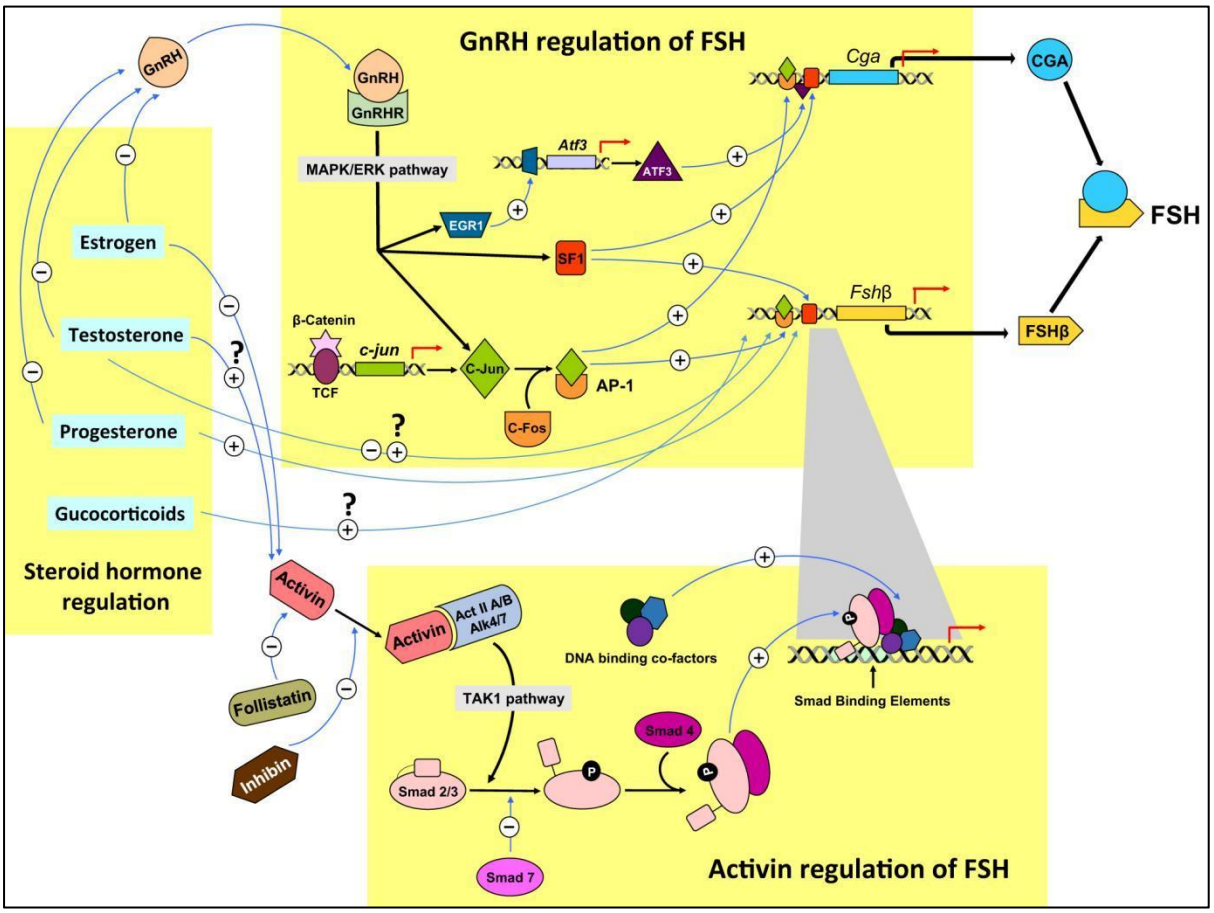

Figure 2. 3 A diagrammatic illustration of Molecular regulation of follicle-stimulating hormone synthesis, secretion and action (Das and Kumar, 2018).

1.2.4 PROLACTIN AND ESTRADIOL

Prolactin (PRL), also known as lactotropin and mammotropin, is a protein best known for its role in enabling mammals to produce milk. It is influential in over 300 separate processes in various vertebrates, including humans (Lopez-Vicchi *et al*., 2020). Prolactin is secreted from the pituitary gland in response to eating, mating, estrogen treatment, ovulation and nursing. It is secreted heavily in pulses in between these events. Prolactin plays an essential role in metabolism, regulation of the immune system and pancreatic development (Ben-Jonathan *et al*., 2006; Ali and Mirza, 2021). In mammals, prolactin is associated with milk production; in fish it is thought to be related to the control of water and salt balance. Prolactin also acts in a cytokine-like manner and as an important regulator of the immune system. It has important cell cycle-related functions as a growth-, differentiating- and anti-apoptotic factor. As a growth factor, binding to cytokine-like receptors, it influences hematopoiesis and angiogenesis and is involved in the regulation of blood clotting through several pathways. The hormone acts in endocrine, autocrine, and paracrine manners through the prolactin receptor and numerous cytokine receptors (Lopez-Vicchi *et al*., 2020). Prolactin has a wide variety of effects. It stimulates the mammary glands to produce milk (lactation): increased serum concentrations of prolactin during pregnancy cause enlargement of the mammary glands and prepare for milk production, which normally starts when levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. Prolactin plays an important role in maternal behavior (Crowley, 2014). The effect of estradiol (and estrogens in general) upon male reproduction is complex. Estradiol is produced by action of aromatase mainly in the Leydig cells of the mammalian testes, but also by some germ cells and the Sertoli cells of immature mammals (Carreau *et al*., 2003). It functions (*in vitro*) to prevent apoptosis of male sperm cells (Lacasse, 2025). While some studies in the early 1990s claimed a connection between globally declining sperm counts and estrogen exposure in the environment, later studies found no such connection, nor evidence of a general decline in sperm counts (Crowley, 2014). Suppression of estradiol production in a subpopulation of subfertile men may improve the semen analysis (Raman and Schlegel, 2002; Zhang *et al*., 2018). Males with certain sex chromosome genetic conditions, such as Klinefelter's syndrome, will have a higher level of estradiol (Visootsak and Graham, 2006; Lacasse, 2025).

1.3 TESTICULAR FUNCTION AND STRUCTURE

1.3.1 SPERMATOGENISIS

Spermatogenesis is the process by which haploid spermatozoa develop from germ cells in the seminiferous tubules of the testicle. This process starts with the mitotic division of the stem cells located close to the basement membrane of the tubules. These cells are called spermatogonial stem cells. The mitotic division of these produces two types of cells. Type A cells replenish the stem cells, and type B cells differentiate into primary spermatocytes. The primary spermatocyte divides meiotically (Meiosis I) into two secondary spermatocytes; each secondary spermatocyte divides into two equal haploid spermatids by Meiosis II. The spermatids are transformed into spermatozoa (sperm) by the process of spermiogenesis. These develop into mature spermatozoa, also known as sperm cells (Sharma *et al*., 2018). Thus, the primary spermatocyte gives rise to two cells, the secondary spermatocytes, and the two secondary spermatocytes by their subdivision produce four spermatozoa and four haploid cells. Spermatozoa are the mature male gametes in many sexually reproducing organisms. Thus, spermatogenesis is the male version of gametogenesis, of which the female equivalent is oogenesis. In mammals it occurs in the seminiferous tubules of the male testes in a stepwise fashion. Spermatogenesis is highly dependent upon optimal conditions for the process to occur correctly, and is essential for sexual reproduction. DNA methylation and histone modification have been implicated in the regulation of this process (Song *et al*., 2011). It starts during puberty and usually continues uninterrupted until death, although a slight decrease can be discerned in the quantity of produced sperm with increase in age. Spermatogenesis starts in the bottom part of seminiferous tubes and, progressively, cells go deeper into tubes and moving along it until mature spermatozoa reaches the lumen, where mature spermatozoa are deposited. The division happens asynchronically; if the tube is cut transversally one could observe different maturation states. A group of cells with different maturation states that are being generated at the same time is called a spermatogenic wave (Schulze, 2009).

1.3.2 LEYDIG CELLS

Leydig cells, also known as interstitial cells of the testes and interstitial cells of Leydig, are found adjacent to the seminiferous tubules in the testicle and produce testosterone in the

presence of luteinizing hormone (LH). They are polyhedral in shape and have a large, prominent nucleus, an eosinophilic cytoplasm, and numerous lipid-filled vesicles. Males have two types of leydig cells that appear in two distinct stages of development: the fetal type and the adult type (Carrasco-Juan *et al*., 2017)**.** The mammalian Leydig cell is a polyhedral epithelioid cell with a single eccentrically located ovoid nucleus. The nucleus contains one to three prominent nucleoli and large amounts of dark-staining peripheral heterochromatin. The acidophilic cytoplasm usually contains numerous membrane-bound lipid droplets and large amounts of smooth endoplasmic reticulum (SER) (Rhoades *et al*., 2022). Besides the abundance of SER with scattered patches of rough endoplasmic reticulum, several mitochondria are also prominent within the cytoplasm. Reinke crystals have lipofuscin pigment and rod-shaped crystal-like structures 3 to 20 micrometres in diameter (Partin *et al*., 2020). Leydig cells release a class of hormones called androgens (19-carbon steroids). They secrete testosterone, androstenedione and dehydroepiandrosterone (DHEA), when stimulated by the luteinizing hormone (LH), which is released from the anterior pituitary in response to gonadotropin releasing hormone which in turn is released by the hypothalamus. LH binds to its receptor (LHCGR) which is a G-protein coupled receptor and consequently increases the production of cAMP. cAMP, in turn through protein kinase A activation, stimulates cholesterol translocation from intracellular sources (primarily the plasma membrane and intracellular stores) to the mitochondria, firstly to the outer mitochondrial membrane and then cholesterol needs to be translocated to the inner mitochondrial membrane by steroidogenic acute regulatory protein, which is the rate-limiting step in steroid biosynthesis. This is followed by pregnenolone formation from the translocated cholesterol via the cholesterol side-chain cleavage enzyme, which is found in the inner mitochondrial membrane, eventually leading to testosterone synthesis and secretion by Leydig cells (Zirkin and Papadopoulos, 2018).

1.3.3 SERTOLI CELLS

Sertoli cells are a type of sustentacular "nurse" cell found in human testes which contribute to the process of spermatogenesis (the production of sperm) as a structural component of

the seminiferous tubules. They are activated by follicle-stimulating hormone (FSH) secreted by the adenohypophysis and express FSH receptor on their membranes. Sertoli cells are specifically located in the convolutions of the seminiferous tubules, since this is the only place in the testes where spermatozoa are produced. As the primary support cell of the tubules, they are generally very large and amorphous, with individual cells stretching from the basal lamina to the lumen; their cytoplasm often completely surrounds the germline cells which they are responsible for nursing. Sertoli cells are easily confused with the other cells of the germinal epithelium when using standard staining techniques; the most distinctive feature of the Sertoli cell is its dark nucleolus. Sertoli cells are required for male sexual development. Sertoli cell proliferation and differentiation is mainly activated by FGF9, with which they also form a feedforward loop (Kim *et al*., 2006; Moniot *et al*., 2009). It has been suggested that Sertoli cells may derive from the fetal mesonephros. After puberty, Sertoli cells begin to elongate. Their nucleoli become larger and tight junctions are completed, creating a fluid-filled lumen space (Shah *et al*., 2021). FSH is responsible for controlling the proliferation of Sertoli cells shortly after birth and stimulates the production of factors derived from Sertoli cells that control the development of the testes and germ cells. FSH, luteinizing hormone. thyroid-stimulating hormone, and hCG are all known to affect Sertoli cell development and male reproductive health. FSH is required for Sertoli cell mitogen, which stimulates the expression of various cell markers (Shah *et al*., 2021). Once fully differentiated, the Sertoli cell is considered terminally differentiated, and is unable to proliferate (Sharpe *et al*., 2003). Therefore, once spermatogenesis has begun, no more Sertoli cells are created, and their population within the seminiferous tubules is finite. Because its main function is to nourish developing sperm cells through the stages of spermatogenesis, the Sertoli cell has also been called the "mother" or "nurse" cell (Rato *et al*., 2012). Sertoli cells also act as phagocytes, consuming the residual cytoplasm during spermatogenesis. Translocation of cells from the basal lamina to the lumen of the seminiferous tubules occurs by conformational changes in the lateral margins of the Sertoli cells. Sertoli cells secrete the following substances:

- anti-Müllerian hormone (AMH), secreted during the early stages of fetal life
- inhibin and activins, secreted after puberty, work together to regulate FSH secretion
- androgen-binding protein (also called testosterone-binding globulin) increases testosterone concentration in the seminiferous tubules to lightly stimulate spermatogenesis

- estradiol - an aromatase converts testosterone to 1,7-beta-estradiol to direct spermatogenesis
- ETS Related Molecule or ERM transcription factor is needed for maintenance of the spermatogonial stem cells in the adult testis
- transferrin, a blood plasma protein for iron ion delivery (Xiong *et al*., 2006)
- testicular ceruloplasmin, a ceruloplasmin-like protein which is immunologically similar to serum ceruloplasmin.

Sertoli cells are also responsible for establishing and maintaining the spermatogonial ste cell niche, which ensures the renewal of stem cells and the differentiation of spermatogonia into mature germ cells that progress stepwise through the long process of spermatogenesis, ending in the release of spermatozoa in a process known as spermiation (O'Donnell *et al*., 2011).

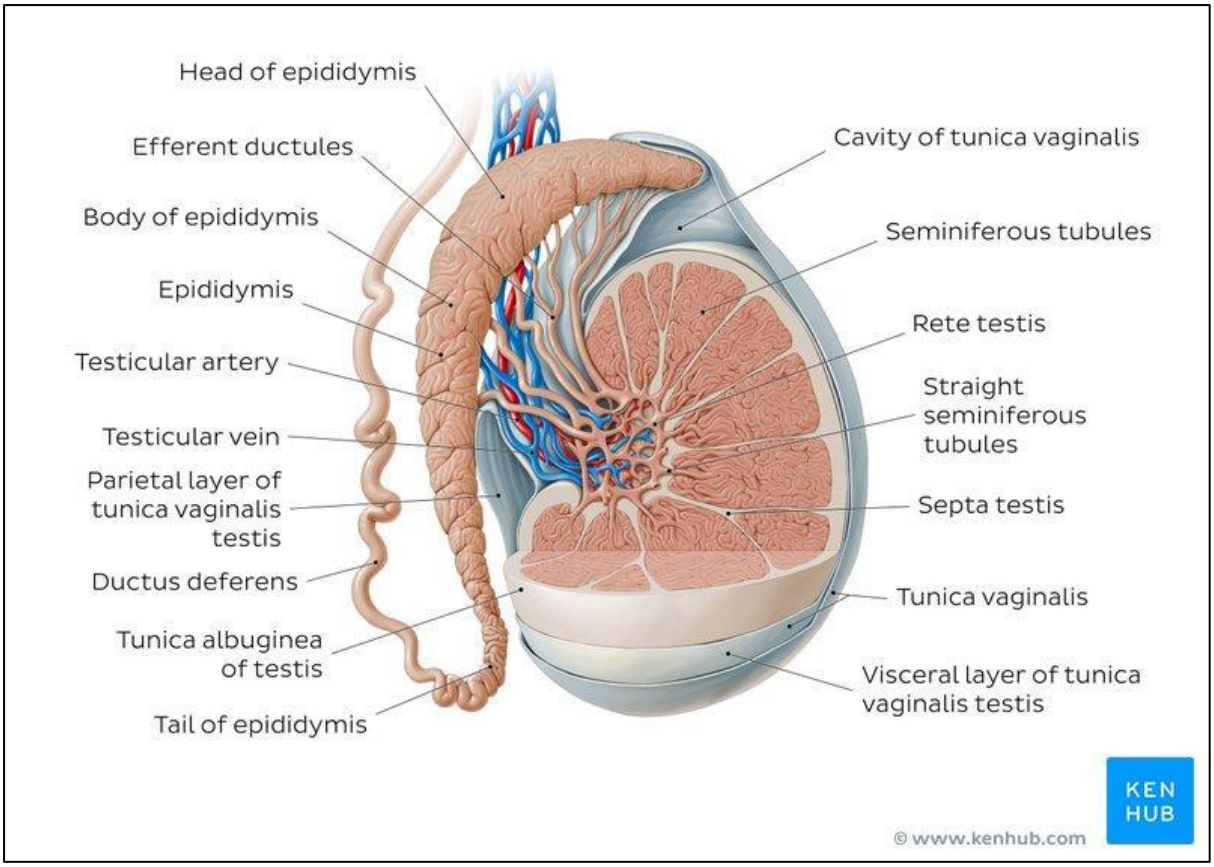

Figure 2. 4 A diagrammatic illustration of the testes.

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1.4 SEMEN ANALYSIS

Semen analysis, also known as a seminogram or spermogram, is the cornerstone of laboratory evaluation for male fertility. It provides a quantitative and qualitative assessment of the seminal fluid ejected during ejaculation and the spermatozoa contained within it (Sunder and Leslie, 2025). The analysis offers critical insights into the functional status of the testes and the accessory sex glands (e.g., seminal vesicles, prostate, bulbourethral glands) (Sapozhkova and Eremin, 2020).

A comprehensive semen analysis evaluates key parameters which are indicative of normal male reproductive function (Chawre *et al*., 2024). These include :

Volume: The total volume of the ejaculate.

Sperm Concentration (Count): The number of spermatozoa per milliliter of semen.

Total Sperm Number: The total number of spermatozoa in the entire ejaculate.

Sperm Motility: The percentage of sperm that are moving, including progressive motility (sperm moving actively) and non-progressive motility.

Sperm Morphology: The percentage of sperm with a normal anatomical structure.

Vitality: The percentage of live sperm in the ejaculate.

Additional Parameters: The analysis also includes assessments of pH, time to liquefaction, and the presence of white blood cells or agglutination

(Chawre *et al*., 2024; Sapozhkova and Eremin, 2020).

Table 2.1: Key Parameters in Semen Analysis and Their Clinical Significance Based on WHO Guidelines (Chawre *et al*., 2024; Sunder and Leslie, 2025).

Parameter	Description	Clinical Significance (Based on WHO Lower Reference Limits)
Volume	Total ejaculate volume	Hyperspermia (>5.0 mL) or hypospermia (<1.5 mL) may indicate accessory gland dysfunction or retrograde ejaculation .
Concentration	Sperm count per mL	Oligozoospermia: < 15 million sperm/mL. Azoospermia: No sperm in the ejaculate .
Total Motility	% of motile sperm (progressive + non-progressive)	Asthenozoospermia: < 40% motile sperm .
Progressive Motility	% of sperm with active movement	< 32% progressively motile is below the reference limit .
Morphology	% of normally shaped sperm	Teratozoospermia: < 4% normal forms (strict criteria) is associated with reduced fertility .
Vitality	% of live sperm	< 58% live sperm is considered below the reference limit .
pH	Acidity/Alkalinity	A pH < 7.2 may indicate an obstruction or congenital absence of the vas deferens .

1.4.1 SPECIMEN COLLECTION AND PROCEDURE

For accurate results, the semen sample is typically collected after 2-7 days of sexual abstinence via masturbation into a sterile container (Sunder and Leslie, 2025). The sample

must be kept at body temperature and delivered to the laboratory within 1 hour of collection to prevent changes in sperm quality (Hofmann and Giwercman, 2023). The sample undergoes liquefaction and is then analyzed using standardized methods described in the World Health Organization (WHO) laboratory manual (Chawre *et al*., 2024).

Clinical Significance and Interpretation: Semen analysis is a primary tool in investigating male factor infertility (Sunder and Leslie, 2025). Abnormal results can guide further diagnostic testing :

Hormonal Analysis: Men with a low sperm count should have initial labs including serum total testosterone and follicle-stimulating hormone (FSH) to identify endocrine pathologies such as hypogonadism (Hofmann and Giwercman, 2023; Sunder and Leslie, 2025).

Genetic Testing: Karyotyping to rule out conditions like Klinefelter's syndrome is recommended in cases of severe oligospermia (Chawre *et al*., 2024).

Imaging: Scrotal or transrectal ultrasound can confirm or rule out obstructions in the genital tract, such as ejaculatory duct obstruction or congenital absence of the vas deferens (Sapozhkova and Eremin, 2020).

Infection Screening: The presence of over 1 million leukocytes per mL (pyospermia) requires evaluation for genital tract inflammation or infection (Sunder and Leslie, 2025).

CHAPTER THREE

2.0 MATERIALS AND METHODOLOGY

2.1 MATERIALS USED

- Test tubes
- Centrifuge
- Oral gavage needles
- Syringes (2ml and 5ml)
- Serum Separator Tube (SST)
- Plain bottles
- Plastic cages
- Sawdust
- Feed
- Water
- Plates
- Latex gloves
- Chloroform
- Cotton wool
- Weighing scale

2.2 STUDY AREA

This study was carried out in the animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Edo State.

2.3 EXPERIMENTAL ANIMALS AND DESIGN

Thirteen (14) healthy male Wistar rats, weighing between 100–150 g, were obtained from the Animal House of the Department of Anatomy, University of Benin, and housed in the same facility. Upon procurement, the animals were subjected to a mandatory two weeks period of acclimatization to the laboratory environment prior to the commencement of the study. During this time, they were maintained under standard animal husbandry conditions: ambient temperature of 25–29 °C, a natural light/dark cycle, and free access to standard rat chow and clean drinking water.

Following acclimatization, the animals were randomly divided into two groups. Group 1 (n = 7) served as the control group and received normal feed and water, while Group 2 (n = 7) served as the test group and was administered the plant extract.

Body weight: Body weights of all animals were precisely recorded using a calibrated digital weighing scale (precision 0.1g) immediately prior to the start of treatment.

Group	Name	Treatment	Number of rats
1	Control group	Distilled water	6
2	Test group	500mg/kg of extract	7

2.4 STUDY DURATION

The study was carried out for a period of six (6) weeks, after which the animals were sacrificed and samples were collected.

2.5 SOURCES OF JATROPHA TANJORENSIS

Fresh leaves of *J. tanjorensis* (Hospital trofan) were procured from a specific local market in Benin City, Edo State, Nigeria. To ensure accuracy and consistency in the phytochemical profile of the material, the plant was botanically identified and authenticated by a specialist in the Department of Plant Biology and Biotechnology, University of Benin. A voucher specimen (UBH-J317) was prepared and deposited in the departmental herbarium for future reference and verification.

2.6 PREPARATION OF JATROPHA TANJORENSIS EXTRACT AND DOSE CONCENTRATION

The fresh leaves were kept in the oven at 800c for ten minutes to stop any enzyme activity and then at 600c for 30minutes. They were collected from the oven, air dried and ground into coarse powder. 50g of the powdered leaves was stirred into 450ml of methanol. The mixture was then kept aside for 48hours to

allow it to infuse. It was then filtered using a filter paper. The filtrate was concentrated to 200ml (1ml of the extract being equivalent to 0.25g of the starting material). The extract was kept in Petri dishes with tight fitting covers in a refrigerator until it was time to use.

The extract was dissolved into a solution with 2g of the extract into 20ml of methanol . The dosage was calculated in this equation;

$$\frac{\text{Weight of experimental animals(in Kg)x dosage}}{\text{Concentration of solution (in ml)}}$$

2.7 SAMPLE COLLECTION

At the end of the experiment, the final body weights of the rats were measured using a calibrated digital scale. The animals were then anaesthetized by placing them in a sealed chamber containing chloroform-soaked cotton wool. After ensuring full anaesthesia, each rat was positioned dorsally on a dissection table. A midline incision was made through the abdomen and thorax using surgical scissors to expose the heart. Blood was collected via cardiac puncture and transferred into a Serum Separator Tube (SST) for subsequent analysis.

The caudal epididymis was carefully identified and dissected from the testis. It was then cleaned of adhering adipose tissue and transferred to a petri dish containing 2 mL of pre-warmed (37°C) normal saline. Several incisions were made into the caudal epididymis with a sharp sterile surgical blade to release spermatozoa into the saline solution. The solution was gently swirled and incubated at 37°C for 10 minutes to allow for adequate sperm dispersion. This sperm suspension was then used for the assessment of sperm motility, count, and morphology.

Immediately after blood collection, both testes were carefully dissected out from each rat. The testes were freed from the epididymides and cleared of any adhering tissues. The right testis from each animal was weighed on a digital balance to determine the gonadosomatic index. It was then fixed by complete immersion in a large volume (approximately 10 times the tissue volume) of Bouin's fluid for 48 hours.

2.8 HORMONAL ANALYSIS

Serum concentrations of Testosterone (T), Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH) and Prolactin were quantified using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits specifically validated for the Wistar rat model. All assays were conducted strictly according to the manufacturers' instructions, adhering to rigorous quality control standards regarding sensitivity and variability. This analysis is critical for determining whether the extract's effects are mediated by direct gonadal toxicity or by central regulation via the hypothalamus/pituitary.

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2.9 TESTICULAR HISTOLOGY

The excised testicular tissue reserved for structural analysis was promptly immersed in 10% Neutral Buffered Formalin (NBF) for fixation for at least 48 hours. The fixed tissues underwent routine histological processing, including dehydration through ascending grades of alcohol, clearing in xylene, and embedding in paraffin wax. Sections of 5mu thickness were cut using a rotary microtome, mounted on glass slides, and stained using the standard Hematoxylin and Eosin (HandE) technique for morphological evaluation. Microscopic examination was conducted under light microscopy. Qualitative analysis assessed the overall micro-architecture, focusing on the integrity of the basement membrane, the organization of the germinal epithelium, the presence of cellular vacuolation, and any signs of germ cell

maturation arrest. Quantitative analysis (morphometry) involved measuring the diameter of at least 20 randomly selected seminiferous tubules per rat using a calibrated ocular micrometer.

2.10 STATISTICAL ANALYSIS

All data were expressed as Mean ± Standard Error of the Mean (SEM). The results were analyzed using a one-way analysis of variance (ANOVA) followed by a post-hoc test (Tukey's test) to determine significant differences between the treatment groups and the control. The statistical analysis was performed using a software package such as GraphPad Prism (version 9.0). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

CHAPTER FOUR

3.0 RESULTS AND DISCUSSION

3.1 RESULTS

Table 4. 1: Comparing the mean values of reproductive hormones in male Wistar rats following the administration of *jatropha tanjorensis* extract.

Parameters	**Control**	***Jatropha tanjorensis***	**p-values**
Prolactin	10.73 ± 0.317	12.18 ± 0.704	0.1095
LH	5.975 ± 1.217	9.450 ± 0.595	0.0426
FSH	9.075 ± 2.04	15.65 ± 0.307	0.0190
Testosterone	3.225 ± 0.312	2.350 ± 0.132	0.0416
Testicular Weight	1.025 ± 0.063	1.075 ± 0.111	0.7084

P< 0.05 indicates significant difference

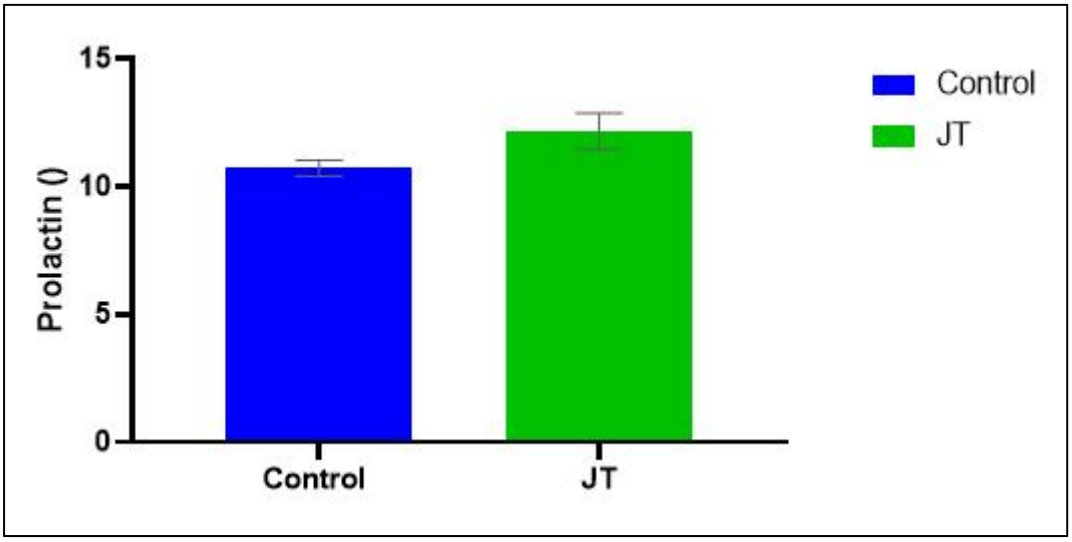

Figure 4.1 Showing the effect of *jatropha tanjorensis* on prolactin hormone of male Wistar rats.

There was no statistically significant differencesin *Jatropha tanjorensis* treated group compared with control.

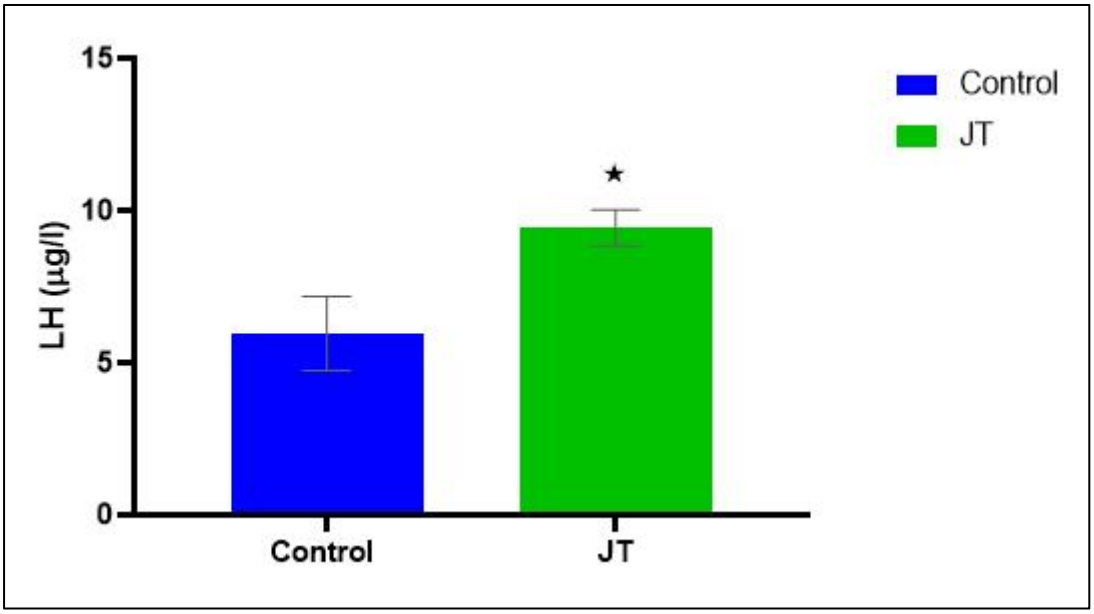

Figure 4.2 Showing the effect of *jatropha tanjorensis* on Luteinizing hormone of adult male Wistar rats.

There was a statistically significant increase in *jatropha tanjorensis* treated group compared with control.

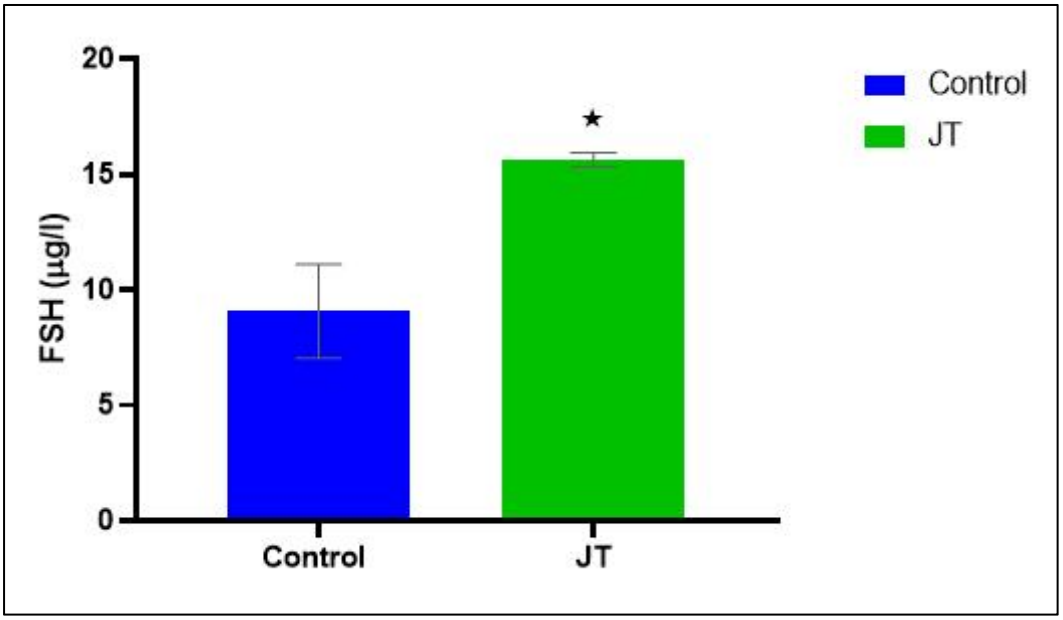

Figure 4.3 Showing the effect of *jatropha tanjorensis* on follicle stimulating hormone of male Wistar rats.

There was a significant increase in *jatropha tanjorensis* treated group compared with control.

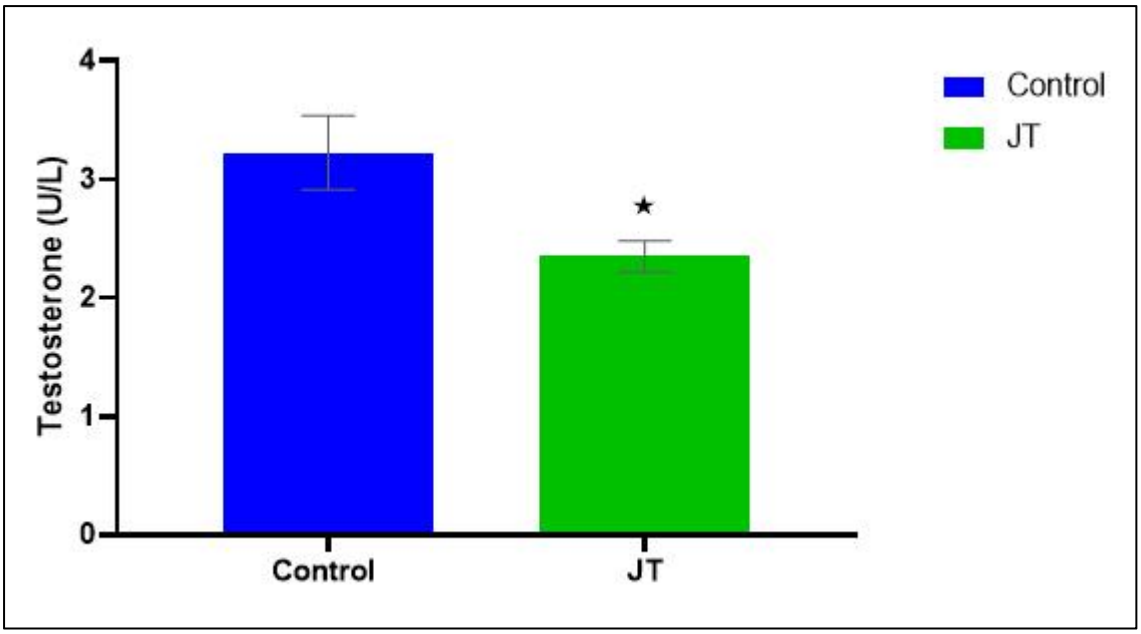

Figure 4.4: Showing the effect of *jatropha tanjorensis* on testosterone hormone of male Wistar rats.

There was a significant decrease in *jatropha tanjorensis* treated group compared with control.

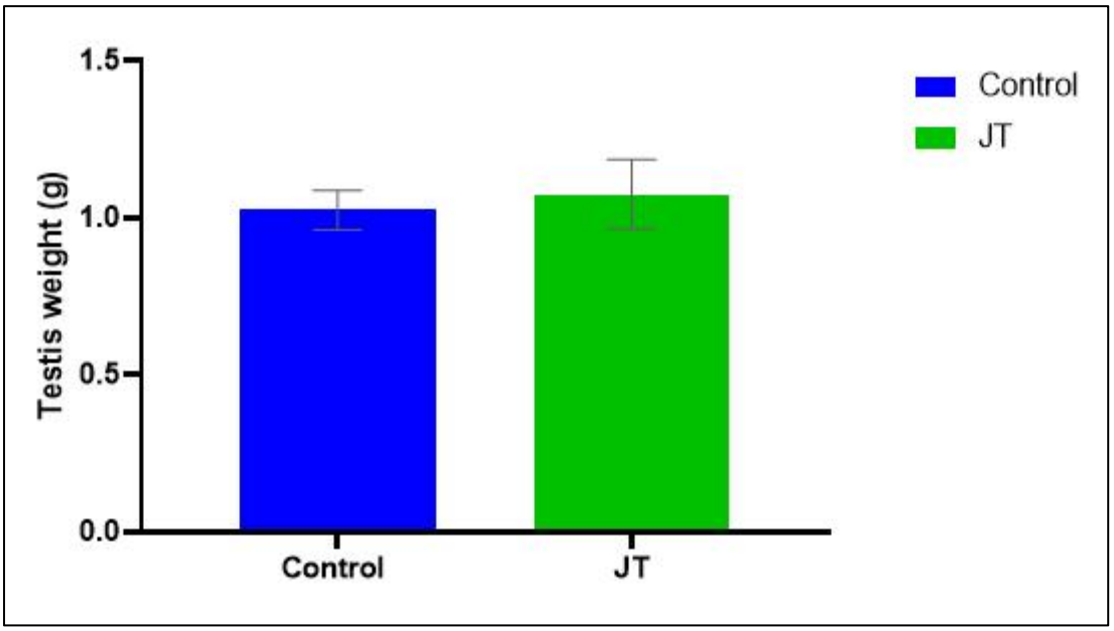

Figure 4.5: Showing the effect of *jatropha tanjorensis* on testicular weight of male Wistar rats.

There was no significant differences in *jatropha tanjorensis* treated group compared with control.

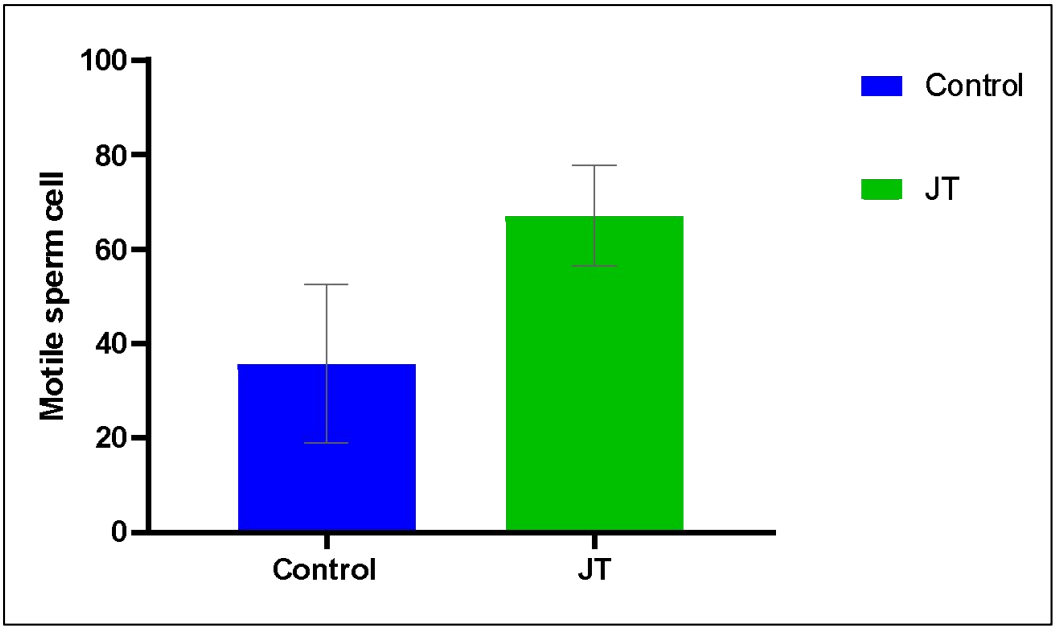

Figure 4. 6: The effect of *jatropha tanjorensis* on motile sperm cells of male Wistar rats.

There was a statistically significant increase in *jatropha tanjorensis* treated group compared with control group.

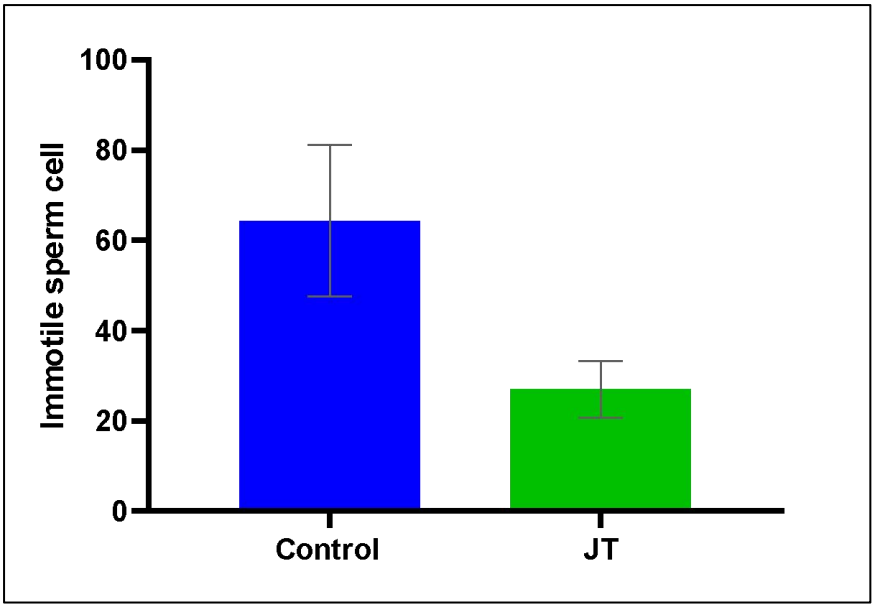

Figure 4. 7: The effect of *jatropha tanjorensis* on non-motile sperm cells of male Wistar rats.

There was a statistically significant decrease in *jatropha tanjorensis* treated group compared with control group.

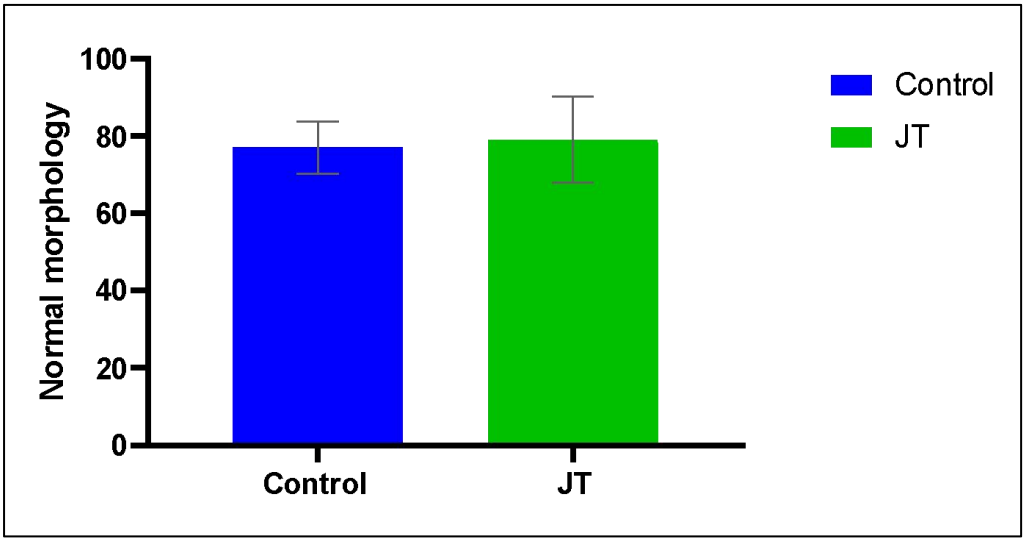

Figure 4. 8: The effect of *jatropha tanjorensis* on normal sperm mophology of male Wistar rats.

There was no statistically significant difference in *jatropha tanjorensis* treated group compared with control group.

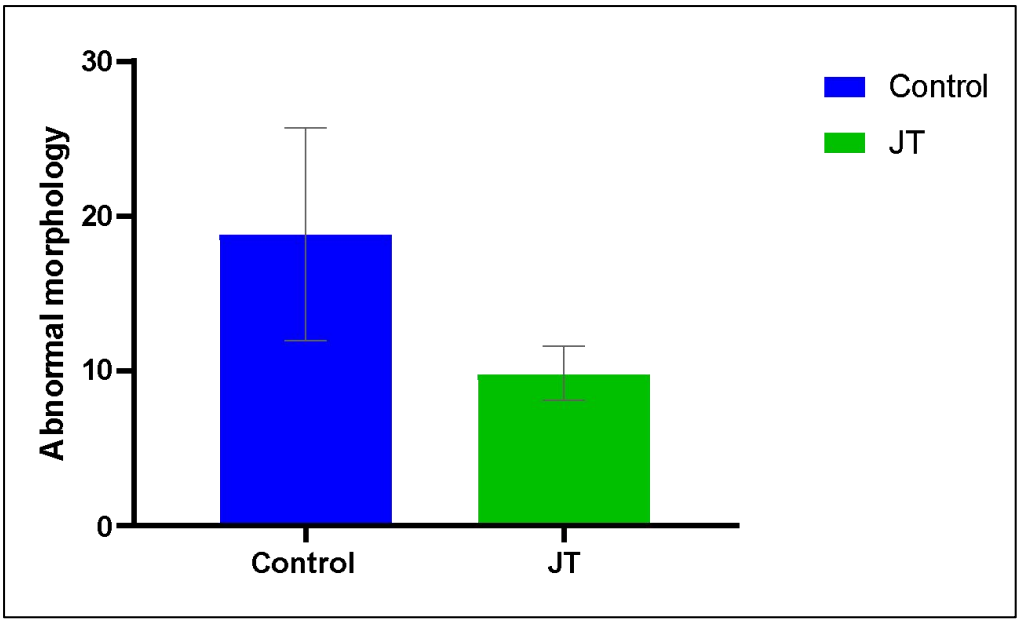

Figure 4. 9: The effect of *jatropha tanjorensis* on abnormal sperm mophology of male Wistar rats.

There was a statistically significant decrease in *jatropha tanjorensis* treated group compared with control group.

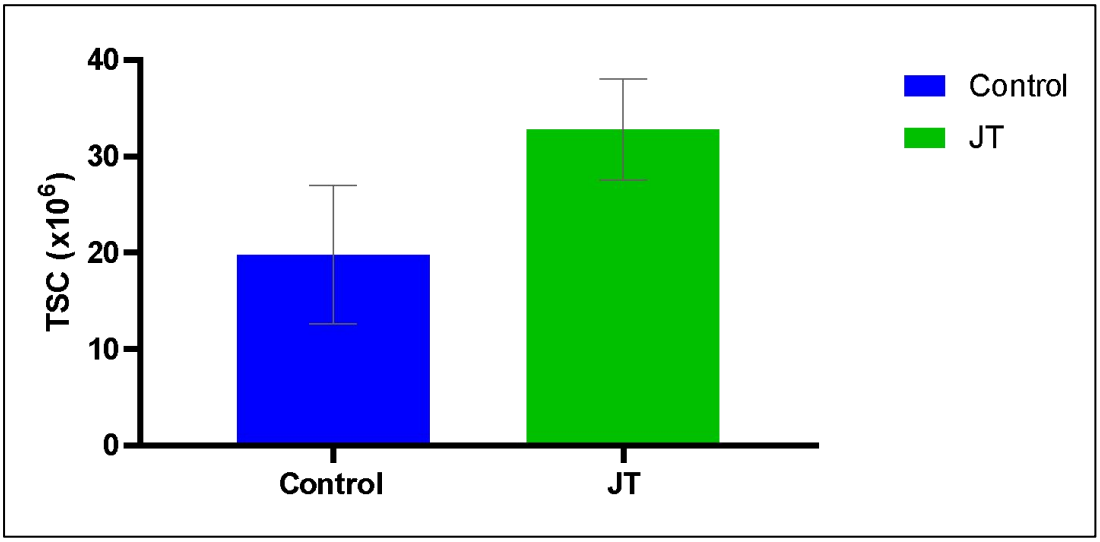

Figure 4. 10: The effect of *jatropha tanjorensis* on total sperm count of male Wistar rats.

There was a statistically significant increase in *jatropha tanjorensis* treated group compared with control group.

CHAPTER FIVE

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

This study aimed to evaluate the effect of the aqueous leaf extract of Jatropha tanjorensis on key fertility hormones and testicular parameters in adult male Wistar rats. The findings indicate that the extract significantly disrupts the normal hormonal balance essential for male reproductive function.

The most prominent finding of this research is the specific pattern of hormonal changes observed: a significant decrease in serum testosterone alongside significant increases in Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). This combination is highly indicative of a condition known as primary hypogonadism or hypergonadotropic hypogonadism (John *et al*., 2020). This means the testicles themselves are failing to function properly, despite receiving strong signals from the pituitary gland in the brain. The brain, detecting low testosterone levels, increases the secretion of LH (to stimulate testosterone production) and FSH (to support sperm production). The fact that testosterone remains low despite high levels of LH strongly suggests that the extract has a direct, inhibitory effect on the testicular cells responsible for hormone production.

These findings are supported by another study by John *et al*., (2020), which isolated specific compounds, phytol and lupeol, from Jatropha tanjorensis leaves. The study also found that these compounds significantly reduced serum levels of FSH, LH, and testosterone in male Wistar rats, and also impaired sperm motility and count. This provides a plausible explanation for the results of this study, identifying the active agents in the plant that could be responsible for the anti-androgenic effects.

However, conflicting evidence in the scientific literature, while data from this study shows a clear anti-fertility effect in male rats, another study concluded that the same plant, at the same dose (500mg/kg), improved female fertility by increasing FSH and estrogen, and enhancing pregnancy outcomes (Ukoh *et al*., 2022). Furthermore, a 2024 study suggested that Jatropha tanjorensis could actually increase testosterone levels and protect against reproductive damage caused by lead poisoning. These contradictions highlight a crucial point: the effects of Jatropha tanjorensis may be highly dependent on gender, physiological state, and the presence of other toxins. A compound that is detrimental to healthy male reproductive function might be beneficial in a diseased state or in females, a phenomenon known as hormesis.

The results revealed that there was no significant change in testicular weight. It suggests that the treatment period was sufficient to disrupt the hormonal function of the testes (a biochemical change) but not long enough to cause gross physical atrophy or major structural damage that would affect overall weight (John *et al*., 2020).

The semen analysis results demonstrate a significant and largely beneficial influence of *Jatropha tanjorensis* leaf extract on key spermatogenic endpoints in the testes of adult male Wistar rats. The observed outcomes,a significant increase in motile sperm count and total sperm count, coupled with a concurrent decrease in non-motile and abnormally shaped sperm cells, collectively suggest that the extract potentiates male fertility by enhancing testicular function and supporting the process of spermatogenesis. The absence of a significant change in normal sperm morphology, while the rate of abnormal forms decreased, indicates a specific action on improving sperm quality rather than inducing a non-specific proliferative effect.

4.2 CONCLUSION

This study demonstrates that the methanol extract of Jatropha tanjorensis leaves exerts significant disruptive effects on the reproductive hormonal milieu of male Wistar rats. The extract acts at multiple levels of the HPG axis, primarily by stimulating the secretion of pituitary gonadotropins (LH and FSH) while simultaneously inhibiting testicular testosterone production. This leads to a state of hypergonadotropic hypogonadism, indicating a direct testicular defect (primary hypogonadism). Therefore, despite its nutritional and therapeutic uses, the consumption of Jatropha tanjorensis may pose a potential risk to male reproductive health by impairing steroidogenesis and disrupting critical hormonal balance.

REFRENCES

Abura G, B. (2025). Hormonal Response of the Reproductive System to Phytochemicals: Targeting Prolactin, Estrogen, and Gonadotropins for Fertility Regulation. *Research Output Journal of Engineering and Scientific Research*, **4**(3): 1–8. https://doi.org/10.59298/ROJESR/2025/4.3.1800

Adeleke, C. A., Gbola O., Ezekiel, O. I., Olanrewaju, R. O., Adeniyi, A.A. and E J Verspohl. (2004). The Antidiabetic Potential of Jatropha tanjorensis Leaves. *Nigerian Journal of Natural Products and Medicine*. **8**(1): 55-58

Agogo, M., Okon, U., Olatunbosun, T., Ben, E., and Agabi, M. (2024). Modulatory Effect of Jatropha tanjorensis Ethanol Leaf Extract and Vitamin E on Some Reproductive Hormone in Lead-Treated Male Wistar Rats. *International Journal of Innovative Science and Research Technology (IJISRT)*, 2148–2155. https://doi.org/10.38124/ijisrt/IJISRT24APR2092

Ahaotu, E. O., Osuji, C. N., Nwabueze, E. U. and Ibeh, C. C. (2013). Benefits of Jatropha gossipifolia in Nigeria. *International Journal of Agriculture and Biosciences*. **2**(6): 349-355.

Akdoğan, M., Tamer, M. N., Cüre, E., Cüre, M. C., Köroğlu, B. K., and Delibaş, N. (2007). Effect of spearmint (Mentha spicata Labiatae) teas on androgen levels in women with hirsutism. *Phytotherapy research: PTR*. **21**(5): 444–447.

Akhtar, M. K., Kelly, S. L., and Kaderbhai, M. A. (2005). Cytochrome b(5) modulation of 17{alpha} hydroxylase and 17-20 lyase (CYP17) activities in steroidogenesis. *The Journal of endocrinology*. **187**(2): 267–274.

Ali, M., and Mirza, L. (2021). Morbid Obesity Due to Prolactinoma and Significant Weight Loss After Dopamine Agonist Treatment. *AACE clinical case reports*. **7**(3): 204–206.

Ando, H. (2021). Luteinizing hormone. *In Elsevier eBooks* (pp. 189–191).

Anhwange, B. A., Agbidye, I. G., Kyenge, B. A., and Ngbede, P. O. (2019). Phytochemical Screening, Antimicrobial Activities and Nutritional content of Jatropha Tanjorensis Leaves. *NIGERIAN ANNALS OF PURE AND APPLIED SCIENCES*, **1:** 108–113.

Andersen, M. L., and Tufik, S. (2008). The effects of testosterone on sleep and sleep-disordered breathing in men: its bidirectional interaction with erectile function. *Sleep medicine reviews*. **12**(5): 365–379.

Armanini, D., Fiore, C., Mattarello, M. J., Bielenberg, J., and Palermo, M. (2002). History of the endocrine effects of licorice. *Experimental and clinical endocrinology and diabetes: official journal, German Society of Endocrinology [and] German Diabetes Association*. **110**(6): 257–261.

Atre, I., Mizrahi, N., Hausken, K., and Levavi-Sivan, B. (2024). In silico insights into intra- and inter-species interactions of piscine gonadotropin hormones and receptor crosstalk. *International Journal of Biological Macromolecules*, **260:** 129524.

Basaria S. (2013). Reproductive aging in men. *Endocrinology and metabolism clinics of North America*. **42**(2): 255–270.

Bassil, N., Alkaade, S., and Morley, J. E. (2009). The benefits and risks of testosterone replacement therapy: a review. *Therapeutics and clinical risk management*. **5**(3): 427–448.

Ben-Jonathan, N., Hugo, E. R., Brandebourg, T. D., and LaPensee, C. R. (2006). Focus on prolactin as a metabolic hormone. *Trends in endocrinology and metabolism: TEM*, **17**(3): 110–116.

Bernard, D. J., Li, Y., Toufaily, C., and Schang, G. (2019). Regulation of gonadotropins. *Oxford Research Encyclopedia of Neuroscience*.

Bole-Feysot, C., Goffin, V., Edery, M., Binart, N., and Kelly PA (1998). Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptor knockout mice. *Endocrine Reviews*. **19** (3): 225–68.

Boulpaep, E. L., and Boron, W. F. (2005). Medical physiology: a cellular and molecular approach. *St. Louis, Mo: Elsevier Saunders*. p. 1125.

Cahoreau, C., Klett, D., and Combarnous, Y. (2015). Structure-function relationships of glycoprotein hormones and their subunits' ancestors. *Frontiers in endocrinology*, **6**, 26.

Carrasco-Juan, J. L., Álvarez-Argüelles Cabrera, H., Martín Corriente, M. C., González-Gómez, M., Valladares Parrilla, R., Gutiérrez García, R., and Díaz-Flores, L. (2017). Ovarian Leydig cells (OLC): A histomorphological and immunohistochemical study. *Histology and histopathology*. **32**(10): 1089–1097.

Carreau, S., Lambard, S., Delalande, C., Denis-Galeraud, I., Bilinska, B., and Bourguiba, S. (2003). Aromatase expression and role of estrogens in male gonad: a review. *Reproductive Biology and Endocrinology*. **1**(1): 35

Čeponis, J., Wang, C., Swerdloff, R. S. and Liu, P. Y. (2017). Anabolic and Metabolic Effects of Testosterone and Other Androgens: Direct Effects and Role of Testosterone Metabolic Products. *Thyroid Diseases*. pp. 1–22.

Chawre, S., Khatib, M. N., Rawekar, A., Mahajan, S., Jadhav, R., and More, A. (2024). A Review of Semen Analysis: Updates From the WHO Sixth Edition Manual and Advances in Male Fertility Assessment. *Cureus*. https://doi.org/10.7759/cureus.63485

Crowley, W. R. (2014). Neuroendocrine regulation of lactation and milk production. *Comprehensive Physiology*, 255–291.

Das, N., and Kumar, T. R. (2018a). Molecular regulation of follicle-stimulating hormone synthesis, secretion and action. *Journal of Molecular Endocrinology*, **60**(3): R131–R155.

De Mattos, K., Pierre, K. J., and Tremblay, J. J. (2023). Hormones and Signaling Pathways Involved in the Stimulation of Leydig Cell Steroidogenesis. *Endocrines*, *4*(3), 573–594. https://doi.org/10.3390/endocrines4030041

Durdiakova, J., Ostatnikova, D., and Celec, P. (2011). Testosterone and its metabolites - modulators of brain functions. *Acta Neurobiologiae Experimentalis*, **71**(4): 434–454.

Ezcurra, D., and Humaidan, P. (2014). A review of luteinising hormone and human chorionic gonadotropin when used in assisted reproductive technology. *Reproductive biology and endocrinology: RBandE*. **12**(1): 95.

Fanis, P., Neocleous, V., Papapetrou, I., Phylactou, L. A., and Skordis, N. (2023). Gonadotropin-Releasing Hormone Receptor (GnRHR) and Hypogonadotropic Hypogonadism. *International Journal of Molecular Sciences*, **24**(21): 15965. https://doi.org/10.3390/ijms242115965.

Fisch, H., and Goldstedin, R. (2003). Environmental estrogens and sperm counts (PDF). *Pure and Applied Chemistry*. **75** (11–12): 2181–2193.

Grant, P. (2010). Spearmint herbal tea has significant anti-androgen effects in polycystic ovarian syndrome. A randomized controlled trial. *Phytotherapy research: PTR*. **24**(2): 186–188.

Hackney, A. C., Moore, A. W., and Brownlee, K. K. (2005). Testosterone and endurance exercise: development of the "exercise-hypogonadal male condition". *Acta physiologica Hungarica*. **92**(2): 121–137.

Håkonsen, L. B., Thulstrup, A. M., Aggerholm, A. S., Olsen, J., Bonde, J. P., Andersen, C. Y., Bungum, M., Ernst, E. H., Hansen, M. L., Ernst, E. H., and Ramlau-Hansen, C. H. (2011). Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reproductive health*. **8**(1): 24.

Handelsman, D. J. (2001). Estrogens and falling sperm counts. *Reproduction, fertility, and development*. **13**(4): 317–324.

Hapsari, R. A. F., and Wulandari, E. (2023). Histological Properties of Male Rats Testicular Organs after Given Jatropha curcas Seed Extract. *International Journal of Infertility and Fetal Medicine*, **14**(1): 1–7. https://doi.org/10.5005/jp-journals-10016-1307

Hofmann, M., and Giwercman, A. (2023). Toward standardization in reporting data on basic human semen examination: A crucial step in advancing the field of andrology and reproductive medicine. *Andrology*, **11**(7): 1223–1224. https://doi.org/10.1111/andr.13518

Hohl, A., Zanela, F. A., Ghisi, G., Ronsoni, M. F., Diaz, A. P., Schwarzbold, M. L., Dafre, A. L., Reddi, B., Lin, K., Pizzol, F. D., and Walz, R. (2018). Luteinizing Hormone and Testosterone Levels during Acute Phase of Severe Traumatic Brain Injury: Prognostic Implications for Adult Male Patients. *Frontiers in Endocrinology*, **9**.

Huhtaniemi, I. (2014). Late-onset hypogonadism: current concepts and controversies of pathogenesis, diagnosis and treatment. *Asian journal of andrology*. **16**(2): 192–202.

Huhtaniemi, I. T. (2014). Andropause--lessons from the European Male Ageing Study. *Annales d'Endocrinologie*. **75** (2): 128–131.

Hulmi, J. J., Ahtiainen, J. P., Selänne, H., Volek, J. S., Häkkinen, K., Kovanen, V. *et al*. (2008). Androgen receptors and testosterone in men—effects of protein ingestion, resistance exercise and fiber type. *The Journal of Steroid Biochemistry and Molecular Biology*. **110** (1–2): 130–37.

Ilahi, S., and Ilahi, T. B. (2022). Anatomy, Adenohypophysis (Pars Anterior, Anterior Pituitary). In *StatPearls*. StatPearls Publishing.

Iwalewa, E. O., Adewunmi, C. O., Omisore, N. O., Adebanji, O. A., Azike, C. K., Adigun, A. O., Adesina, O. A., and Olowoyo, O. G. (2005). Pro- and antioxidant effects and cytoprotective potentials of nine edible vegetables in southwest Nigeria. *Journal of medicinal food*. **8**(4): 539–544.

Izzi, A., Benie, T., Thieulant, M.-L., Men-Olivier, L., and Duval, J. (1992). Stimulation of LH Release from Cultured Pituitary Cells by Saponins of *Petersianthus macrocarpus*: A Permeabilizing Effect. *Planta Medica*, **58**(03): 229–233. https://doi.org/10.1055/s-2006-961441

Jiang X, Liu H, Chen X, Chen PH, Fischer D, Sriraman V, *et al*. (2012). Structure of follicle-stimulating hormone in complex with the entire ectodomain of its

receptor. *Proceedings of the National Academy of Sciences of the United States of America*. **109** (31): 12491–6.

Jiang, X., Dias, J. A., and He, X. (2014). Structural biology of glycoprotein hormones and their receptors: insights to signaling. *Molecular and cellular endocrinology*. **382**(1): 424–451.

Kavoussi, P. K., Hudson, K., Machen, G. L., Barsky, M., Lebovic, D. I., and Kavoussi, S. K. (2021). FSH levels and testicular volumes are associated with the severity of testicular histopathology in men with non-obstructive azoospermia. *Journal of Assisted Reproduction and Genetics*, **38**(11): 3015–3018. https://doi.org/10.1007/s10815-021-02313-y

Kim, Y., Kobayashi, A., Sekido, R., DiNapoli, L., Brennan, J., Chaboissier, M. C., Poulat, F., Behringer, R. R., Lovell-Badge, R., and Capel, B. (2006). Fgf9 and Wnt4 act as antagonistic signals to regulate mammalian sex determination. *PLoS biology*. **4**(6): e187.

Koehler, K., Parr, M. K., Geyer, H., Mester, J., and Schänzer, W. (2009). Serum testosterone and urinary excretion of steroid hormone metabolites after administration of a high-dose zinc supplement. *European journal of clinical nutrition*. **63**(1): 65–70.

Koskenniemi, J. J., Virtanen, H. E., and Toppari, J. (2017). Testicular growth and development in puberty. *Current Opinion in Endocrinology Diabetes and Obesity*, **24**(3): 215–224.

Kumar, V., Kural, M. R., Pereira, B. M., and Roy, P. (2008). Spearmint induced hypothalamic oxidative stress and testicular anti-androgenicity in male rats - altered levels of gene expression, enzymes and hormones. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association*. **46**(12): 3563–3570.

Lacasse, P. (2025). Interactions between prolactin and local regulation of the mammary gland. *Journal of Dairy Science*.

Laszczyńska, M. (2015). Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging. *Environmental Geochemistry and Health*, **38**(3): 749–761.

le Cotonnec, J. Y., Porchet, H. C., Beltrami, V., and Munafo, A. (1998). Clinical pharmacology of recombinant human luteinizing hormone: Part I. Pharmacokinetics after intravenous administration to healthy female volunteers and comparison with urinary human luteinizing hormone. *Fertility and sterility*. **69**(2): 189–194.

Lei, T., Yang, Y., and Yang, W. (2025). Luteinizing hormone regulates testosterone production, leydig cell proliferation, differentiation, and circadian rhythm during spermatogenesis. *International Journal of Molecular Sciences*, **26**(8): 3548.

Liu, L., Kang, J., Ding, X., Chen, D., Zhou, Y., and Ma, H. (2015). Dehydroepiandrosterone-Regulated Testosterone Biosynthesis via Activation of the ERK1/2 Signaling Pathway in Primary Rat Leydig Cells. *Cellular Physiology and Biochemistry*. **36** (5): 1778–1792.

Livera, G., Rouiller-Fabre, V., Pairault, C., Levacher, C. and Habert R (2002). Regulation and perturbation of testicular functions by vitamin A. *Reproduction*. **124** (2): 173–180.

Lopez-Vicchi, F., De Winne, C., Brie, B., Sorianello, E., Ladyman, S. R., and Becu-Villalobos, D. (2020). Metabolic functions of prolactin: Physiological and pathological aspects. *Journal of Neuroendocrinology*, **32**(11).

Lucas, B. K., Ormandy, C. J., Binart, N., Bridges, R. S., and Kelly, P. A. (1998). Null mutation of the prolactin receptor gene produces a defect in maternal behavior. *Endocrinology*. **139**(10): 4102–4107.

Luetjens, C. M. and Weinbauer, G., F (2012). Testosterone: Biosynthesis, transport, metabolism and (non-genomic) actions. *Testosterone: Action, Deficiency, Substitution*. **4**(2): 15–32.

MacDonald, A. A., Herbison, G. P., Showell, M., and Farquhar, C. M. (2010). The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Human reproduction update*. **16**(3): 293–311.

Martin, L. J., and Touaibia, M. (2020). Improvement of Testicular Steroidogenesis Using Flavonoids and Isoflavonoids for Prevention of Late-Onset Male Hypogonadism. *Antioxidants*, **9**(3): 237. https://doi.org/10.3390/antiox9030237

Millar, A. C., Faghfoury, H., and Bieniek, J. M. (2021). Genetics of hypogonadotropic hypogonadism. *Translational Andrology and Urology*, **10**(3): 1401–1409. https://doi.org/10.21037/tau.2020.03.33

Minnetti, M., De Alcubierre, D., Bonaventura, I., Pofi, R., Hasenmajer, V., Tarsitano, M. G., Gianfrilli, D., Poggiogalle, E., and Isidori, A. M. (2022). Effects of licorice on sex hormones and the reproductive system. *Nutrition*, **103**–104: 111727.

Monageng, E., Offor, U., Takalani, N. B., Mohlala, K., and Opuwari, C. S. (2023). A Review on the Impact of Oxidative Stress and Medicinal Plants on Leydig Cells. *Antioxidants*, **12**(8): 1559. https://doi.org/10.3390/antiox12081559

Moniot, B., Declosmenil, F., Barrionuevo, F., Scherer, G., Aritake, K., Malki, S., Marzi, L., Cohen-Solal, A., Georg, I., Klattig, J., Englert, C., Kim, Y., Capel, B., Eguchi, N., Urade, Y., Boizet-Bonhoure, B., and Poulat, F. (2009). The PGD2 pathway, independently of FGF9, amplifies SOX9 activity in Sertoli cells during male sexual differentiation. *Development*. **136** (11): 1813–1821

Monson, N. R., Klair, N., Patel, U., Saxena, A., Patel, D., Ayesha, I. E., and Nath, T. S. (2023). Association between vitamin D deficiency and testosterone levels in adult males: a systematic review. *Cureus.*

Motl, R. W., Suh, Y., Balantrapu, S., Sandroff, B. M., Sosnoff, J. J., Pula, J., Goldman, M. D., and Fernhall, B. (2012). Evidence for the different physiological significance of the 6- and 2-minute walk tests in multiple sclerosis. *BMC Neurology*, **12**(1). https://doi.org/10.1186/1471-2377-12-6

Nicholls, P. K., Stanton, P. G., Chen, J. L., Olcorn, J. S., Haverfield, J. T., Qian, H., Walton, K. L., Gregorevic, P., and Harrison, C. A. (2012). Activin signaling regulates Sertoli cell differentiation and function. *Endocrinology*. **153** (12): 6065–6077.

O'Donnell, L., Nicholls, P. K., O'Bryan, M. K., McLachlan, R. I., and Stanton, P. G. (2011). Spermiation: The process of sperm release. *Spermatogenesis*. **1**(1): 14–35.

Omoregie, E. S. and Sisodia, B. S. (2011). In vitro anti-plasmodial activity and cytotoxicity of leaf extract from Jatropha tanjorensis. *Pharmacology Online*. **2**: 656-673.

Omoregie, E.S. and Osagie, A.U. (2007). Phytochemical screening and anti-anemic effect of Jatropha tanjorensis leaf in protein malnourished rats. *Plant Arch*. **7**(2): 509-516.

Oseghale, I. D., Ikokwu, G. M., Ralph-Okhiria, O. H., Odunvbun, P., and Adedotun, A. M. (2025). Comprehensive Ethnomedicinal and Phytopharmacological Review of Jatropha tanjorensis. *Asian Journal of Research in Biochemistry*, **15**(4): 62–76.

Oyewole, O. (2011). Phytochemical Analysis and Hypolipidemic Properties of Jatropha tanjorensis Leaf Extract. *European Journal of Medicinal Plants*, **1**(4): 180–185.

Partin, Alan W., Wein, Alan J., Kavoussi, Louis R., Peters, Craig A., and Dmochowski, Roger, R. (2020). Campbell Walsh Wein Urology. *Elsevier Health Sciences*. p. 1124.

Pentikäinen, V., Erkkilä, K., Suomalainen, L., Parvinen, M., and Dunkel L (2000). Estradiol acts as a germ cell survival factor in the human testis in vitro. *The Journal of Clinical Endocrinology and Metabolism*. **85** (5): 2057–67.

Pilz, S., Frisch, S., Koertke, H., Kuhn, J., Dreier, J., Obermayer-Pietsch, B., Wehr, E., and Zittermann, A. (2011). Effect of vitamin D supplementation on testosterone levels in men. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolism*. **43**(3): 223–225.

Pitteloud, N., Dwyer, A. A., DeCruz, S., Lee, H., Boepple, P. A., Crowley, W. F., Jr, and Hayes, F. J. (2008). Inhibition of luteinizing hormone secretion by testosterone in men requires aromatization for its pituitary but not its hypothalamic effects: evidence from the tandem study of normal and gonadotropin-releasing hormone-deficient men. *The Journal of clinical endocrinology and metabolism*. **93**(3): 784–791.

Plant, T. M., and Marshall, G. R. (2001). The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocrine reviews*. **22**(6): 764–786.

Raman, J. D., and Schlegel, P. N. (2002). Aromatase inhibitors for male infertility. *The Journal of urology*. **167**(2 Pt 1): 624–629.

Rastrelli, G., Corona, G., Mannucci, E., and Maggi, M. (2014). Factors affecting spermatogenesis upon gonadotropin-replacement therapy: a meta-analytic study. *Andrology*. **2**(6): 794–808.

Rato, L., Alves, M. G., Socorro, S., Duarte, A. I., Cavaco, J. E., and Oliveira, P. F. (2012). Metabolic regulation is important for spermatogenesis. *Nature reviews. Urology*. 9(6), 330–338.

Rhoades, Rodney A., and Bell, David R. (2022). Medical Physiology: Principles for Clinical Medicine. *Lippincott Williams and Wilkins*. p. 2031.

Rotter, I., Kosik-Bogacka, D. I., Dołęgowska, B., Safranow, K., Kuczyńska, M., and Laszczyńska, M. (2015). Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging. *Environmental Geochemistry and Health*, **38**(3): 749–761.

Salonia, A., Rastrelli, G., Hackett, G., Seminara, S. B., Huhtaniemi, I. T., Rey, R. A., Hellstrom, W. J. G., Palmert, M. R., Corona, G., Dohle, G. R., Khera, M., Chan, Y.-M., and Maggi, M. (2019). Paediatric and adult-onset male hypogonadism. *Nature Reviews. Disease Primers*, **5**(1): 38. https://doi.org/10.1038/s41572-019-0087-y

Sapozhkova, Zh. Yu., and Eremin, K. I. (2020). UPDATES IN PROTOCOL FOR HUMAN SEMEN EXAMINATION. *Russian Clinical Laboratory Diagnostics*, **65**(2): 106–110. https://doi.org/10.18821/0869-2084-2020-65-2-106-110

Schultheiss, O. C., Campbell, K. L., and McClelland, D. C. (1999). Implicit power motivation moderate men's testosterone responses to imagined and real dominance success. *Hormones and behavior*. **36**(3): 234–241.

Schulze, W. (2009). Evidence of a Wave of Spermatogenesis in Human Testis. *Andrologia*. **14** (2): 200–207.

Sfetcu, N. (2014). Health and Drugs: Disease, Prescription and Medication.

Shah, W., Khan, R., Shah, B., Khan, A., Dil, S., Liu, W., Wen, J., and Jiang, X. (2021). The Molecular Mechanism of Sex Hormones on Sertoli Cell Development and Proliferation. *Frontiers in endocrinology*. **12**: 648141.

Sharma, S., Hanukoglu, A., and Hanukoglu, I. (2018). Localization of epithelial sodium channel (ENaC) and CFTR in the germinal epithelium of the testis, Sertoli cells, and spermatozoa. *Journal of molecular histology*. **49**(2): 195–208.

Sharma, T. P., Nett, T. M., Karsch, F. J., Phillips, D.J., Lee, J. S., Herkimer, C., *et al*. (2012). Neuroendocrine control of FSH secretion: IV. Hypothalamic control of

pituitary FSH-regulatory proteins and their relationship to changes in FSH synthesis and secretion. *Biology of Reproduction*. **86** (6): 171.

Sharpe, R. M., McKinnell, C., Kivlin, C., and Fisher, J. S. (2003). Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction (Cambridge, England)*. **125**(6): 769–784.

Sheffield-Moore, M (2000). Androgens and the control of skeletal muscle protein synthesis. *Annals of Medicine*. **32** (3): 181–186.

Silva, M. A., Villaseñor, R. M. V., Márquez, S. R., González, M. H., Jaime, H. B., García, X. G., and Montiel, J. L. C. (2013). Testosterone Levels and Development of the Penile Spines and Testicular Tissue during the Postnatal Growth in Wistar Rats. *Advances in Sexual Medicine,* **03**(03): 1–9.

Singh, M. K., Nair, N., and Bedwal, R. S. (2015). Prepubertal to postpubertal development of testes in Wistar rats. *The Egyptian Journal of Histology*, **38**(1): 116–125.

Smith, T., and Batur, P. (2020). Prescribing testosterone and DHEA: The role of androgens in women. *Cleveland Clinic Journal of Medicine*, **88**(1): 35–43.

Song, N., Liu, J., An, S., Nishino, T., Hishikawa, Y., and Koji, T. (2011). Immunohistochemical Analysis of Histone H3 Modifications in Germ Cells during Mouse Spermatogenesis. *Acta histochemica et cytochemica*. **44**(4): 183–190.

Stamatiades, G. A., and Kaiser, U. B. (2018). Gonadotropin regulation by pulsatile GnRH: Signaling and gene expression. *Molecular and cellular endocrinology*. **463**: 131–141.

Stoléru, S. G., Ennaji, A., Cournot, A., and Spira, A. (1993). LH pulsatile secretion and testosterone blood levels are influenced by sexual arousal in human males. *Psychoneuroendocrinology*. **18**(3): 205–218.

Strickland, T. W., Parsons, T. F., and Pierce, J. G. (2019). Structure of LH and HCG. In CRC Press eBooks (pp. 1–15).

Sunder, M., and Leslie, S. W. (2025). Semen Analysis. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK564369/

Tilbrook, A. J., and Clarke, I. J. (2001). Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. *Biology of reproduction*. **64**(3): 735–742.

Torjesen, P. A. and Sandnes, L. (2004). Serum testosterone in women as measured by an automated immunoassay and a RIA. *Clinical Chemistry*. **50** (3): 678-679.

Tuck, S. P. and Francis, R.M. (2009). "Testosterone, bone and osteoporosis". Advances in the Management of Testosterone Deficiency. *Frontiers of Hormone Research*. **37**:123–32.

Ubuka, T., Son, Y. L., Tobari, Y., Narihiro, M., Bentley, G. E., Kriegsfeld, L. J., and Tsutsui, K. (2014). Central and direct regulation of testicular activity by gonadotropin-inhibitory hormone and its receptor. *Frontiers in endocrinology*. **5**: 8.

Ulloa-Aguirre, A., Reiter, E., and Crépieux, P. (2018). FSH Receptor Signaling: Complexity of Interactions and Signal Diversity. *Endocrinology*. **159**(8): 3020–3035.

Ukraintseva, Y., Liaukovich, K., Polishchuk, A., Martynova, O., Belov, D., Simenel, E., Cruz, M. M. E., and Nizhnik, A. (2018). Slow-wave sleep and androgens: selective slow-wave sleep suppression affects testosterone and 17α-hydroxyprogesterone secretion. *Sleep Medicine*, **48:** 117–126.

Vingren, J. L., Kraemer, W. J., Ratamess, N. A., Anderson, J. M., Volek, J. S., and Maresh, C. M. (2010). Testosterone physiology in resistance exercise and training: the up-stream regulatory elements. *Sports medicine (Auckland, N.Z.)*. **40**(12): 1037–1053.

Visootsak, J., and Graham, J. M., Jr (2006). Klinefelter syndrome and other sex chromosomal aneuploidies. *Orphanet journal of rare diseases*. **1**(1): 42.

Wallace, E. M., Groome, N. P., Riley, S. C., Parker, A. C., and Wu, F. C. W. (1997). Effects of Chemotherapy-Induced Testicular Damage on Inhibin, Gonadotropin, and Testosterone Secretion: A Prospective Longitudinal Study. *The Journal of Clinical Endocrinology and Metabolism*, **82**(9): 3111–3115. https://doi.org/10.1210/jcem.82.9.4238

Xiong, X., Wang, A., Liu, G., Liu, H., Wang, C., Xia, T., Chen, X., and Yang, K. (2006). Effects of p,p'-dichlorodiphenyldichloroethylene on the expressions of transferrin and androgen-binding protein in rat Sertoli cells. *Environmental Research*. **101** (3): 334–339.

Xu, W., Morford, J., and Mauvais-Jarvis, F. (2019). Emerging role of testosterone in pancreatic β cell function and insulin secretion. *Journal of Endocrinology*, **240**(3): R97–R105.

Zanato, V. F., Martins, M. P., Anselmo-Franci, J. A., Petenusci, S. O., and Lamano-Carvalho, T. L. (1994). Sexual development of male Wistar rats. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas*. **27**(5): 1273–1280.

Zirkin, B. R., and Papadopoulos, V. (2018). Leydig cells: formation, function, and regulation. *Biology of reproduction*. **99**(1): 101–111.Chawre, S., Khatib, M. N., Rawekar, A., Mahajan, S., Jadhav, R., & More, A. (2024). A Review of Semen Analysis: Updates From the WHO Sixth Edition Manual and Advances in Male Fertility Assessment. *Cureus*. https://doi.org/10.7759/cureus.63485

Hofmann, M., & Giwercman, A. (2023). Toward standardization in reporting data on basic human semen examination: A crucial step in advancing the field of andrology and reproductive medicine. *Andrology*, *11*(7), 1223–1224. https://doi.org/10.1111/andr.13518

John, A., Bawa, I., John, A., Yakubu, O. E., & Richard, O. I. (2020). Effects of Selected Secondary Metabolites in Leaf Extract of Jatropha Tanjorensis on Some Gonadal Hormones in Male Wistar Rats. *Journal of Biotechnology and Biomedical Science*, *2*(3), 31–42. https://doi.org/10.14302/issn.2576-6694.jbbs-20-3466

Sapozhkova, Zh. Yu., & Eremin, K. I. (2020). UPDATES IN PROTOCOL FOR HUMAN SEMEN EXAMINATION. *Russian Clinical Laboratory Diagnostics*, *65*(2), 106–110. https://doi.org/10.18821/0869-2084-2020-65-2-106-110

Sunder, M., & Leslie, S. W. (2025). Semen Analysis. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK564369/

Ukoh, I., Sunday, B., Antai, A., Arikpo, S., Nsa, H., & David, I. (2022). Consumption of Aqueous Leaf Extract of Jatropha tanjorensis Improves Fertility Potential and Gestational Outcome in Virgin Female Wistar Rats. *Nigerian Journal of Physiological Sciences: Official Publication of the Physiological Society of Nigeria*, *37*(2), 247–253.

Zhang, S., Qi, H., Wen, X., Li, P., Gao, X., and Ao, J. (2018). The phosphorylation of Tudor-SN mediated by JNK is involved in the regulation of milk protein synthesis induced by prolactin in BMECs. *Journal of Cellular Physiology*, **234**(5): 6077–6090.