EFFECTS OF BILATERAL ORCHIDECTOMY ON HORMONE PROFILE OF MALE WISTAR RATS

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A PROJECT SUBMITTED TO THE DEPARTMENT OF ANATOMY,

SCHOOL OF BASIC MEDICAL SCIENCES,

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CERTIFICATION

This is to certify that this work was carried out under my supervision by **KUALE, OKIEMUTE OGHENETEGA,** of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfilment of the award of Bachelor of Science Degree (B.Sc.)

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EXTERNAL EXAMINER **DATE**

DEDICATION

I dedicate this work to God for his mercy, grace, provision and strength throughout my Undergraduate programme. I also dedicate this work to my family.

ACKNOWLEDGEMENT

First and foremost, I acknowledge God almighty for his mercy and grace. To my parents whose support, love, sacrifices and counsel were always in abundance.

To my supervisor, Dr. C. L. Sakpa who was there to provide guidance and assistance throughout this project. To my loving Family members, Grandma, Uncle Sunny, Aunty Bero, Aunty Amina and Aunty Dede and friends, for their love, advise and support.

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ABSTRACT

Bilateral orchidectomy is a surgical procedure usually carried out for males with prostate cancer and other diseases affecting both testes. This study was carried out to observe the effect of bilateral orchidectomy on the gonadotropin hormones, (LH, FSH), PRL and the steroid hormones, (Progesterone, Testosterone and Oestrogen). Ten (10) male Wistar rats were used for the experiment. The rats were divided into two experimental groups: 1- control (Co) (n=5), 2 - Treatment group (Tr) (n=5). The rats in the treatment group were bilaterally orchidectomized under chloroform anaesthesia. The rats were sacrificed after 4 weeks. Blood samples were collected from the IVC and assayed for LH, FSH, Prolactin, Progesterone, Testosterone and Oestrogen hormones. The study showed that bilateral orchidectomy increased serum levels of LH and FSH concentrations (3.247±0.152 and 1.177±0.039) which were statistically significant ($p<0.05$). There was no change in the serum level of Prolactin ($p>0.05$). However there was statistically significant decrease ($p<0.05$) in the serum concentrations of Progesterone, Testosterone and Oestrogen. This study showed that bilateral orchidectomy increased serum level of LH and FSH, the two hormones principally required for stimulating Testosterone production and spermatogenesis respectively. The sex steroids, Progesterone, Testosterone and Oestrogen were decreased. Testosterone is needed for spermatogenesis and in conjunction, FSH results in spermiation. The decrease in testosterone therefore may lead to infertility in the castrated rats.

CHAPTER ONE

INTRODUCTION

Castration includes any action by which an individual loses the use of his testes (Abraham, 2018). Castration can be performed either surgically or chemically (Sarin *et al.,* 2020). However, Christine (2018) asserted that surgical castration is usually bilateral orchidectomy, that is, removal of both testes and epididymis, but may also include other surgical measures that result in loss of testicular function, while chemical castration comprises the local, intratesticular use of pharmaceutical drugs and substances that cause coagulation and subsequent degeneration of testicular tissue**.**

An orchidectomy is done to treat cancer or, for other reasons, to lower the level of testosterone, the primary male sex hormone, in the body. Surgical removal of a testicle is the usual treatment if a tumour is found within the gland itself, but an orchidectomy may also be performed to treat prostate cancer or cancer of the male breast, as testosterone causes these cancers to grow and metastasize. An orchidectomy is sometimes done to prevent cancer when an undescended testicle is found in a patient who is beyond the age of puberty**.** There are three types of orchidectomy;

- Simple Orchidectomy
- Subcapsular Orchidectomy
- Inguinal or Radical Orchidectomy (Frey, 2004)

1. Simple orchidectomy; is approached through the scrotum, and historically has been a significant means of hormonal manipulation in the management of patients with locally advanced prostate cancer. This indication has, over time, been replaced by the less invasive hormonal medications such as gonadotropin-releasing hormone agonists or antagonists. This approach to therapy has limited the indications of simple orchiectomy to the removal of an atrophic testis and non-viable testis due to trauma, torsion, or infection (Okoye *et al.*, 2021).

2. Subcapsular Orchidectomy; this can either be unilateral or bilateral. A subcapsular orchiectomy is also performed for treatment of prostate cancer. The operation is similar to a simple orchiectomy, with the exception that the glandular tissue is removed from the lining of each testicle rather than the entire gland being removed (Frey., 2004). In 1942, Riba described subcapsular bilateral orchiectomy as a surgical method of avoiding the "empty scrotum" without damaging the oncological effectiveness (Riba, 1942)**.** This type of orchiectomy is done primarily to keep the appearance of a normal scrotum (Frey, 2004).

3. Radical/Inguinal Orchidectomy; the standard‐of‐care for the removal and treatment of testis cancer includes a radical inguinal orchiectomy (Nerli *et al.*, 2010)**.** This is the most common operation performed for testis cancer world wide. It may be either unilateral, involving only one testis, or bilateral. This procedure is

called an inguinal orchiectomy because the surgeon makes the incision, which is about 3 in (7.6 cm) long, in the patient's groyne(grion) area rather than directly into the scrotum. It is called a radical orchiectomy because the surgeon removes the entire spermatic cord as well as the testicle itself. The reason for this complete removal is that testicular cancer frequently spreads from the spermatic cord into the lymph nodes near the kidneys. A long non-absorbable suture is left in the stump of the spermatic cord in case later surgery is necessary (Frey, 2004).

1.2 INDICATIONS FOR ORCHIDECTOMY

- Undescended testis (UDT); this is a common condition in childhood, and it is related to endocrine disorders, sperm damage, and testicular deterioration. It is estimated to affect 1 to 4% of full term and up to 30% of preterm male neonates (Wei Yi *et al*., 2018**).** Terms such as undescended testis, retentio testis, cryptorchidism, and maldescended testis describe a testis that is not normally located at the bottom of the scrotum (Jerzy *et al.*, 2016). Cryptorchidism is the absence of at least one testicle from the scrotum. It is the most common birth defect involving the male genitalia. About 3% of full-term and 30% of premature male infants are born with one or both testicles undescended. Approximately 80% of cryptorchid testes descend by the third month of life. This makes the true incidence around 1%. Cryptorchidism may occur on one or both sides but more commonly affects the right testicle. The testicle may be anywhere along the "path

of descent," such as: Located high in the retroperitoneal abdomen to the inguinal ring; In the inguinal canal; ectopic from the path of descent; hypoplastic; dysgenetic; missing or absent; unilateral (two-thirds). The undescended testicle can usually be palpated in the inguinal canal. In a minority of patients, the missing testicle may be located in the abdomen or be nonexistent. Undescended testicles are associated with decreased fertility (bilateral cases), increased testicular germ cell tumours (overall risk under 1%), testicular torsion, inguinal hernias, and psychological problems. To reduce risks, undescended testes may be brought into the scrotum with an orchiopexy (Leslie *et al.,* 2022). Unlike cryptorchidism in children, postpubertal cryptorchidism is associated with an increased probability of neoplasms, which has led orchiectomy to be the recommended treatment (Jae *et al.,* 2015).

- Testicular torsion; this refers to the twisting of the spermatic cord structures with the subsequent loss of perfusion to the homolateral testicle. It is a urological emergency; early diagnosis and surgery are vital to saving the testicle and preserving future fertility (Alain *et al.,* 2020). In patients with testicular torsion, the blood flow in the symptomatic testis is decreased or absent compared with the asymptomatic testis, 4-8% of cases of testicular torsion are as a result of trauma**.** Once the diagnosis of testicular torsion is confirmed, the rapid restoration of blood flow to the testis is critical, other factors predisposing patients to testicular torsion

include an increase in testicular volume (often associated with puberty), testicular tumour, testicles with horizontal lie, a history of cryptorchidism, and a spermatic cord with a long intrascrotal portion (Erika *et al.,* 2005). Delay in care may necessitate orchiectomy which has been associated with reduced fertility (Jonathan *et al.,* 2005)**.** Testicular torsion accounts for approximately 10% to 15% of acute scrotal disease in children, and results in an orchiectomy rate of 42% in boys undergoing surgery for testicular torsion. Orchiectomy is performed if the affected testicle appears grossly necrotic or nonviable, delay in treatment may be associated with decreased fertility (Victoria *et al.,* 2013).

- Inflammatory diseases of the testis; orchitis is an inflammation of the testicle unilaterally or bilaterally usually caused by viruses and bacteria, bacterial infections of the prostate and urinary tract infection can cause orchitis, bacteria that can cause sexually transmitted infections can also cause orchitis in sexually active males**.** Orchitis caused by bacteria is treated with antibiotics and anti inflammatory, antibiotics are not necessary for viral causes of the disease, instead there are suggestive therapies like bed rest, antipyretics, analgesics, scrotal support, and hot & cold packs for analgesia (Azmat *et al.,* 2021).

- Benign conditions of the testis; diseases that affect the testis can span from being non- malignant or benign to life threatening and agonising, benign testicular

disorders (BTDs) are the most common non-cancerous testicle problems that present as painless lumps or swellings in the scrotum (Dua *et al.*, 2017). Testicular microlithiasis (TM), which usually affects both testes, is diagnosed primarily by ultrasound. TM has been found to be associated with benign conditions but has also been reported in association with testicular cancer (Robert *et al.*, 1996). Testicular microlithiasis is found in approximately 5% of asymptomatic young men, studies report TM in association with conditions that increase the risk for developing testicular germ cell cancer (TGCT), such as cryptorchidism intratubular germ cell neoplasia of unclassified type (ITGCNU). There also are several case reports of the interval development of testicular tumours in patients who had a previous sonographic diagnosis of TM (Lain *et al.*, 2010).

Cases of cancer

1. Intratesticular cancer; this can also be called testicular cancer, affects the testis. More than half of painless solid swellings of the body of the testis are malignant, with a peak incidence in men aged 30 to 34 years. Most testicular cancers are germ cell tumours and half of these are seminomas, which tend to affect older men and have a good prognosis (Peter *et al* ., 2016). Cryptorchidism increases the risk of developing testicular cancer in the affected testicle from four to sixfold. The risk factors for the development of testicular cancer are generally associated with testicular dysgenesis syndrome. The list of risk factors for testicular cancer

includes cryptorchidism, hypospadias, decreased spermatogenesis evidenced by subfertility or infertility, a positive familial history of testicular tumours in first degree relatives, childhood inguinal hernias, paediatric atrophic testis, germ cell neoplasia in situ (GCNIS), and a history of a contralateral testicular malignancy (Nauman *et al* ., 2022).

2. Extratesticular cancer; The extratesticular scrotal contents consist of the epididymis, spermatic cord, and fascia. The fascia is derived from the embryologic descent of the testis through the abdominal wall; most extratesticular masses are benign (Paula *et al* ., 2003).
    - The Epididymis; it lies over the testis and receives the spermatozoa produced by the testis. Epididymis tumours are commonly soft-tissue or mesothelial neoplasm in origin. Cystadenomas, papillary tumours and adenomatoid tumours are the most common tumours (Lijian *et al.*, 2015). Malignant neoplasms of the epididymis are rare; statistics have shown that the epididymal tumour makes up about 0.03% of all male cancers (Ching-hei *et al* ., 2012). The most common extratesticular neoplasm found in the epididymis is the adenomatoid tumour. Extratesticular lesions can occur on the epididymis as epididymitis which is benign, most frequently it can lead to an acute scrotum, which can be acute or chronic and may develop into epididymo-orchitis or scrotal abscess. Symptoms include, epididymal

enlargement, skin thickening, hydroceles, and hyperemia (Paula *et al* ., 2003).

- The Spermatic cord; they are rare tumours, usually present as unilaterally, hard, firm slow-growing masses (weeks or more than 10 years) of the inguinal canal or the scrotum, whose size varies between 1.5 and 30 cm. These are frequently irregular masses that are clearly distinct from the testis, and usually do not transilluminate light during physical exam evaluation. They may be accompanied by pain, a hydrocele, or symptoms secondary to metastasis. Apparently most of these tumours are benign and approximately 25% constitute potentially life-threatening malignant tumours. The most common reported malignant histological types include liposarcomas, leiomyosarcomas, rhabdomyosarcomas, malignant fibrous histiocytoma, and fibrosarcomas. A large percentage of the spermatic cord neoplasia (70-80%) are benign and they are composed primarily of lipoma (Dayron *et al* ., 2012).

Generally extratesticular malignant neoplasms despite their relative rarity do occur, they include; rhabdomyosarcoma, liposarcoma, leiomyosarcoma, malignant fibrous histiocytoma, mesothelioma, and lymphoma (Paula *et al* ., 2003).

3. Prostate Cancer; this cancer is the second most frequent malignancy in men and the fifth leading cause of death worldwide. It may be asymptomatic at the early stage. However, the most frequent complaint is difficulty with urination, increased frequency, and nocturia, all symptoms that may also arise from prostatic hypertrophy. More advanced stages of the disease may present with urinary retention and back pain, as axis skeleton is the most common site of bony metastatic disease. Many prostate cancers are detected on the basis of elevated plasmatic levels of prostate-specific antigen (PSA > 4 ng/mL), a glycoprotein normally expressed by prostate tissue. (Prashanth 2019). Androgen deprivation therapy in its surgical form (bilateral orchiectomy) rather than its medical form (using luteinizing hormone-releasing hormone (LHRH) analogues/antagonists, anti-androgens and others) is the mainstay of treatment in these patients due to the fact that it is a much cheaper option as most patients cannot afford regular injections needed to maintain castrate serum testosterone level (Dubem *et al* ., 2018)**.** Testosterone is not the cause of prostate cancer, but is considered essential for the growth of these tumours. Normal prostate cells and malignant prostate cancer cells at least initially rely on androgen stimulation via androgen receptors for growth and proliferation, the prostate cancer is a hormonally responsive tumour. Androgen withdrawal causes a retardation of prostate cell growth, thought to be from programmed cell death and ischemic injury from anoxia. Thus, manipulation of the hormonal milieu plays a role in the treatment of prostate cancer and often decreases morbidity and increases survival (Leonard *et al.,* 2009).

1.3 AIM

The aim of this study is to determine the effect of bilateral orchidectomy on the hormone profile of male wistar rats.

Specific Objectives

Specifically the study sort to;

- Determine the level of gonadotropic hormones via LH, FSH and Prolactin following bilateral orchidectomy.
- Determine the level of male steroid hormones via progesterone, testosterone and oestrogen following bilateral orchidectomy.

1.4 STATEMENT OF PROBLEM

Impotence after Bilateral Orchidectomy which is the first line of treatment. A lot of people have come up with increased incidence of prostate cancer.

1.5 SIGNIFICANCE OF STUDY

Significance of this study is that bilateral orchidectomy affects the pituitary gonadal hormone as well as the testicular hormones.

CHAPTER TWO

LITERATURE REVIEW

2.1 BILATERAL OCHIDECTOMY

Bilateral orchidectomy has been performed for over 2,000 years. Reference to orchidectomy occurs in Biblical and ancient mythological texts, for concrete evidence exists for it to have been practised in Rome and China. The inguinoscrotal hernia provided early surgeons with a challenge that resulted in many surgeries (orchidectomy), and at the same time, some boys were being castrated to provide operatic entertainment for the nobility of time. Over the years orchidectomy has been used to treat male aggression and more recently it has been proposed as a treatment for carcinoma of the prostate. Bilateral orchidectomy is now reserved for patients with prostate cancer and over the last 20 years, urologists have developed ways of chemically castrating the individual to avoid the need of mutilative surgery. The word "castration" is usually understood as meaning the removal of both testicles, but it has often meant the removal of the penis as well. A discussion on orchidectomy would be incomplete without referencing eunuchs. The term eunuch is derived from two Greek words, εὐνοῦχος (*eunoukhos*) which literally translates as guardian bedchamber. It is completely interchangeable with the word chamberlain (Glass *et al.,* 1997). A eunuch is a castrated human male. From remote antiquity, eunuchs were employed in the Middle East and in China in two main functions: as guards and servants in harems or other women's

quarters, and as chamberlains to kings. Nevertheless it appears from biblical and Roman references that not all eunuchs had their testicles completely removed (Glass *et al.,* 1997).

2.2 TESTIS

The testis are firm mobile organs, a typical male has two testes approximately 5 cm long, 3cm wide and 2.5cm thick. Weighing 10-15g each, the testes are suspended outside the body in a fleshy sac called scrotum. The left testes lie slightly lower than the right testes (Keith *et al*., 2014). The primary function of the testes is the production of sperm. The testes is made up of three components that play a significant role in sperm production, they are;

- Seminiferous tubules
- Sertoli
- Leydig cells

The testes also contains series of ducts and tissues which perform the role of sperm transportation, they are;

- Tunica
- Tunica Vasculosa
- Tunica Albuginea
- Tunica Vaginalis

2.3 EPIDIDYMIS

A crucial part of the male reproductive system is the epididymis. Sperm are matured and stored there until they are released into the ductus deferens or vas deferens. The process of ejaculation in males involves the movement of sperm from the epididymal tail to the vas deferens. The latter's peristaltic muscle contractions advance the sperm. Before they are ejaculated, they are combined with the seminal fluid from the accessory glands, and the resulting mixture is known as semen (Koene et al., 2010). The second situation where epididymal sperm numbers have been reported to decline is following bilateral castration. In keeping with other male accessory sex glands, the epididymis is androgen dependent and, following castration, the epithelium regresses rapidly, especially in the initial segment and proximal caput epididymal regions, which receive an additional supply of testosterone and growth factors from testicular fluid. As a consequence, sperm maturation and survival are adversely affected, and, after several weeks, only a few degenerated spermatozoa are present in the cauda epididymis and vas deferens (Jones, 2004).

2.4 SPERMATOGENESIS

Spermatogenesis is a highly coordinated complex cellular event that takes place in the epithelium of seminiferous tubules of mammalian testes (rat, mouse or man). This process involves mitotic division leading to the conversion of diploid spermatogonia (2n) to type B spermatogonia which undergoes cellular transformation to spermatocytes. Spermatocytes enter meiosis to form haploid spermatids (1n), which finally develop to

spermatozoa in a process called spermiogenesis (Hess and de Franca, 2008). The phases of Spermatogenesis are composed of mitotic renewal of spermatogonial stem cells and spermatogonia, transformation of type B spermatogonia to preleptotene spermatocytes, transit of preleptotene spermatocytes across the blood–testis barrier (BTB), meiosis, differentiation of round spermatids to spermatozoa via spermiogenesis, and spermiation. However, spermatogenesis is a continuum, which results in transitional areas being observed between two stages (Hess and de Franca, 2008).

Phases of Spermatogenesis

Mitosis

In this phase of spermatogenesis diploid (2n) germ cells (spermatogonia stem cells and spermatogonia) which reside in the basement membrane of the seminiferous tubule undergo a series of mitotic cell division in which the resulting daughter cells are diploid (identical with parent cell without significant genetic alterations both in shape, number, content and composition of the gene). These cells are characterised based on the presence and distribution of heterochromatin (Chiarini-Garcia and Russell, 2001). In rats, three different types of spermatogonia are recognized. They are type-A, intermediate, and type-B spermatogonia. These are further characterised into four classes: undifferentiated type A spermatogonia [A single (As), A paired (Apr), A aligned (Aal)]; differentiated type A spermatogonia (A1, A2, A3, A4); intermediate spermatogonia (In); and type B

spermatogonia (B) (Russell et al., 1990; de Rooij and Russell, 2000). The undifferentiated spermatogonia, including stem cells, are located in niches of the seminiferous epithelium, which are regulated by the sertoli cell and are not easily identified in light microscopy (Chen et al., 2005; Oatley and Brinster, 2006; Ogawa et al., 2005).

Meiosis

B-spermatogonia will undergo mitotic division to form two preleptotene spermatocytes. These later cells will represent the onset of meiotic prophase. These preleptotene spermatocytes rest on the basement membrane while leptotene and zygotene spermatocytes move through the BTB (Russell, 1977; Russell, 1980). These spermatocytes are located in specific stages and are identifiable by light microscopy, but leptotene and zygotene spermatocytes appear to be attached to the basement membrane. Spermatocytes are found in all the stages of spermatogenesis, because meiosis is a prolonged period of spermatogenesis that extends over approximately 14 days in the mouse (Hess and de Franca, 2008). Spermatocytes are the cells of meiosis and it occurs in and defines a single stage (XII). In the mouse, this is found in approximately 10% of the seminiferous tubular cross section and it goes through three categories, all occurring in stage XII:

(a) Meiosis I: the division of 4n cells.

(b) Formation of secondary spermatocytes (2n), which are larger than step 1 spermatids, but rarely are found as the only spermatocyte in a tubular cross section.

(c) Meiosis II, the division of 2n secondary spermatocytes to form haploid (1n) round spermatids. In rats, there is an increase in size for primary spermatocytes, from preleptotene to diplotene (Franca et al., 2005). This increase is followed by a significant decrease in cell size during spermiogenesis in such a way that, due to changes in chromatin and nuclear condensation, spermatid nuclear volume reaches only 1/50th of its initial volume.

Spermiogenesis

This involves the process of transformation of round, haploid spermatids into elongate, highly condensed spermatozoa that are released into the seminiferous tubule lumen. The differentiation of spermatids proceeds through at least four prolonged steps: Golgi step, capping step, acrosome step, and maturation step (Hess and de Franca, 2008). Golgi vesicles and granules secreted by the Golgi apparatus (Steps 1-3) contain acrosome enzymes that cover the developing sperm nucleus during the early stages of spermiogenesis. Capping (steps 4-8) involves steps in formation of round spermatids, where the acrosomal granule touches the nuclear envelope and the vesicle begins to flatten into a small cap over the nuclear surface. The acrosomal vesicle becomes very thin and the granule flattens. The acrosome flattens over approximately 1/3 of the nuclear

surface. In the late stage the nuclei begin to change shape (Hess and de Franca, 2008). Acrosomal steps (9-14) involve migration of the acrosomal system over the ventral surface of the elongating spermatid nucleus. This step also involves condensation of chromatin, as the chromosomes are packed more tightly and stain more intensely with hematoxylin (Hess and de Franca, 2008). Maturation (steps 15-16) shows fewer changes in nuclear shape and acrosomal migration. The nucleus continues to condense and the acrosome matures into a thin PAS positive structure that protrudes at the apex but covers the entire surface of the nucleus except the portion connected to the tail. Excess cytoplasm is removed resulting in the formation of prominent cytoplasmic lobes and residual bodies, which contain unused mitochondria, ribosomes, lipids, vesicles and other cytoplasmic components (Hess et al., 1993). The mature, elongated spermatids undergo a further complex remodelling during the process of spermiation, which is the process by which the mature spermatids are released from the sertoli cells prior to their passage to the epididymis (O'Donnell et al., 2011). This remodelling includes the removal of specialised adhesion junctions that have ensured tight adhesion of the spermatid to the sertoli cell during its elongation process, further remodelling of the spermatid head and acrosome and removal of the extensive cytoplasm to produce streamlined spermatozoon. The cytoplasm of the spermatid migrates to a caudal position around the tail and is markedly reduced in volume. The morphological features of spermiation are relatively conserved between species, particularly among mammals (Russell, 1993b). Spermiation is highly susceptible to perturbation by pharmacological modulators and by agents that suppress gonadotropins (O'Donnells et al., 2011).

2.4 HORMONAL REGULATION OF SPERMATOGENESIS

Spermatogenesis is supported by two hormones of the anterior pituitary gland, namely the Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). While FSH exerts its effects on sertoli cells, LH regulates steroidogenesis in leydig cells in the testes interstitium (Cheng and Mruk, 2010) which are involved in the production of testosterone and estrogens to regulate spermatogenesis (Shaha, 2008; Carreau and Hess, 2010; Carreau et al., 2010). The Hypothalamo-Pituitary Gonadal (HPG) axis is the central regulator of reproductive function in both male and females. The hypothalamus secretes Gonadotropin-Releasing Hormone (GnRH), a decapeptide secreted from the arcuate nucleus of the hypothalamus (Falardeau et al., 2008; Kim et al., 2008) which stimulates the adenohypophysis (Anterior Pituitary gland) to secrete two gonadotropins: FSH and LH. These hormones are major regulators of testicular function. Steroid hormones secreted by the testes, testosterone and oestrogen, along with other testicular hormones, such as inhibin and activin, control GnRH and gonadotropin release from the hypothalamus and pituitary gland respectively (Asimakopoulos, 2012). GnRH is solely responsible for the release of gonadotropins (Hayes and Crowley 1998). However, the release of GnRH is under the control of the kisspeptin/ GPR54 system. The kisspeptin/GPR54 system mediates the effects of the peripheral steroids on GnRH secretion. Testosterone and its metabolites (DHT or estradiol) is the major steroid hormone regulating GnRH secretion. It is also responsible for inhibiting gonadotropin secretion through a negative feedback mechanism at the hypothalamic and pituitary levels (Hayes and Crowley 1998). Testosterone and DHT act mainly at the hypothalamic

level by decreasing the frequency of GnRH release, whereas estrogens decreases gonadotropin secretion by reducing the threshold of LH and FSH peaks at the pituitary level (Dufourny et al., 2005).

2.4.1 Role of Gonadotropins in Spermatogenesis

GnRH stimulates the secretion of LH and FSH by binding to gonadotrophs of the anterior pituitary (Manetti and Honig, 2010). The Stimulus for GnRH can, under certain conditions, cause one of the two gonadotropins to be released. For example, Low GnRH pulse frequency causes FSH to be released preferentially probably due to differential expression of the FSH receptor (Ferris and Shupnik, 2006). LH and FSH are glycoprotein hormones secreted by the pituitary gland and play a role in the development, maturation and function of the gonad. They consist of two polypeptide chains, α and β. The α-subunit is common to all members of the glycoprotein hormone family but the β subunit differs in each hormone and confers specificity of action. The regulation of gene expression of LH and FSH has been extensively studied in experimental animals, especially rodents, and involves a complex interplay between hypothalamic GnRH and gonadal steroids and peptides acting at the hypothalamic and pituitary level (Burger et al., 2004). Regulation of gonadotropin expression, synthesis and secretion by steroid hormones is rather complex depending on the experimental model. Generally, gonadal steroids exert negative control on gonadotropins mainly at the hypothalamic level, resulting in depressed GnRH release via kisspeptin/GPR54 system (Novaira et al., 2009).

2.4.2 Prolactin

Prolactin is a peptide hormone secreted by the pituitary lactotrophs and the prostate gland (Gerlo et al., 2006). Prolactin is involved in the control of testosterone secretion (Gill-sharma, 2009) and sexual behaviour and activity in men (Galdiero *et al.,* 2012). Prolactin receptors expressed in the hypothalamus and choroid plexuses are suggestive of its involvement in the regulation of male fertility (Labrie *et al.,* 2005). Dopamine release by the hypothalamic neurons regulates the secretion of prolactin via the hypothalamic–hypophyseal portal system; therefore, any impairment of dopamine release results to hyperprolactinemia (Bachelot and Binart, 2007). Hyperprolactinemia inhibits testosterone synthesis indirectly through prolactin-induced hypersecretion of adrenal corticosteroids which in turn inhibits GnRH secretion and consequently secretion of LH and FSH, arrest of spermatogenesis, impaired sperm motility and altered sperm quality (Masud *et al.,* 2007; Kaiser, 2012). Studies suggest that prolactin is a crucial hormone for the regulation of male fertility with a defined ontogenetic pattern of development of the hypothalamic releasing and inhibitory factors that determine its level in circulation (Freeman *et al.,* 2000; Andrews and Grattan, 2004). The hypothalamus contains prolactin-responsive dopaminergic neurons for auto-regulation as well as stimulation of dopaminergic neurons involved in the inhibition of GnRH neurons. In addition to the hypothalamic regulatory factors, adenohypophyseal lactotrophs at express a testosterone-responsive inhibitory mechanism for prolactin synthesis and oestrogen dependent stimulatory mechanisms for prolactin secretion, whereas leydig cells in the testis express a prolactin dependent regulatory mechanism for testosterone synthesis. The prolactin responsive mechanisms

constitute the biological substrates for the pituitary-hypothalamic-gonadal feedback system in the male mammals, which relies on the gonadal steroids, testosterone and estradiol for long-loop, and prolactin for short-loop feedback mechanisms (Dutt *et al.*, 1986; Gill-Sharma *et al.*, 2003; Grattan *et al.*, 2007).

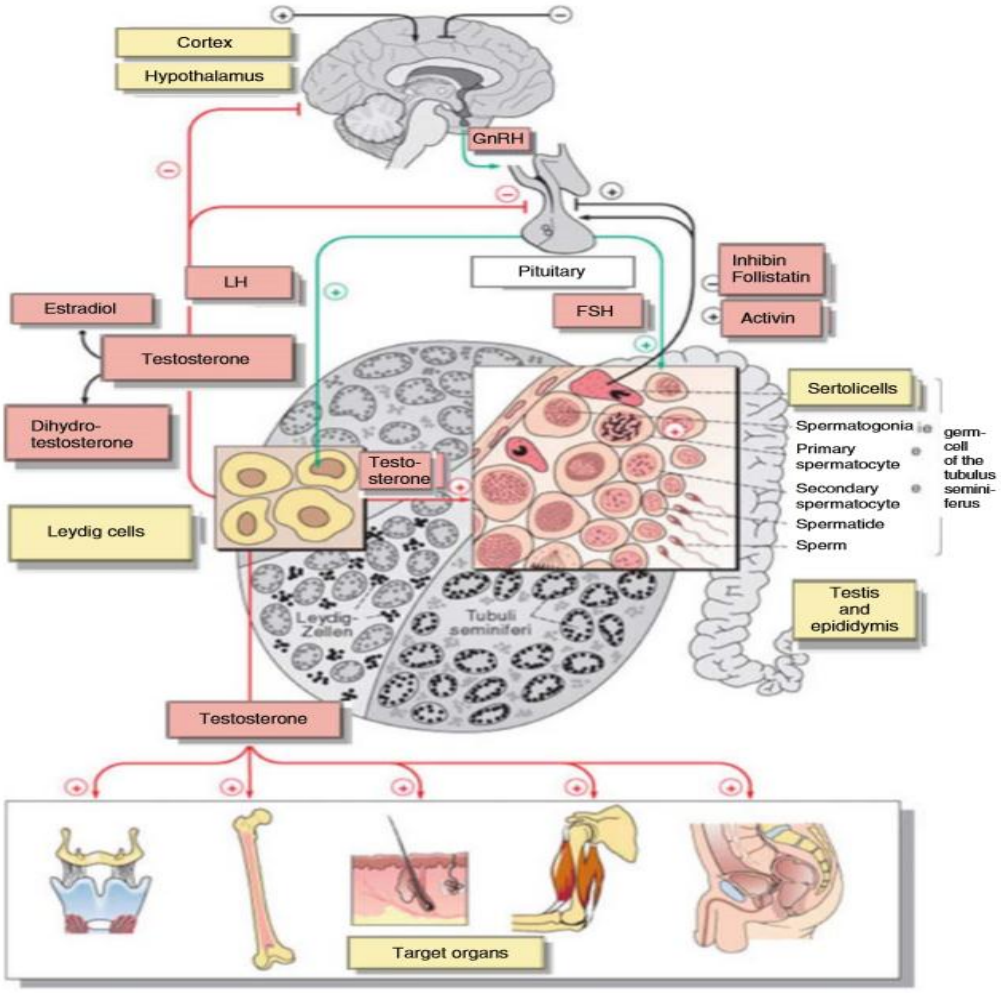


Figure 2.4: Hormonal regulation of the testicular function and effects of androgens (Nieschlag *et al.*, 2008).

These mechanisms operate to contain the adverse effects of reproductive stress and ensure that serum levels of prolactin remain within the physiological range, because even mild-to-moderate hyperprolactinemia, if allowed to become chronic, affects the quality of mature spermatozoa and their fertilising potential. Hyperprolactinemia also affects reproductive behaviour in spite of normal testosterone levels. Importantly, whereas the effects of acute hyperprolactinemia appear to be mediated via testosterone inhibition, those due to moderate hyperprolactinemia would be a consequence of FSH deficits (Aleem *et al.,* 2006; Aleem *et al.,* 2008).

This effect of gonadal steroid at the pituitary level is more complex, but available evidence shows that estrogens inhibit GnRH-stimulated gonadotropin synthesis and secretion at this level. In rodents, testosterone has a specific stimulatory effect on FSH gene expression, synthesis and secretion directly at the pituitary level. Available experimental evidence shows that there is increase of serum FSH in the presence of normal testosterone levels in oligozoospermia implying that FSH secretion is under the control of some other factor(s) which is/are related to the efficiency of spermatogenesis (Boepple et al.,2008). Research has shown that there is significant inverse correlation between serum concentrations of inhibin B and serum levels of FSH, testis size and sperm numbers (von Eckardstein et al., 1999). As a consequence serum levels of inhibin B directly reflect the integrity of the germinal epithelium and of the sertoli cells (Boepple et al., 2008). LH and FSH exert their functions via specific receptors (Simoni et al., 1997). The gonadotropin receptors also belong to the family of the G protein-coupled

receptors (Kossack et al., 2008; Gromoll and Simoni, 2005). FSH acts on FSH-receptor on testicular sertoli cells to form FSH- FSHR complex to drive spermatogenesis (Fan and Hendrikson, 2005). In rats, FSH stimulates sertoli cell proliferation during prepubertal development (Heckert and Griswold, 2002). As a consequence, sertoli cell number correlates positively with the number of germ cells. Studies have shown that LH stimulates the secretion of testosterone by acting on LH receptors on Intestitial leydig cells (Eblen et al., 2001; Lei et al., 2001). Testosterone is secreted in pulses by leydig cells in response to LH stimulation. An increase in testosterone or its metabolites, dihydrotestosterone (DHT) and oestradiol, secretion inhibits secretion of LH through a negative feedback effect on the hypothalamus and pituitary gland (Chimento et al., 2014). Negative feedback on FSH secretion occurs through inhibin B produced by sertoli cells and estradiol which is derived from aromatization of testosterone but the major feedback inhibition comes from estradiol. The major negative feedback on FSH comes from estradiol, which is derived from aromatization of testosterone (Hayes et al., 2001).

2.4.3 Role of Steroid Hormones in Spermatogenesis

Testosterone and its metabolites have a major role in regulation of spermatogenesis. The presence of endogenous testosterone and its receptor, androgen receptor (AR), in the testes are very important for spermatogenesis. Cells of the testes harbouring ARs are targets for testosterone. These receptors are found in sertoli cells, peritubular cells, leydig cells (Kotitschke et al., 2009) and a few scattered in germ cells (Hu et al., 2008). The most

important target cells for testosterone are sertoli cells, which are involved in spermatogenesis. The relationship between leydig and sertoli cells through testosterone is the underlying mechanism of spermatogenesis. The absence of testosterone and/or ARs leads to a defective blood–testis barrier (BTB). The implication is that germ cells will not progress beyond meiosis (Kotitschke et al., 2009). In these instances, immature germ cells are displaced from sertoli cells, while mature germ cells cannot be released. Therefore absence or low testosterone levels in serum will compromise the integrity of sertoli cells and the BTB. Consequently, postmeiotic cells will be exposed to autoimmune attack and gonadotoxic agents such as alcohol, tobacco, illicit drugs, chemotherapeutic agents, antihypertensive agents, hormonal agents, psychotherapeutic drugs and antibiotics (Nudell et al., 2002) which easily gain access into the seminiferous tubules, where sperm production takes place. Testosterone concentration is about 25-fold–125-fold higher in the testes than in serum (Smith & Walker, 2014) and about 20% of testicular testosterone is converted to oestrogens by aromatase while 80% of conversion to oestrogen takes place in peripheral tissues such as adipose tissue and striated muscle cells (Kaufmann & Vermeulen, 2005). At its origin, testosterone also has a local effect on the interstitium and seminiferous tubules indirectly, facilitating spermatogenesis and sperm maturation by virtue of its action on sertoli cells (Smith & Walker, 2014). The important role of oestrogen in spermatogenesis (mostly in meiotic and postmeiotic stages) is indicated by the presence of oestrogen receptors and aromatase in various parts of the testes. The level of Sex Hormone binding Globulin (SHBG) in plasma is a determinant for regulating the presence of free and albumin-bound androgens and oestrogens (Hautanen, 2000). Free

testosterone and albumin bound testosterone in the serum are readily available for biological actions. Other androgens include DHT, androstenedione, dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEAS). Testosterone is converted to a more potent DHT in peripheral tissues such as the liver, scalp, genital skin and the prostate gland, by α-reductase isozymes type 1 and type 2 (Russell and Wilson, 1994). The exact role of DHT in spermatogenesis is not clear. Research has shown that the inhibition α-reductase activity blocks the conversion of testosterone to DHT with a consequent reduction in sperm motility (Amory et al., 2007). DHEA and DHEAS are adrenal steroids synthesised by adrenal cortex and in the brain and are also converted to more potent testosterone by 17β-hydroxysteroid dehydrogenase. DHEA and DHEAS are also produced in the testes and serve as precursors to androgens and oestradiol (Kroboth et al., 1999). They are involved in neuroprotection, catecholamine synthesis and have antioxidant, anti-inflammatory and antiglucocorticoid effects (Labrie et al., 2005; Manigner et al., 2009). Kisspeptins, produced by clusters of discrete hypothalamic nuclei encoded by KISS1, and their G-protein-coupled receptor (GPR54) play an important role in the production and secretion of GnRH and in the negative feedback effects of testosterone and oestradiol on the hypothalamus (Novaire et al., 2009). A study carried out on human volunteers has shown that plasma levels of LH, FSH and testosterone were elevated following acute intravenous administration of kisspeptin, indicating major role of the kisspeptin–GPR54 system in regulating reproductive hormones by stimulating testicular function and spermatogenesis (Ratnasabapathy and Dhillo, 2013). Other factors that influence and regulate the HPG axis and gonadotropins include glucocorticoids, leptin

and opioid peptides. Increased activity of the HPA axis has a direct inhibitory effect on GnRH secretion mediated through glucocorticoids (Kirby et al., 2009). Opioid peptides including enkephalins, endorphins and dynorphins are found in the central nervous system and peripheral tissues (Froehlich, 1997). Opioid peptides inhibit the HPG axis. Usage of exogenous opioids (such as morphine) over a long period of time leads to hypogonadism and low serum testosterone concentrations (Yen et al., 1985). Leptin is a protein that is synthesised and secreted by adipocytes in white adipose tissue (Kelesidis et al., 2010) which binds to specific receptors in the brain and peripheral tissues (Cunningham et al., 1999). Circulating leptin levels decrease during fasting period in humans, indicating that leptin is involved in regulating changes in metabolic status (Ahima et al., 1996). Reduced leptin signalling leads to reduced GnRH neuronal activity (Teerds et al., 2011). This phenomenon could be caused by decreased hypothalamic KISS1 expression (a potent regulator of GnRH, LH and FSH release) as kisspeptin neurons express leptin receptors. Increased HPA axis activity stimulates leptin synthesis, and leptin exerts negative feedback on CRH (Heiman et al., 1997).

2.4.4 Hypothalamo-Pituitary Adrenal Axis

Stimulation of the HPA axis inhibits the HPG axis via inhibition of GnRH at the hypothalamic level. The main hormones of the HPA axis include CRH, glucocorticoids, AVP and ACTH. CRH receptors have been identified in the testes. Therefore the HPA axis may have a direct effect on the testes (Kalantaridoua et al., 2010). Glucocorticoid receptors

are located in hypothalamic neurons, the pituitary gland and the testes. Therefore, the actions of glucocorticoid on the HPG axis are mediated at multiple levels (Whirledge & Cidlowski, 2010).

In the hypothalamus, Glucocorticoid downregulates GnRH resulting in impairment of pulsatile release of LH and FSH from the pituitary gland (Whirledge & Cidlowski, 2010). At the pituitary level, glucocorticoids regulate the GnRH receptor gene (GNRHR), (Kotitschke et al., 2009) thereby modulating the effect of GnRH on the release of gonadotropins. Glucocorticoid also reduces the testicular response to LH and the concentration of LH receptors in both animals and humans, which leads to reduced testosterone secretion (Hu et al., 2008; Whirledge & Cidlowski, 2010). Glucocorticoid receptors are localised in leydig cells, macrophages, fibroblasts, smooth muscle cells and endothelial cells of blood vessels in the testes (Hu et al., 2008). They are also present in zygotene and early pachytene primary spermatocytes during stages I–III and XIII–XIV of the spermatogenic cycle. These receptors are also localised in male reproductive accessory tissues, such as the epididymis, the vas deferens, and the prostate (Schultz *et al.*, 1993) although their functional role in these organs is not known. Studies have shown that glucocorticoids directly inhibit testosterone production from leydig cells and this inhibition is probably caused by direct activation of glucocorticoid receptors in leydig cells through genomic and non-genomic mechanisms (Hu *et al.*, 2008; Whirledge and Cidlowski, 2010).

2.4.5 Gonadotropin-Inhibiting Hormone

Gonadotropin-inhibiting hormone (GnIH) is a decapeptide which belongs to the RFamide peptides family. It was first isolated from the quail hypothalamus but has been reported in mammals **(**Osuji et al., 2014**).** The effect of GnIH is mediated via the GPR147 receptors located in the GnRH neurons in the hypothalamus (Ubuka et al., 2014). In mammals, GnIH neurons are located in the dorsomedial hypothalamic area where their axon terminates in the preoptic area in association with GnRH neurons (Ubuka et al., 2014). This connection suggests direct inhibition of GnRH by GnIH thereby causing impairment in the pulsatile release of GnRH (Ubuka et al., 2014).

GnIH is released into the hypothalamic–hypophyseal portal system where it inhibits the secretion of LH and FSH by gonadotrophs. The mammalian testes also express the GnIH receptors which is indicative of direct inhibitory effect on testosterone secretion and spermatogenesis (Chowdhury et al., 2010).

Achieving and maintaining effective suppression of serum testosterone levels in men is an essential strategy in the management of prostate cancer. The American Society of Clinical Oncology (ASCO) 2007 guidelines and National Comprehensive Cancer Network (NCCN) 2009 guidelines recommend either luteinizing hormone-releasing hormone (LHRH) agonists or bilateral orchiectomy as first-line therapy for men with advanced prostate cancer *(Loenard et al., 2009).* According to *Leonard Gomella et al,* bilateral removal of the testes is traditionally the gold standard for androgen ablation. The

current use of androgen ablation therapy in prostate cancer includes treatment based on serum prostate-specific antigen (PSA), if the PSA level is high it is a likely indication for prostate cancer. Medical or chemical castration is almost exclusively performed by the use of injectable LHRH analogues or LHRH antagonists. Intermittent hormonal therapy (IHT) is being investigated as an alternative to continuous hormonal therapy. Although intermittent therapy may rely upon restoring a normal testosterone level, it is believed that the testosterone level should be as low as possible when the patient is on treatment, thus generating the lowest serum PSA level possible and likely improving outcome.

However the data on IHT are promising, trials reported thus far are relatively small and somewhat underpowered, and it is likely that its use will increase in the future as trials mature. Surgical castration through bilateral orchiectomy is infrequently used today because the procedure is irreversible, making the potential use of Intermittent hormonal therapy (IHT) impossible. It is also associated with significant psychological impact. Subcapsular orchiectomy, with maintenance of the tunica albuginea and epididymis, may provide psychological benefit to some men who must undergo orchiectomy.

Normal prostate cells and malignant prostate cancer cells at least initially rely on androgen stimulation via androgen receptors for growth and proliferation. Androgen withdrawal causes a retardation of prostate cell growth, thought to be from programmed

cell death and ischemic injury from anoxia. Thus, manipulation of the hormonal milieu plays a role in the treatment of prostate cancer and often decreases morbidity and increases survival. Testosterone is not the cause of prostate cancer, but is considered essential for the growth of these tumours. Simon et al., (1999) affirmed that the production of male gametes depends on the concerted action of the two gonadotropins FSH and LH on the testis. The action of LH is mediated through the production of testosterone by the Leydig cells. Since male germ cells possess neither FSH nor androgen receptors, the action of FSH and testosterone occurs through the Sertoli cells.

Although the precise function of these two hormones remains elusive, the existing evidence suggests that both FSH and testosterone are able to stimulate all phases of spermatogenesis. In the male FSH is required for the determination of Sertoli cell number, and for induction and maintenance of normal sperm production. Emmanuel et al. (1996) stated that many studies have consistently shown that castration induces a prompt increase in serum levels and pituitary content of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as a concomitant rise in steady state levels of the messenger RNAs directing their synthesis. In 1990, Kitahara et al. examined the effects of castration on the release of LH and FSH under basal conditions.

The pituitary cells were prepared from 7-week-old intact rats and rats orchidectomized 2 weeks prior; castration increased FSH secretion. However, the pituitary FSH contents were similar in cells from intact and castrated rats. There was an adequate increase in LH secretion, the pituitary LH content in both intact and castrated rats were not the same, that of the castrated rats rose by 3- folds. Prof. Luiz et al. (2016) pointed out that in men, testicular Leydig cells secrete more than 95% of the total circulating testosterone in male prostate is controlled by steroid hormones(sex steroids/ sex hormone) which encounter changes after castration. Prolactin is involved in the regulation of the male prostate, having already been identified in the tissue, acting through its receptor PRLR. The peptide hormone prolactin (PRL) is secreted mainly by the pituitary gland and, to a lesser extent, by peripheral tissues, such as the breast, decidua, prostate, and the brain, and it is involved in a broad spectrum of physiological processes in vertebrates. The principal stimulatory and inhibitory control of prolactin secretion is a hypothalamic hormone that inhibits the prolactin secretion of dopamine. However, in the male, serum prolactin levels increased after castration (Mariana et al., 2021).

Sarin et al., (2020) stated that testosterone, an androgen responsible for male secondary sexual characteristics, is produced primarily in the testes (95%) and also in small amounts by the adrenal glands (5%). Testosterone is the main sex hormone for men, regulating various functions such as libido, bone density, red blood cell count, male characteristics, and male behaviours. Testosterone is also the fundamental hormone in the regulation of

prostate cancer growth. Prof. Luiz et al., (2016) pointed out that in men, testicular Leydig cells secrete more than 95% of the total circulating testosterone, and the rest is produced by the fasciculate and reticular zones of the adrenal glands. According to Boodman et al. (1992), castrated men frequently have an erection and may be capable of intercourse.

Their sex drive is diminished because the testicles are no longer present to produce testosterone. But the drive is not eliminated. A small amount of testosterone is also produced in the adrenal glands; if the supply from the testicles is reduced, the adrenal glands compensate and produce more of the hormone. Tsutomu (2014) stated that after surgical castration (orchidectomy) serum testosterone levels usually drop to less than 20 ng/dl (0.69 nmol/l).

CHAPTER THREE

MATERIALS AND METHOD

3.1 ANIMAL CARE AND MANAGEMENT

Twenty (10) male Wistar rats were bought from the animal holdings of the Department of Anatomy,University of Benin. The rats were kept in plastic cages and allowed to acclimatise for two weeks before castration. They were allowed access to pellets (manufactured by Premier Feed Mill Co.Ltd, a subsidiary of Flour Mills of Nigeria Plc.), and water ad libitum throughout the experimental period.

3.2 EXPERIMENTAL DESIGN

The animals were kept under similar conditions and housed in cages under a temperature of (23°C) and light (12 hours light and dark cycles) controlled room and received drinking water and normal rodent pellets ad libitum throughout the entire period. The rats were divided into two experimental groups: 1 - control (Co) (n=5); 2 - Treatment group (Tr) (n=5). The rats in the treatment group were bilaterally orchidectomized under ketamine anaesthesia .

3.3 ANIMAL HORMONAL ASSAY

The rats were sacrificed after 4 weeks. At the end of the experimental period, the rats were sacrificed under chloroform anaesthesia. A midline ventical incision was made

through the ventral abdominal wall and developed into the peritoneal cavity. The inferior vena cava was identified and blood samples collected into plain bottles for hormonal studies with a 5ml syringe and needle. The blood sample was then centrifuged for 5 minutes at a speed of 5000 revolutions per minute for 15 minutes. The supernatant was collected, refrigerated at -20°C and later assayed LH, FSH, prolactin, progesterone, testosterone and oestradiol using Enzyme Linked Immunosorbent Assay (ELISA) Hormone Test Kits (Accu-Bind ELISA Microwells manufactured by Monobind Inc. Lake Forest, CA 92630, USA).

Procedure for LH

- The microplate wells (Streptavidin coated microplate wells packaged in an aluminium bag with a drying agent) were formatted for each serum reference, control, and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.
- Fifty microliters (50 µl) of the appropriate serum references control and specimen put into the assigned wells.
- One hundred microliters (100 µl) of LH-Enzyme (one vial contains enzyme labelled purified antibody, biotinylated monoclonal mouse IgG in buffer, dye and preservative) were added to all wells.

- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plate were blotted dry with absorbent paper.
- Three hundred and fifty microliters (350 µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes.
- The wash decanted and the procedure repeated two additional times.
- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Five hundred microliters (500 µl) 0.050ml of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30) minutes of adding the stop solution (Uotila, 1981).

Procedure for FSH

- The microplate wells (Streptavidin coated microplate wells packaged in an aluminium bag with a drying agent) were formatted for each serum reference, control, and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.
- Fifty microliters (50 µl) of the appropriate serum references control and specimen put into the assigned wells.
- One hundred microliters (100 µl) of LH-Enzyme (one vial contains enzyme labelled purified antibody, biotinylated monoclonal mouse IgG in buffer, dye and preservative) were added to all wells.
- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plate were blotted dry with absorbent paper.
- Three hundred and fifty microliters (350 µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes.
- The wash decanted and the procedure repeated two additional times.

- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Five hundred microliters (500 µl) 0.050ml of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30) minutes of adding the stop solution (Uotila, 1981).

The procedure is the same as that for LH except for the specific enzyme labelled antibody biotinylated monoclonal mouse IgG in buffer, dye and preservative (Uotila, 1981).

Procedure for Prolactin

- The microplate wells (Streptavidin coated microplate wells packaged in an aluminium bag with a drying agent) were formatted for each serum reference, control, and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.
- Fifty microliters (50 µl) of the appropriate serum references control and specimen put into the assigned wells.

- One hundred microliters (100 µl) of LH-Enzyme (one vial contains enzyme labelled purified antibody, biotinylated monoclonal mouse IgG in buffer, dye and preservative) were added to all wells.
- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plates were blotted dry with absorbent paper.
- Three hundred and fifty microliters (350 µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes.
- The wash decanted and the procedure repeated two additional times.
- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Five hundred microliters (500 µl) 0.050ml of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30) minutes of adding the stop solution (Uotila, 1981).

The procedure is the same as that of LH except for the specific enzyme labelled antibody biotinylated monoclonal mouse IgG in buffer, dye and preservative (Uotila, 1981).

Procedure for Progesterone

- The microplate wells (Streptavidin coated microplate wells packaged in aluminium bags with a drying agent) were formatted for each serum reference, control and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.
- Ten microliters (100 µl) of the appropriate serum references control and specimen put into the assigned wells.
- Fifty microliters (50µl) of working testosterone enzyme (one (1) vial contains testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilising matrix with green dye) were added to all wells.
- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plate was blotted dry with absorbent paper.
- Three hundred and fifty microliters (350µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes. The wash decanted and the procedure repeated two additional times.

- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Fifty microliters (50 µl) of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30)minutes of adding the stop solution (Tietz, 1986).

The procedure is same as testosterone and oestradiol except the vial of working substrate for progesterone contains progesterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilising matrix with red dye and the vial working substrate for estradiol contains

Procedure for Testosterone

- The microplate wells (Streptavidin coated microplate wells packaged in aluminium bags with a drying agent) were formatted for each serum reference, control and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.

- Ten microliters (100 µl) of the appropriate serum references control and specimen put into the assigned wells.
- Fifty microliters (50µl) of working testosterone enzyme (one (1) vial contains testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilising matrix with green dye) were added to all wells.
- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plate was blotted dry with absorbent paper.
- Three hundred and fifty microliters (350µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes. The wash decanted and the procedure repeated two additional times.
- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Fifty microliters (50 µl) of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30)minutes of adding the stop solution (Tietz, 1986).

Procedure for Estradiol

- The microplate wells (Streptavidin coated microplate wells packaged in aluminium bags with a drying agent) were formatted for each serum reference, control and test specimen.
- The unused microwell strips were replaced back into the aluminium bag, seal and store at 2-8 o C.
- Ten microliters (100 µl) of the appropriate serum references control and specimen put into the assigned wells.
- Fifty microliters (50µl) of working testosterone enzyme (one (1) vial contains testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilising matrix with green dye) were added to all wells.
- The microplate was swirled gently for 20-30 seconds, covered and incubated for 60 minutes at room temperature.
- The contents of the microplate were discarded by decantation and the plate was blotted dry with absorbent paper.
- Three hundred and fifty microliters (350µl) of wash buffer (one (1) vial contains surfactant in buffered saline with preservative) was added to the decant. This was repeated two (2) times for a total of three (3) washes. The wash decanted and the procedure repeated two additional times.

- One hundred microliters (100 µl) of working substrate solution (one (1) vial contains tetramethylbenzidine in the buffer) was added to all wells. It was incubated at room temperature for fifteen (15) minutes.
- Fifty microliters (50 µl) of stop solution (one (1) vial contains a strong acid, 1N HCl) was added to each well and gently mixed for 15-20 seconds.
- The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630nm to minimise well imperfections) in a microplate reader within thirty (30)minutes of adding the stop solution (Tietz, 1986).

3.4 STATISTICAL ANALYSIS

The data generated would be analysed using descriptive statistics. Values would be presented as mean ± Standard Error of Means (S.E.M.) on tables and Bar charts. All statistical analysis would be carried out using Graph Pad Prism software manufactured by Graph Pad Software Inc. 7825 Fay Avenue, Suite 230 La Jolla, CA 92037, USA. The significance difference in the means of all parameters would be determined using one-way analysis of variance (ANOVA; 95 % confidence interval).

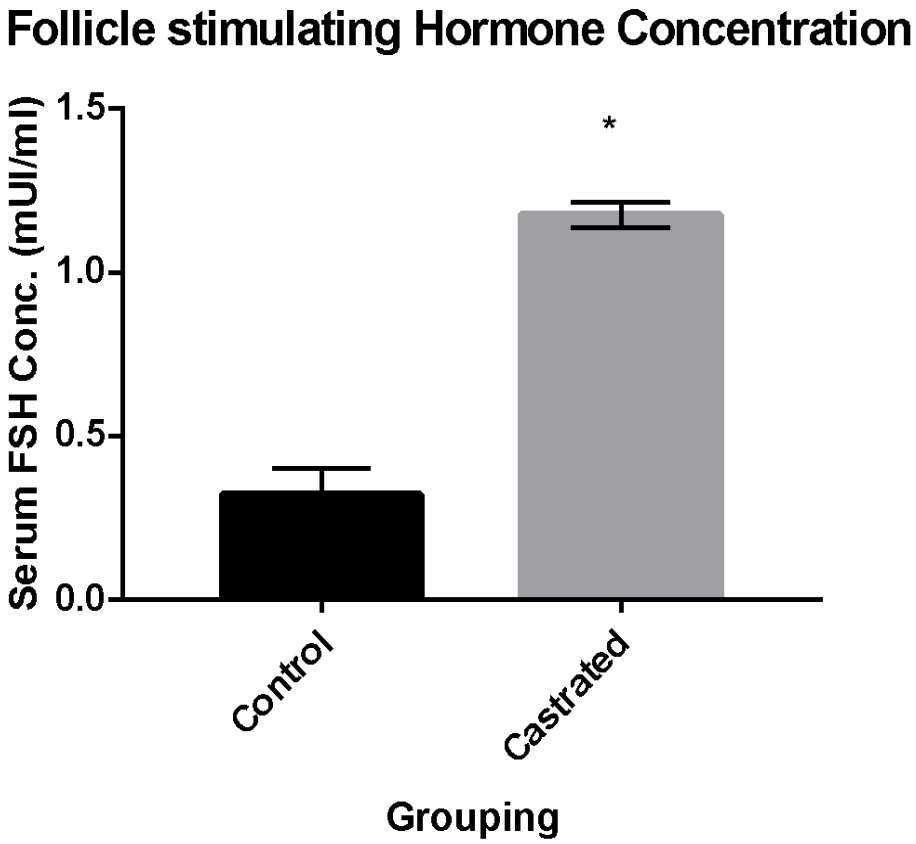
CHAPTER FOUR

RESULTS

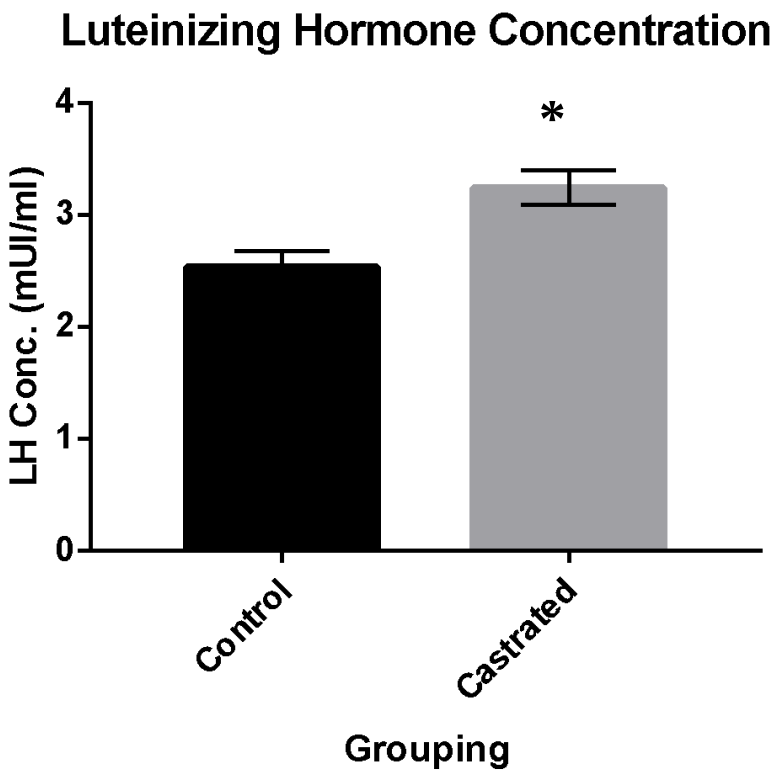
Table 1: Comparison of hormonal profile of rats in control and castrated groups

Parameters	**Control**	**Castrated**	***P*-value**
	N=5	**N=5**	
Follicle Stimulating Hormone (mUI/ml)	0.320 ± 0.046	1.177 ± 0.039	0.0001
Luteinizing Hormone (mUI/ml)	2.537 ± 0.145	3.247 ± 0.152	0.0278
Prolactin (ng/ml)	1.027 ± 0.027	0.777 ± 0.136	0.146
Progesterone (ng/ml)	2.427 ± 0.064	0.717 ± 0.056	0.0001
Oestrogen (pg/ml)	26.930 ± 1.658	5.833 ± 1.304	0.0006
Testosterone (ng/ml)	0.533 ± 0.033	0.067 ± 0.033	0.00006

Values are represented as Mean ± SEM for each group; n=5/group; **P*<0.05 indicates significant difference compared with control.



* represents a statistical significant difference (P<0.05). Chart showing serum FSH concentration, there was a statistical significant increase (P<0.05) in the level of serum FSH concentration (mUI/ml) when compared with control.



* represents a statistical significant difference (P<0.05). Chart showing serum LH concentration. There was a statistical significant increase (P< 0.05) in the level of LH concentration (mUI/ml) when compared with control.

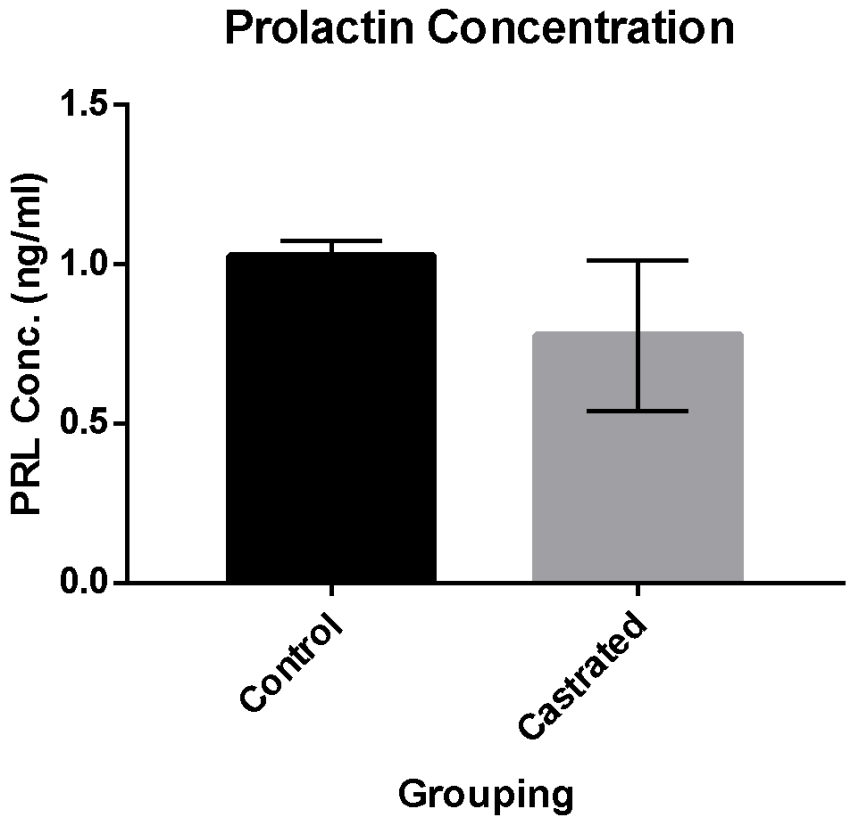
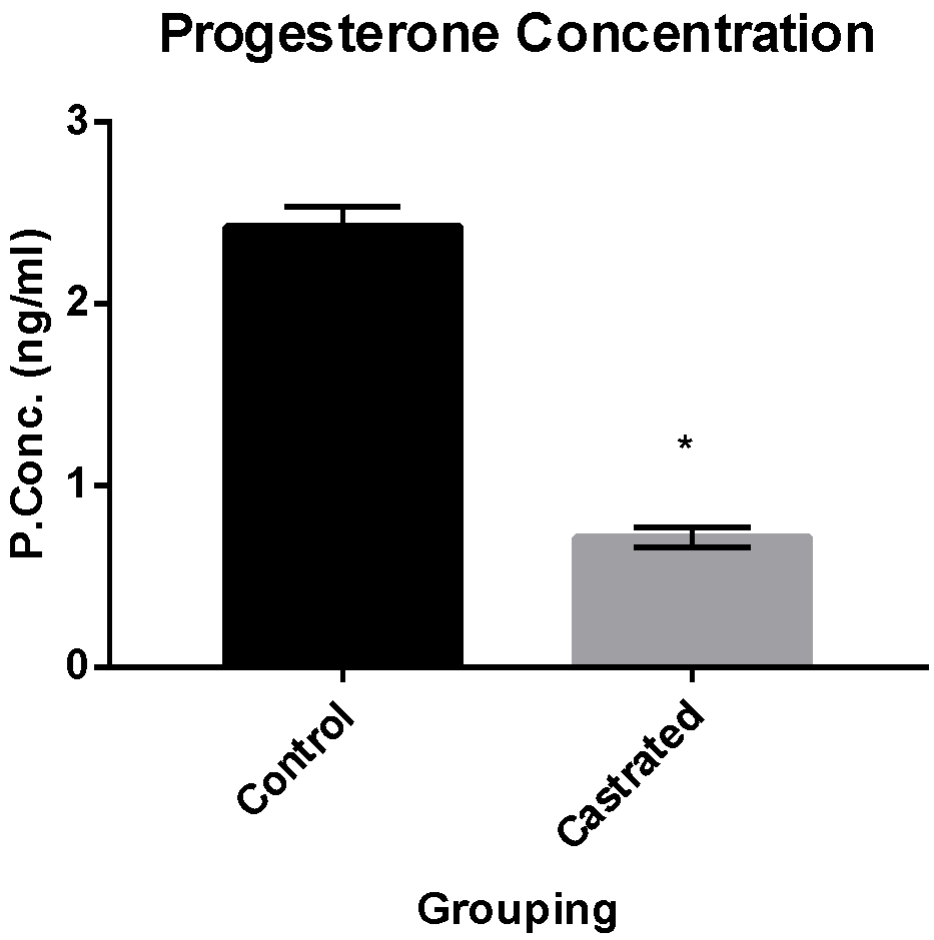
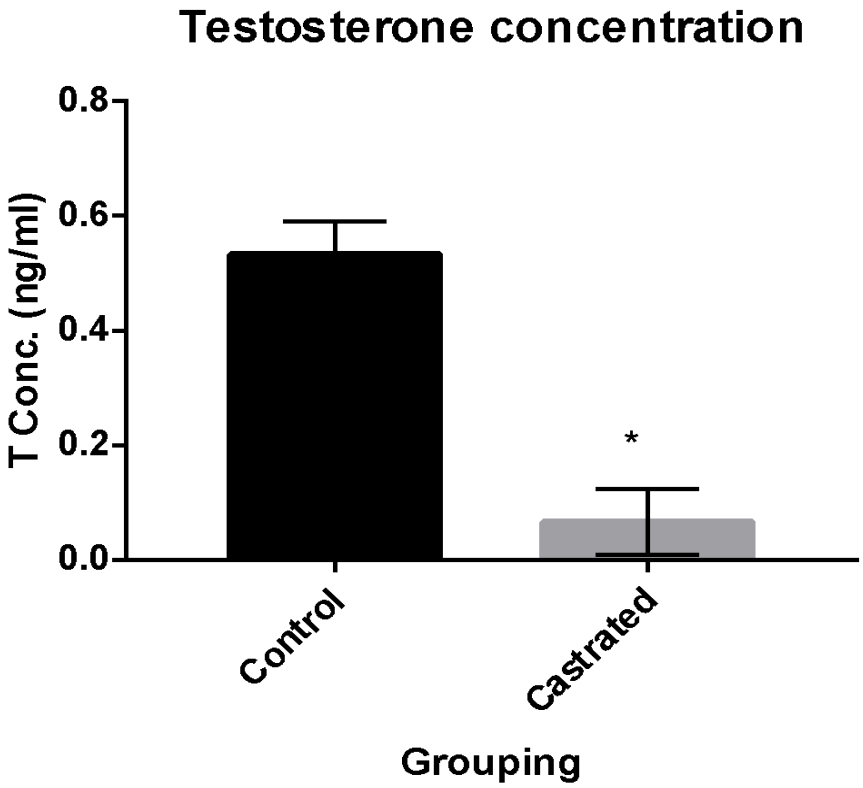


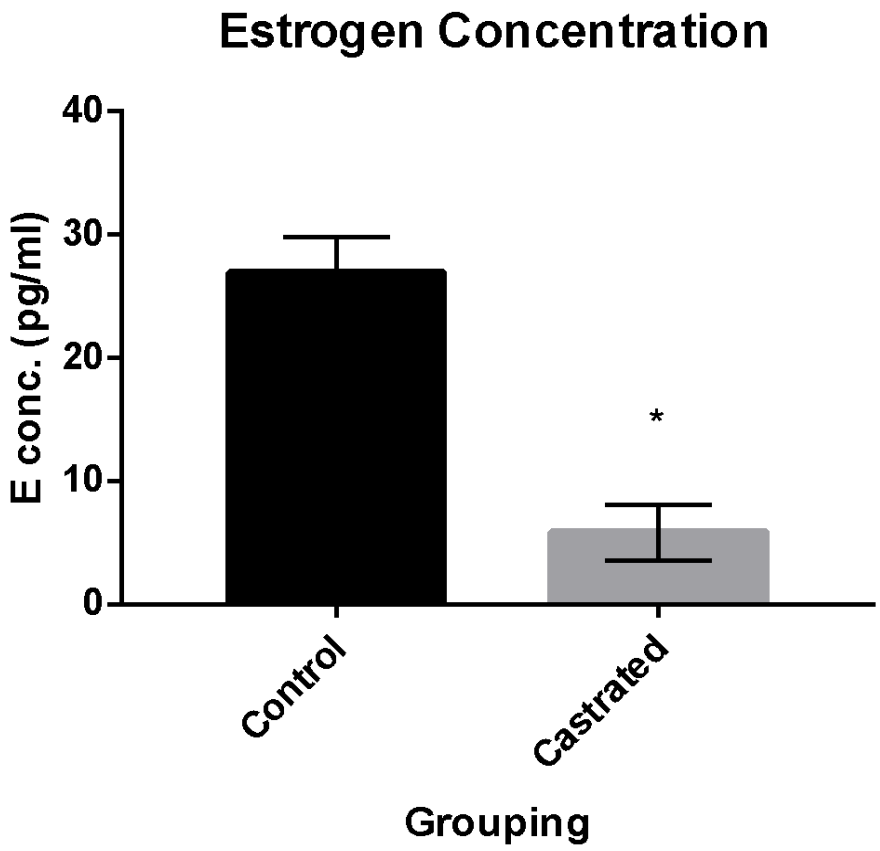
Chart showing the level of prolactin hormone concentration. There was no statistical significant difference ($P<0.05$) in the level of prolactin concentration (ng/ml)) when compared with control.



* represents a statistical significant difference ($P < 0.05$). Chart showing serum progesterone concentration. There was a statistical significant decrease ($P < 0.05$) in the level of serum progesterone (ng/ml) when compared with the control.



* represents a statistical significant difference ($P < 0.05$). Chart showing serum progesterone concentration. There was a statistical significant decrease ($P < 0.05$) in the level of serum testosterone (ng/ml).



Represents a statistical significant difference ($P < 0.05$). Chart showing serum oestrogen concentration. There was a statistical significant decrease ($P < 0.05$) in the level of serum oestrogen (pg/ml) when compared with control.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Spermatogenesis is regulated by two anterior pituitary hormones, FSH and LH. While FSH exerts its effects on sertoli cells, LH regulates spermatogenesis in Leydig cells in the testes, which are involved in the production of testosterone and estrogen to regulate spermatogenesis (Cheng and Mruk, 2010). Other sources of testosterone production in males include the adrenal gland. For this reason castration does not usually result in absence of testosterone and estrogen in the blood of castrated males due to the extra testicular sources (Mabrouk et al,.2014). Low testosterone levels usually stimulate GnRH in the hypothalamus to secrete LH and FSH. Prolactin levels was unaffected in this study. This may be due to the fact that prolactin does not directly affect the production of testosterone but rather through some other unknown mechanism. Resulting in increased levels of these hormones following castration (bilateral orchidectomy). In the present study, the serum concentrations of LH and FSH were increased significantly while the levels of testosterone, progesterone and oestrogen were reduced. These findings support previous work done (Vrouwe et al., 2022; Sulaiman et al., 2019; Attah et al., 2019; Weichno et

al., 2017).

5.2 CONCLUSION

This study showed that bilateral orchiectomy increased serum level of LH and FSH, the two hormones principally required for stimulating testosterone production and spermatogenesis respectively. The sex steroids, progesterone, testosterone and oestrogen were decreased is needed for spermatogenesis and in conjunction, FSH results in spermiation. Prolactin levels remain unchanged in this study. The decrease in testosterone therefore, may lead to infertility in the castrated rats.

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