

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

**EFFECT OF GLUCOCORTICOIDS ON THYROID AND  
GONADAL FUNCTION IN DOMESTIC RABBITS**

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# **DEDICATION**

To the Soul of my Parents

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## ABSTRACT

The effect of multiple intramuscular injections of the synthetic glucocorticoid, prednisolone (2.2 mg/kg body weight) every alternate day, on serum thyroxine ( $T_4$ ), triiodothyronine ( $T_3$ ), thyroid stimulating hormone (TSH) and leuteinzing hormone (LH) concentrations in male domestic rabbits were assessed.

Results revealed that serum  $T_4$  and  $T_3$  concentrations decreased significantly after 6 injections (day 12 of the experiment) of prednisolone were made. However, with 3 injections (day 6 of the experiment) of prednisolone there were non-significant differences in serum  $T_4$  and  $T_3$  values between the control and the treated animals. Prednisolone treatment also resulted in non-significant reduction in the concentration of TSH despite the concomitant profound decrease of  $T_4$  and  $T_3$ .

Moreover, serum concentration of LH - which is indispensable for ovulation and normal gonadal endocrine function - was significantly reduced in the treated group compared to the control when 3 injections and 6 injections of prednisolone were administered to the domestic rabbits.



(T<sub>3</sub>) / 2.2 (Prednisolone)  
(T<sub>4</sub>) :  
(LH) (TSH)

12) Prednisolone

(

( 6) Prednisolone

Prednisolone

( )

-

-

.Prednisolone

## INTRODUCTION

The adrenal cortex synthesizes a variety of steroids from cholesterol and releases them into the circulation. The steroids with effects on intermediary metabolism are termed "glucocorticoids". The major glucocorticoid in most domestic animals is cortisol (Adams, 2001).

Several synthetic glucocorticoids that are more potent than cortisol have been developed. Two examples of that are in common clinical use are prednisolone and dexamethasone (Laurence *et al.*, 1999).

Glucocorticoids are potent anti-inflammatory and immunosuppressive agents. The majority of therapeutic applications for these agents fall into these classifications. However, the adverse metabolic effects are difficult to separate pharmacologically from the therapeutic benefits, making glucocorticoids potent, yet potentially dangerous compounds. They are among the most widely used (and misused) class of drugs in human and veterinary medicine. Despite this, scientific information on glucocorticoid therapy in most domestic species is scarce particularly with respect to endocrine side effects (Adams, 2001).

It should be recognized that local applications also have similar systemic effects (Adams, 2001). Unfortunately, in our country so many imported and locally blended cosmetic creams containing glucocorticoids are used by women. Furthermore, some are taken orally for the purpose of depositing fat. Cutaneously applied gluco-

corticoids causing prompt and profound adrenocortical suppression is reported in man and animal (Zenoble and Kemppainen, 1987).

An excess of endogenous and exogenous glucocorticoids, causes many well-documented biochemical endocrine effects (DeNovo and Prasse, 1983; Dandona *et al.*, 1985; Meyer *et al.*, 1990; Kemppainen *et al.*, 1991; Kurtdede *et al.*, 2004). Such alterations will result in erroneous misleading interpretation of laboratory results.

Considering these adverse effects of glucocorticoids. Therefore this study was designed to determine the effect; if any, induced by prednisolone on thyroid metabolic hormones, thyroid stimulating hormone and lutenizing hormone levels in domestic rabbits.

# CHAPTER ONE

## LITERATURE REVIEW

### 1.1 The adrenal glands:

Each of the adrenal glands weighs about 4 grams, lies at the superior poles of the two kidneys and is composed of two distinct parts, the adrenal medulla and the adrenal cortex (Guyton and Hall, 2006).

The central 20 percent of the gland, the adrenal medulla is functionally related to the sympathetic nervous system; it secretes the hormones epinephrine and norepinephrine in response to sympathetic stimulation. The adrenal cortex secretes an entirely different group of hormones, called corticosteroids. These hormones are all synthesized from the steroid cholesterol. Two major types of adrenocortical hormones, the mineralocorticoids and the glucocorticoids, are secreted by the adrenal cortex. In addition to these, small amounts of sex hormones are secreted, especially androgenic hormones (Guyton and Hall, 2006).

More than 30 steroids have been isolated from the adrenal cortex, but two are of exceptional importance to the normal endocrine function of the human body: aldosterone, which is the principal mineralocorticoid and cortisol, which is the principal glucocorticoid (Guyton and Hall, 2006).

It was not until the 1920s that the vital importance of the adrenal cortex was appreciated and the distinction between the hormones of adrenal cortex and medulla was made (Laurence *et al.*, 1999).

## **1.2 Synthetic pathways of adrenal steroids:**

Fig. (1) gives the principal steps in the formation of the important steroid products of the adrenal cortex: aldosterone, cortisol and androgens (Guyton and Hall, 2006).

The precursor for all the steroids is cholesterol, which can be either taken up from the circulating lipoproteins or synthesized endogenously from acetate. Conversion of cholesterol to steroid hormones requires a series of enzymatic modifications that occur both in the mitochondria and the endoplasmic reticulum. Most of these enzymes are members of the cytochrome P<sub>450</sub> super family of oxidases (Montgomery *et al.*, 1996).

### **1.2.1 Pregnenolone:**

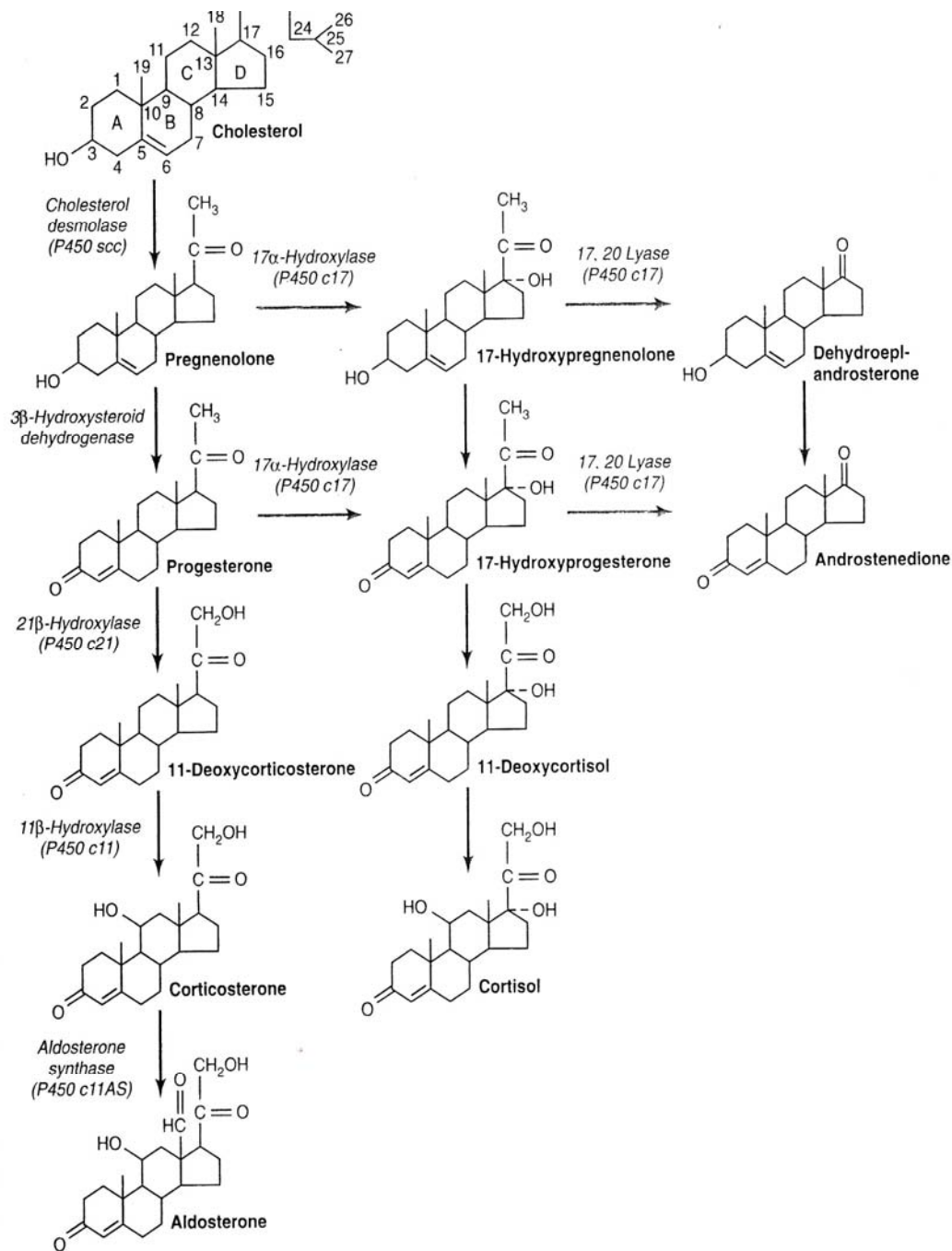
It is the precursor of all steroid hormones in mammals, and conversion of cholesterol to pregnenolone is the rate-limiting step in steroid hormones synthesis. This is accomplished in mitochondria by a multi-enzyme side-chain cleavage (SCC) complex. In humans, complete deficiency of SCC activity is fatal without hormone replacement therapy, because it causes an inability to synthesize any glucocorticoids or mineralocorticoids (Montgomery *et al.*, 1996).

### **1.2.2 21-Hydroxylase deficiency:**

Interestingly, 21-hydroxylase deficiency is associated with a tendency to waste salt in urine, whereas, 11  $\beta$ -hydroxylase deficiency is associated with hypertension. This difference is due to the mineralocorticoid effects of 11-deoxycorticosterone (DOC), (Montgomery *et al.*, 1996). Patients with the classic form of congenital adrenal hyperplasia due to 21-hydroxylase deficiency show excess androgens

# Fig (1): Synthetic Pathways of adrenal Steroids

(From Guyton and Hall , 2006)



(Volkl *et al.*, 2006). This is because, cortisol secretion is reduced and feedback leading to the increase of adrenocorticotrophic hormone (ACTH) secretion to maintain adequate cortisol. This in turn leads to diversion of the steroid precursors into the androgenic steroid pathways (Kumar and Clark, 2002). The suppression of gonadotropin secretion leading to failure in spermatogenesis, due to 21-hydroxylase was also reported (Tiitinen and Välimäki, 2002).

### **1.3 Glucocorticoids:**

#### **1.3.1 Secretion and transport:**

Acute and chronic regulation of adrenal glucocorticoids synthesis is under the control of ACTH. Once synthesized cortisol diffuses from the adrenal cortex and binds to a specific plasma-binding protein called transcortin, or cortisol binding globulin (CBG). About 90% of cortisol in the plasma is bound (Montgomery *et al.*, 1996).

#### **1.3.2 Excretion of cortisol:**

Excretion of cortisol by the kidneys occurs in proportion to the concentration of unbound hormone in plasma. By measuring the amount of cortisol excreted in 24 hours, a test called urinary free cortisol, one has an index of the average free cortisol in the plasma. This has become one of the most accurate measures of both excessive and deficient cortisol states (Montgomery *et al.*, 1996).

#### **1.3.3 Individual glucocorticoids:**

##### **1.3.3.1 Cortisol (hydrocortisone)**

It is the principal naturally occurring steroid; it is given orally, a soluble salt can be given intravenously for rapid effect in emergency (Laurence *et al.*, 1999).

Cortisol has one of the most pronounced circadian rhythms in nature. Most of the cortisol for the day is made in the few hours preceding awakening. For a person with a normal daily schedule, relatively little cortisol remains in the plasma by 4 pm. Diurnal variation of plasma cortisol level is related to the sleep-work pattern rather than to any light-dark pattern. It generally requires about 2 weeks to alter this circadian rhythm (Montgomery *et al.*, 1996). The adrenal gland produces cortisol at about 1 mg/kg B.wt. in most domestic species (Adams, 2001).

#### **1.3.3.2 Prednisolone:**

Synthetic, predominantly anti-inflammatory, biologically active, and has little sodium-retaining activity (Laurence *et al.*, 1999). It is four times as potent as cortisol, and can be used for alternate-day therapy (Adams, 2001).

#### **1.3.3.3 Prednisone:**

It is a prodrug i.e. it is biologically inert and converted into prednisolone in the liver (Laurence *et al.*, 1999).

#### **1.3.3.4 Methylprednisolone:**

It is similar to prednisolone; it is used intravenously (Laurence *et al.*, 1999).

#### **1.3.3.5 Dexamethasone and betamethasone**

They are longer acting than prednisolone. They are 30 times as potent as cortisol (Adams, 2001).

#### **1.3.3.6 Beclomethasone and budesonide:**

They are used by inhalation for treating asthma (Laurence *et al.*, 1999).



Inhaled corticosteroids are integral part of the management of asthma, beclomethasone dipropionate twice daily (500 µg) have shown decreased insulin sensitivity and significant rises in both total and high density lipoprotein concentration (Balfour, 1988). Also a delay in puberty and growth retardation were observed in children (Stead and Cooke, 1989).

#### **1.4 Hypothalamic-pituitary adrenal axis (HPAA):**

In most domestic species, as in humans, cortisol is the main glucocorticoid produced by the adrenal glands under the influence of ACTH with diurnal variation (Adams, 2001).

Glucocorticoids production is under hypothalamic control, corticotropin releasing hormone (CRH) is secreted by the hypothalamus in response to circadian rhythm, stress and other stimuli. CRH travels down the portal system to stimulate ACTH release from the anterior pituitary (Kumar and Clark, 2002).

Circulating ACTH stimulates cortisol production in the adrenal. The cortisol secreted (or any other synthetic glucocorticoid administered to the patient) causes negative feedback on the hypothalamus and the pituitary to inhibit further CRH release (Kumar and Clark, 2002). Long term use of supra-physiologic doses of glucocorticoids may lead to adrenocortical atrophy and decreased adrenal secretory reserve (Adams, 2001).

##### **1.4.1 Physiologic stress:**

Almost any type of physical or mental stress can lead within minutes to greatly enhanced secretion of ACTH and consequently cortisol as well, often increasing cortisol secretion as much as 20-fold

(Guyton and Hall, 2006). It is reported by Hamilton-Fairley and Taylor (2003) that stress is one of the causes of anovulation in women.

The association between stressful life events and the development of cancer is supported by a large body of anecdotal clinical evidence that has been collected since the eighteenth century. It is suggested that a prognostic association between severe life stressors and recurrence of breast cancer (Ramirez *et al.*, 1989).

### **1.5 Adrenal insufficiency (Addison's disease);**

Adrenal insufficiency is common in communities with high prevalence of tuberculosis, but too rapid withdrawal of glucocorticoids is another cause (Clayton, 1989).

Rifampicin induces cytochrome P<sub>450</sub> enzymes and accelerates the metabolism of cortisol and can cause adrenal crisis early in the course of antituberculous chemotherapy. Hypoglycaemia strongly support the diagnosis of Addison's disease (Clayton, 1989).

Ideal replacement therapy should mimic the adrenal glands' hormonal output under basal conditions, with doses increasing if the animal is stressed by illness or surgery. Practically, this ideal is never achieved (Adams, 2001).

### **1.6 Cushing's syndrome:**

It is a clinical disorder which results from the exposure of body tissues to sustained high blood levels of glucocorticoids (Jain, 2005). The syndrome is still associated with substantial morbidity and mortality (Boscaro *et al.*, 2001).

The most common form, which is termed Cushing's disease and accounts for 60 – 80% of cases in most series, is generally due to

overproduction of ACTH from the pituitary (Boscaro *et al.*, 2001; Kumar and Clark, 2002).

Long-term treatment with glucocorticoids (e.g. dexamethasone or prednisone) may produce clinical features of hypercortisolism (Boscaro *et al.*, 2001).

The disease is characterized by truncal obesity (96%), facial fullness (82%), diabetes or glucose intolerance (80%), gonadal dysfunction (74%), acne (72%), hypertension (68%), mood disorders (58%) and osteoporosis (38%), in patients who suffered from Cushing's syndrome (Boscaro *et al.*, 2001). In the dog iatrogenic Cushing's syndrome has resulted from topical ophthalmic glucocorticoids preparation (Murphy *et al.*, 1990; Jeffers *et al.*, 1991).

The most common cause of Cushing's syndrome is the use of supraphysiological amounts of exogenous glucocorticoids, including topical or inhaled corticosteroids (iatrogenic Cushing's syndrome). Thus, adequate knowledge of an individual's medication history is essential for diagnosis. Rarely, patient might present with factitious Cushing's syndrome, with hidden use of glucocorticoids, which can be a substantial diagnostic challenge, especially if hydrocortisone is being taken, since the use of this substance will cause raised concentrations of circulating cortisol (Newell-Price *et al.*, 2006).

New data, suggest that Cushing's syndrome is more common than had previously been thought. In screening studies of obese patients with type 2 diabetes, especially those with poor blood glucose control and hypertension, the reported prevalence of Cushing's syndrome is between 2 and 5%. These data suggest that more widespread screening for Cushing's syndrome in such patients is

warranted. Researchers still need to prove that control of cortisol excess is more beneficial than the attention to more specific abnormalities of metabolic and cardiovascular risk. Biochemical confirmation of the hypercortisolaemic state must be established before any attempt at differential diagnosis: failure to do so will result in misdiagnosis, inappropriate treatment and poor management (Newell-Price *et al.*, 2006). Diabetes mellitus and hyperadrenocortism are both well documented and commonly recognized endocrinopathies of dogs (Blaxter and Gruffyad-Joes, 1991).

Reproductive abnormalities frequently associated with Cushing's syndrome in the dog. Moreover a substantial reductions in basal levels of T<sub>4</sub> and T<sub>3</sub> have been observed in human patients with Cushing's syndrome (Kemppainen *et al.*, 1983).

### **1.7 The thyroid gland:**

In the normal adult, the thyroid gland weight is about 25 g and consists of two connected lobes, closely associated with the trachea. The gland is divided by connective tissue into clusters of follicles or acini. Each follicle consists of a single layer of cells surrounding a colloid containing the protein thyroglobulin; there are about a million follicles in the human gland. This colloidal thyroglobulin is the source of the two thyroid hormones: thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) (Gillham *et al.*, 2001).

#### **1.7.1 Thyroid hormones:**

Thyroid hormones are vital signaling molecules in both highly develop and primitive organisms. In-contrast to many signals that fluctuate with relative rapidity, the signals of thyroid hormones are

remarkably stable as a result of the long half-life (6 to 9 days) of  $T_4$ . On the other hand  $T_3$  is more active than  $T_4$  and has a half-life of 24 to 36 hours (Montgomery *et al.*, 1996).

Thyroid hormones regulate energy and heat production; facilitate healthy development of the central nervous system, somatic growth and puberty; and regulate synthesis of protein important in hepatic, cardiac, neurological and muscular function. These diverse effects account for numerous and varied symptoms of hyperthyroidism (Cooper, 2003).

### **1.7.2 Biosynthesis of $T_4$ and $T_3$ :**

Iodide absorbed from the diet or drinking water is transported in the blood to the thyroid gland, where it is very efficiently transferred into the follicular cells of the thyroid gland by an 'iodide pump' system which is dependant on ATP (Gillham *et al.*, 2001). The first step in synthesis of thyroid hormone is the accumulation of iodine (Montgomery *et al.*, 1996).

The next step in thyroid hormone synthesis involves the covalent attachment ("Organification") of the iodine to thyroglobulin (Montgomery *et al.*, 1996). Thyroglobulin is a typical secretory protein. The vesicles segregate to the apical plasma membrane of the follicular cells and it is this stage that thyroglobulin undergoes iodination prior to its release and storage in the colloid (Gillham *et al.*, 2001). Iodide is oxidized by hydrogen peroxide to form a positively charged iodine ion that reacts with specific tyrosine residues on the thyrogloulin to produce monoiodotyrosines within the thyroglobulin. This reaction is catalyzed by the enzyme thyroperoxidase. Monoodotyrosines undergo a similar second reaction to form

diiodotyrosine. This reaction is also catalyzed by thyroperoxidase (Montgomery *et al.*, 1996).

After this step comes the coupling of iodotyrosines to form the thyroid hormones. The coupling of two 3/5 diiodotyrosines yields  $T_4$ , and the coupling of 3 monoiodotyrosine to diiodotyrosine forms  $T_3$  with the removal of alanine side chain (Montgomery *et al.*, 1996).

Under normal conditions,  $T_4$  makes up the majority of hormones produced by the thyroid gland, relatively little  $T_3$  is synthesized. Instead, most  $T_3$  in plasma is formed in extrathyroidal tissue by 5'-deiodinase, which catalyzes the removal of an iodine from the outer ring of  $T_4$ . Many tissues also have the enzyme 5-deiodinase which catalyzes the removal of an iodine from the inner ring to form reverse  $T_3$  ( $rT_3$ ), which is an inactive compound. Formation of  $rT_3$  is thought to be an adaptive mechanism to lower the basal metabolic rate during starvation or systemic illnesses (Montgomery *et al.*, 1996).

### **1.7.3 Release of $T_4$ and $T_3$ from the thyroid gland:**

Thyroglobulin itself is not released into the circulating blood in measurable amounts; instead,  $T_4$  and  $T_3$  must first be cleaved from the thyroglobulin molecule and then these free hormones are released. This process occurs as follows: The apical surface of the thyroid cells sends out pseudopod extensions that close around small portions of the colloid to form pinocytic vesicles that enter the apex of the thyroid cell, then lysosomes in the cell cytoplasm immediately fuse with these vesicles to form digestive vesicles containing digestive enzymes from the lysosomes mixed with the colloid. Multiple proteases among the enzymes digest the thyroglobulin molecules and release  $T_4$  and  $T_3$  in free form. These then diffuse through the base of the thyroid cells into

the surrounding capillaries. Thus, the thyroid hormones are released into the blood (Guyton and Hall, 2006).

About three quarters of the iodinated tyrosine in the thyroglobulin never become thyroid hormones but remains monoiodotyrosine and diiodotyrosine. During the digestion of the thyroglobulin molecule to cause release of  $T_4$  and  $T_3$ , the iodinated tyrosines also are freed from the thyroglobulin molecules. However, they are not secreted into the blood. Instead, their iodine is cleaved from them by a deiodinase enzyme that makes virtually all this iodine available again for recycling within the gland for forming additional thyroid hormones. In the congenital absence of this deiodinase enzyme, many persons become iodine-deficient because of the failure of this recycling process (Guyton and Hall, 2006).

#### **1.7.4 Control of thyroid hormones secretion:**

Stimulation of follicle cells by thyroid stimulating hormone (TSH) activates a cAMP-dependant response which leads to the reuptake of the iodinated thyroglobulin from the colloid by a process of pinocytosis. The pinocytotic vesicles then fuse with lysosomes where proteolysis of the protein occurs to release  $T_3$  and  $T_4$  (Gillham *et al.*, 2001).

The secretion of thyroid hormones is controlled by the pituitary gland and the hypothalamus. Thyrotropin-releasing hormone (TRH), secreted by the hypothalamus, stimulates secretion of TSH by the pituitary gland. TSH, in turn stimulates the secretion of  $T_4$  and  $T_3$  (Evinger and Nelson, 1984).

### **1.7.5 Thyroid diseases:**

Diseases of the thyroid gland are a common type of endocrine dysfunction. They may be as an excess of thyroid hormones, called thyrotoxicosis or hyperthyroidism, or as lack of thyroid hormones, called hypothyroidism (Montgomery *et al.*, 1996).

#### **1.7.5.1 Hyperthyroidism:**

It is a common disease in young women. It is usually caused by an immune alteration that leads to the production of auto-antibodies called, thyroid-stimulating immunoglobulins (TSIG). These antibodies are capable of binding to the TSH receptor and stimulating adenyl cyclase. The thyroid gland is stimulated both to produce thyroid hormones and to grow, but the thyroid hormone T<sub>4</sub> (or T<sub>3</sub>) produced is not capable of shutting off the production of the TSIG. No negative feedback occurs. Hyperthyroidism can occasionally be caused by tumors or autonomously functioning nodules of the thyroid gland (Montgomery *et al.*, 1996).

Hyperthyroid patients manifest symptoms of increased metabolism, including weight loss despite an augmented appetite, heat intolerance, rapid heart rate, tremors, and nervousness. The plasma levels of the thyroid hormones are elevated, but levels of TSH are suppressed because T<sub>4</sub> and T<sub>3</sub> inhibit pituitary synthesis and release of TSH. Hyperthyroidism caused by elevated TSIG level is called Graves' disease and is often associated with goiter and protuberance of the eyes (Montgomery *et al.*, 1996). This Graves' disease is treated with methylprednisolone (Dandona *et al.*, 1989) and this predisposes individuals to glucocorticoid adverse effects.



### **1.7.5.2 Hypothyroidism:**

Normal secretion of thyroid hormone depends on the intact feedback loop of the hypothalamic-pituitary-thyroid axis. Hypothyroidism is the term for deficient thyroid hormones secretion by the thyroid gland (Lindsay and Toft, 1997).

Thyroid hormones have an important role in intellectual development which makes screening programmes essential. Untreated, the hypothyroid newborn infant will lose an estimated 3 – 5 IQ points every month for the first 6 – 16 months (Lindsay and Toft, 1997).

Primary hypothyroidism is the most common form, and implies dysfunction of the thyroid gland. Secondary hypothyroidism results from dysfunction of the pituitary gland with resultant inability to secrete TSH. Histological evaluation of a thyroid gland biopsy specimen reveals lack of active resorption of colloid. Tertiary hypothyroidism involves dysfunction of the hypothalamus with impaired secretion of the thyroid releasing hormone. Histological evaluation of thyroid tissue should resemble secondary hypothyroidism (Evinger and Nelson, 1984).

There are several factors that may alter the basal plasma thyroid hormone concentration (e.g. corticosteroids) and result in an erroneous assessment of thyroid gland function (Evinger and Nelson, 1984).

It is emphasized that both secondary and tertiary hypothyroidism are rare, although important to diagnose; the pattern of subnormal T<sub>4</sub> and thyrotropin is more likely to be due to non-thyroidal illness, than to either of these disorders (Lindsay and Toft, 1997).

In-contrast to hyperthyroid patients, patients with hypothyroidism have a lowered metabolic rate. They are usually cold and gain weight despite normal or diminished caloric intake and may be more susceptible to over dosage of medications because they metabolize them slowly. Severely hypothyroid patients become hypothermic, sluggish, and lapse into unconsciousness, a state called myxedema coma. If untreated, myxedema coma is fatal (Montgomery *et al.*, 1996).

### **1.8 The luteinizing hormone (LH):**

The gonads (testes and ovaries) synthesize hormones necessary for physical development and reproduction. A single hypothalamic releasing factor, gonadotropin-releasing hormone (GnRH), stimulates the anterior pituitary to release the glycoproteins, luteinizing hormone and follicle-stimulating hormone (FSH). LH stimulates the testes to produce androgens and ovaries to produce estrogens and progesterone. FSH regulates the growth of ovarian follicles and stimulates testicular spermatogenesis. For maximum effect on the male or female gonad, FSH also requires the presence of LH (Champe and Harvey, 1994).

The ovarian changes that occur during the sexual cycle depend completely on the gonadotropic hormones, FSH and LH. In the absence of these hormones, the ovaries remain inactive, which is the case throughout childhood when almost no pituitary gonadotropic hormones are secreted (Guyton and Hall, 2006).

As shown in Fig. (2) LH is necessary for final follicular growth and ovulation. Without this hormone, even when large quantities of FSH are available, the follicle will not progress to the stage of ovulation. The LH also has a specific effect on the granulosa and

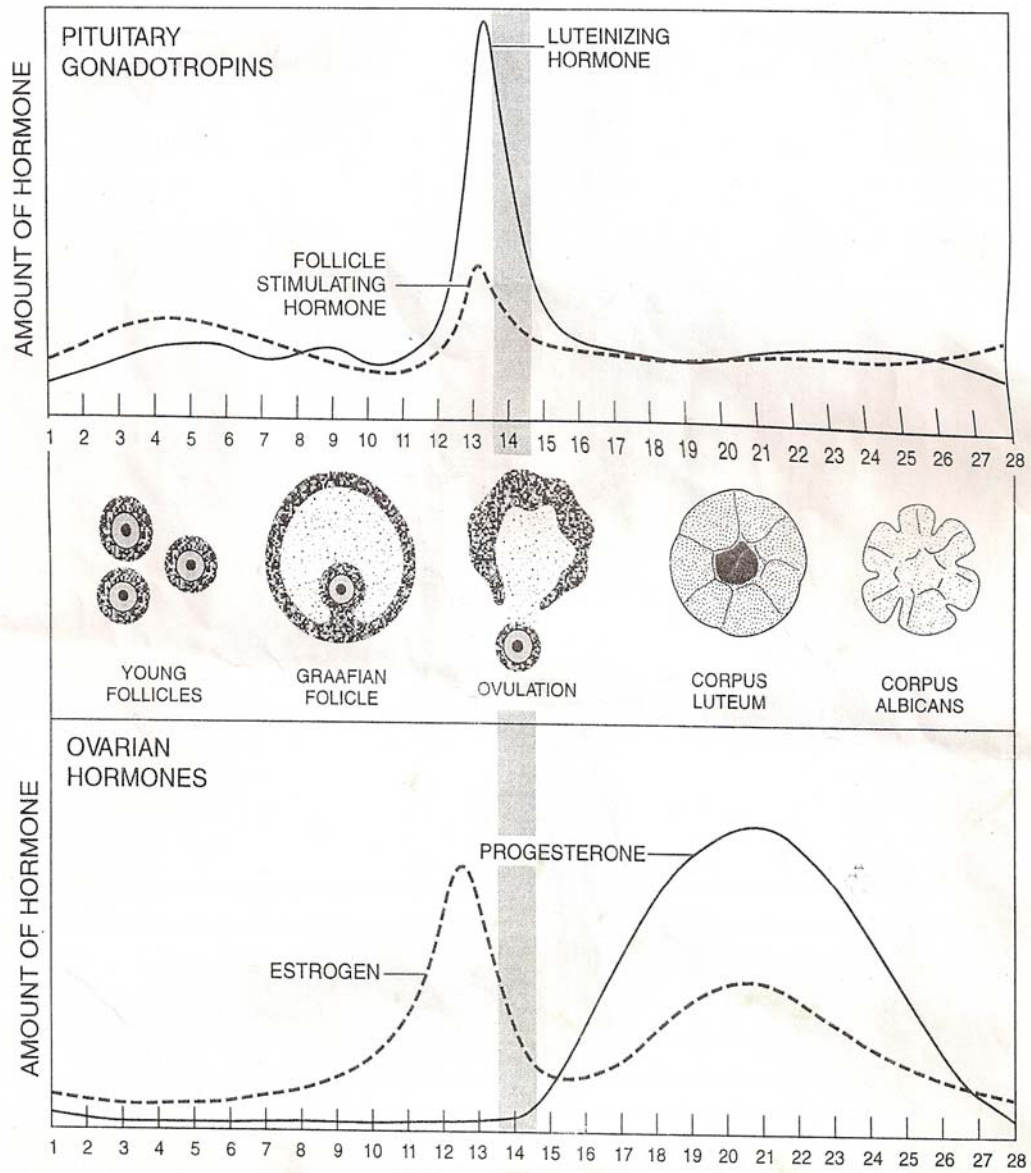
theca cells, converting them mainly to progesterone-secreting cells. Therefore, the rate of secretion of estrogen begins to fall about 1 day before ovulation, while increasing amounts of progesterone begin to be secreted. It is in this environment of rapid growth of the follicle, diminishing estrogen secretion (after a prolonged phase of excessive estrogen secretion) and initiation of secretion of progesterone that ovulation occurs. Without the initial pre-ovulatory surge of LH, ovulation will not take place (Guyton and Hall, 2006).

If the pre-ovulatory surge of LH is not of sufficient magnitude, ovulation will not occur and the cycle is said to be "anovulatory" and there is almost no secretion of progesterone during the later portion of the cycle (Guyton and Hall, 2006).

The first cycles after the onset of puberty are usually anovulatory, as are the cycles occurring several months to years before menopause presumably because the LH surge is not potent enough at these times to cause ovulation (Guyton and Hall, 2006).

It has long been known that administration of either estrogen or progesterone "the pill", if given in appropriate quantities during the first half of the monthly cycle, can inhibit ovulation. Because these hormones can prevent the preovulatory surge of LH secretion by the pituitary gland, which is essential for causing ovulation (Guyton and Hall, 2006).

**Fig. (2): Changes in the levels of gonadotropic hormones and ovarian hormones during the menstrual cycle (from Jain, 2005).**



Testosterone is the major sex steroid in men, it is produced by Leyding cells of the testes. The major stimulus for synthesis and secretion of testosterone is LH, which binds to specific receptor on the surface of Leyding cells, stimulating the production of cyclic AMP. This results in a cascade of events that culminates in an increase in SCC enzyme, 17  $\alpha$ -hydroxylase and 17, 20-Lyase activities. Testosterone is present in very high concentration in the testes, and such concentration is necessary for normal spermatogenesis (Montgomery *et al.*, 1996). The amount of testosterone secreted increases approximately in direct proportion to the amount of LH available (Guyton and Hall, 2006).

Mature Leyding cells are normally found in a child's testes for a few weeks after birth but then disappear until after the age of about 10 years. However, either injection of purified LH into a child at any age or secretion of LH at puberty cause testicular interstitial cells that look like fibroblasts to evolve into functioning Leyding cells (Guyton and Hall, 2006).

### **1.9 Adverse effect of glucocorticoids:**

Exposure to excessive glucocorticoids causes so many adverse effects: Adrenocortical suppression was reported by Newton *et al.* (1978) in workers manufacturing synthetic glucocorticoids (in spite of their adherence to the safety precautions), invasive fungal infection (Lionakis and Kontoyiannis, 2003), growth retardation in asthmatic children (Balfour, 1988), osteoporosis (Shewring *et al.*, 1991; Spector, 1993), cataract (Kumar and Clark, 2002), obesity (Haslam and James, 2005), hypertension and glucose intolerance (Newell-Price *et al.*,

2006) and others mentioned below to reveal the hazards of using glucocorticoids.

### **1.9.1 Effects of glucocorticoids on the thyroid gland:**

Six men were given a single dose of 8 mg betamethasone intravenously (i/v). Immediately after the i/v administration of betamethasone, both plasma TSH and cortisol levels began to fall, and there was a significant reduction of TSH after 12 hr. There were significant decrements in plasma T<sub>3</sub> levels 16 to 40 hr. after betamethasone administration, while plasma T<sub>4</sub> levels didn't show any significant changes. Although the hypothesis that peripheral conversion of T<sub>4</sub> to T<sub>3</sub> is selectively inhibited by the glucocorticoids is intriguing, some of the T<sub>3</sub> decrement may have resulted from the suppression of TSH which is known to induce the thyroidal secretion of T<sub>3</sub> in preference to T<sub>4</sub>. This data indicate that the effects of a single injection of a large dose of synthetic corticosteroid persist for at least 5 days. Therefore, in clinical situation, measurement of plasma thyroid hormones, TSH and cortisol should be avoided at least a week after such treatment (Azukizawa *et al.*, 1979).

In another study in dogs, the effect of multiple intramuscular injections of prednisone (2.2 mg/kg Bwt) every alternate day on thyroid morphology and plasma thyroxine and triiodothyronine concentrations were investigated. Plasma T<sub>4</sub> and T<sub>3</sub> concentrations decreased significantly after 3 injections of prednisone were made. The reduced basal plasma T<sub>4</sub> and T<sub>3</sub> values in dogs given multiple prednisone treatments were similar to reduction in circulating iodothyronines levels after glucocorticoids administration in persons and rats (Woltz *et al.*, 1983).

In-contrast, a single injection of prednisone did not significantly reduce plasma T<sub>4</sub> concentration. The significant reduction in plasma T<sub>3</sub> values when plasma T<sub>4</sub> values were non-significantly reduced implies that prednisone either inhibited thyroid secretion of T<sub>3</sub> or inhibited the conversion of T<sub>4</sub> to T<sub>3</sub>. It has been proposed that glucocorticoids specifically inhibit the 5' deiodinase enzyme which function to convert T<sub>4</sub> to T<sub>3</sub> in the periphery (Woltz *et al.*, 1983).

The impairment of thyroid function due to prednisolone administration is confirmed by morphologic examination of the gland. Indeed, inactive areas characterized by enlarged follicles with accumulation of colloid droplets in epithelial cells were frequently observed in thyroids of treated dogs (Kurtdele *et al.*, 2004). The remarkable low number of lysosomes in thyroids from treated dogs suggested that the inhibition of the lysosomal hydrolysis of colloids in follicle cells induced severe decrease of the hormone release into the blood (Kemppainen *et al.*, 1983; Woltz *et al.*, 1983; Kurtdele *et al.*, 2004). Furthermore, previous studies in humans have reported that steroid anti-inflammatory drugs such as prednisolone directly acted on hypothalamo-hypophyseal axis by reducing the hypophyseal hormones, and notably of TSH and consequently by inducing a secondary hypothyroidism (Kurtdele *et al.*, 2004).

The repeated administration of the synthetic glucocorticoid, prednisone was clearly associated with substantial reduction in basal levels of T<sub>4</sub> and T<sub>3</sub>. Similar reduction in the levels of both hormones have been observed in human patients who suffered from Cushing's syndrome (Visser and Lamberts, 1981).

It is suggested that glucocorticoids induced suppression of T<sub>4</sub> and T<sub>3</sub> levels is a dose-related phenomenon, which may also be affected by the route of administration and the chemical form of the agent (Kemppainen *et al.*, 1983; Woltz *et al.*, 1983).

### **1.9.2 Effects of glucocorticoids on gonadotrophic hormones secretion:**

Short term high dose of dexamethasone administration decreases basal serum levels of LH and FSH in women with normal menstrual cycles (Sowers *et al.*, 1979). In another study it was observed that administration of paramethasone acetate 5 mg daily to young women with normal menstrual cycles resulted in significantly depressed basal LH levels on the second day of administration and that this suppression persisted throughout most of the menstrual cycle (Cortis-Gallegos *et al.*, 1975).

Not only did short-term administration of high doses of dexamethasone suppress basal levels of LH and FSH, but also the gonadotropin response to GnRH was blunted. It has also been reported that women on long-term glucocorticoid therapy had significantly lower LH responses to GnRH than female controls receiving no glucocorticoids. The authors of that study interpreted their results as indicating that glucocorticoids suppress LH release at the anterior pituitary level by diminishing responsiveness to GnRH (Sowers *et al.*, 1979; Insler and Lunenfeld, 1994).

It has been observed that patients with Cushing's disease have suppressed basal level of LH and FSH (Luton *et al.*, 1977). Furthermore pregnancy is a rare occurrence in women with Cushing's syndrome as a result of suppression of gonadotropin secretions primarily by excess glucocorticoids (Aron *et al.*, 1990).



Continuous infusion of intact heifers with ACTH beginning on day 2 of the estrous cycle was able to inhibit the small spikes in plasma LH that normally occur on day 3 and 4. The depression of plasma progesterone is probably due to insufficient circulating LH which resulted in an incomplete development and/or function of corpus luteum. It was also demonstrated that implantation of dexamethasone into the preoptic area was as effective as systemic injections in blocking ovulation in rats (Li and Wagner, 1983).

The effect of glucocorticoids on gonadal steroid and gonadotropic hormone concentrations and subsequent follicular activity in cows undergoing normal estrous cycle was evaluated by administration of dexamethasone (DXM) during the middle of the luteal phase using 2 mg 1/M of DXM twice daily from day 13 through day 17. Results indicated that high glucocorticoid concentrations in the middle of the cycle may delay the onset of the next estrus. This delay appears to be attributable to prolonged luteal activity that may be associated with reduction in pulsatile estradiol secretion as a consequence of suppressed LH concentration in the middle of the cycle. Administration of DXM during the follicular phase delays or interferes with ovulation in cows (Vighio and liptrap, 1990).

When bulls were given 20 mg DXM (I/M), the concentration of LH and testosterone were significantly ( $P < 0.001$ ) lower in the treated group compared to the control on day 2 of the experiment. DXM is a very potent depressant of the levels of both testosterone and LH in bovine plasma and probably acts through pituitary responsiveness to GnRH (Chantarapruteep and Thibier, 1978).

LH secretion in dogs is inhibited by prednisone administration, which consequently results in reduced testosterone concentration (Kemppainen *et al.*, 1983).

### **1.9.3 Effect of glucocorticoids on the liver:**

Healthy dogs were given 1 ml of an otic product containing dexamethasone administered twice daily to both ears of each dog for 21 days. Increase in alkaline phosphatase (ALP),  $\gamma$ -glutamyl transferase (GGT) and alanine transaminase (ALT) activity was progressive until day 21. GGT, ALP and ALT activity increased 58-fold, 11-fold and 18-fold, respectively (Meyer *et al.*, 1990). Dogs given ophthalmic medications containing glucocorticoids have had abnormal liver test results and adrenal suppression (Zenoble *et al.*, 1987; Glaze *et al.*, 1988). Seemingly, glucocorticoids are absorbed from the ear canal of dogs, and even small amounts of glucocorticoids in contact with a limited surface area result in multi-system effects. Unfortunately, results of ALP and GGT are used as sensitive indicator of cholestatic disorders (Meyer *et al.*, 1990). Prednisone injections of 4 mg/kg B.wt./day given for dogs resulted in hepatomegaly due primarily to hepatocellular accumulation of glycogen (Fittschen and Bellamy, 1984).

Serum ALP, ALT and GGT activities increased significantly ( $P < 0.05$ ) in all dogs subjected to hepatic duct ligation and those treated with dexamethasone (DeNovo and Prasse, 1983).

### **1.9.4 Effect of glucocorticoids on the pancreas:**

Thirty seven patients with acute severe Graves' ophthalmopathy were treated with methylprednisolone infusions. There were consistent

subclinical side effects, which consist of an increase in serum activity of exocrine pancreatic enzymes trypsin, amylase and lipase after methylprednisolone treatment (Dandona *et al.*, 1989). This is suggestive of pancreatitis (Dandona *et al.* 1985; Dandona *et al.*, 1989; Kumar and Clark, 2002).

#### **1.9.5 Effect of glucocorticoids on blood glucose concentration:**

Glucocorticoids stimulate gluconeogenesis and cause a moderate decrease in the rate of glucose utilization by most of the cells in the body. These cause the blood glucose to rise. The rise in blood glucose in turn stimulates secretion of insulin. The increase plasma levels of insulin, however, are not as effective in maintaining plasma glucose as they are under normal conditions. Glucocorticoids reduce the sensitivity of many tissues, especially skeletal muscles and adipose tissue to the stimulatory effects of insulin on glucose uptake and utilization (Guyton and Hall, 2006).

The increase in blood glucose concentration is occasionally great enough (50% or more above normal) that the condition is called adrenal diabetes. Administration of insulin lowers the blood glucose concentration only to a moderate amount in adrenal diabetes – not nearly as much as it does in pancreatic diabetes – because the tissues are resistant to the effects of insulin (Guyton and Hall, 2006).

## **CHAPTR TWO**

### **MATERIALS AND METHODS**

#### **2.1 Experimental animals:**

Ten adult intact males, domestic rabbits (*Lepus cuniculus*) ranging in weight from 1.1 – 1.65 kg were used in this study. The animals were purchased from the local market and they were in good health.

#### **2.2 Housing and management:**

##### **2.2.1 Housing:**

The animals were brought to the Department of Biochemistry, Faculty of Veterinary Medicine, Khartoum University, where they were kept in a calm room, provided with adequate ventilation under natural light. The rabbits were penned singly in wire net cages (60 x 60 x 60 cm) to avoid social stress (Szeto *et al.*, 2004) and because intact bucks fight and inflict severe injuries (Flecknel, 2000). They were kept for an adaptation period of two weeks before experimentation so that they were accustomed to handling, collection of blood and experimental conditions.

##### **2.2.2 Feeding:**

The animals were fed equal amounts of fresh Lucerne (*Medicago sativa*) 0.4 kg and sorghum grains (*Sorghum vulgare cundatum*) 90 g with water available ad lib.

### **2.2.3 Hygiene:**

The cages were cleaned everyday using soap and water to avoid high ammonia levels that predispose to respiratory infections. Black phenolic plus water were used for cleaning during the experimental period, to disinfect the whole area.

### **2.2.4 Clinical examination:**

The animals were daily examined and they remained healthy throughout the study.

## **2.3 The experimental procedure:**

### **2.3.1 Animals grouping:**

The rabbits were randomly assigned into treated and control groups (five rabbits per group).

### **2.3.2 The treated group:**

This group was given prednisolone 1% (VMD, Belgium); 2.2 mg/kg body weight (Bwt) intramuscularly 1/M (occipital muscle) every other day at 9 a.m. using one ml syringe of 28 needle gauge, for a total of 6 doses. Day 1 of the experiment is referred to the day of the first injection.

### **2.3.3 The control group:**

This group was given an equivalent volume of prednisolone 1% - vehicle solution 1/M (occipital muscle), which was obtained from (VMD Belgium) via the express mail.

### **2.3.4 Handling and restraint:**

Animals were approached vertically (from above), grasping by the 'scruff' of the withers to lift the animal from the cage, supporting

the rump with the other hand. The rabbit was then placed in a small plastic cage so that it was confined and easily injected.

### **2.3.5 Blood sampling:**

The hair over the jugular vein was shaved. Blood for separation of serum was obtained by venipuncture of the jugular vein using 24 gauge needle and 5 ml disposable syringe. Blood was then transferred to plain tubes with clot activator and allowed to clot at room temperature for 3 hours. The clotted blood was centrifuged at 3000 rpm for ten minutes. The separated serum was decanted using a micropipette and immediately stored at -20°C until analysis.

## **2.4 Analytical methods:**

### **2.4.1 Stat Fax 2100 Micro- plate Reader (USA):**

It is fully automatic, reads and prints absorbance of 96 wells in about 2 minutes. It has a large non-volatile memory stores at least 50 user entered test, light source is tungsten lamp with lamp saver feature. Calculation modes includes; single-point calibration, uptake mode, point to point curve fit, polynomial regression linear regression, cut off absorbance and multipoint % absorbance. Additional features includes: complete user prompting flags and error massager, automatic interpretation options, control locator, self-check system and curve plotting and editing.

### **2.4.2 Determination of serum thyroxine (T<sub>4</sub>) concentration using enzyme immunoassay (EIA):**

#### **Principle of the test:**

Monoclonal antibodies specific to T<sub>4</sub> are immobilized on micro well plates. The label used is T<sub>4</sub> conjugated to horseradish Peroxidase

(HRP). In the assay  $T_4$  is released from its binding proteins by ANS (8-Anilino-naphthalen-1-sulfonic acid) present in the assay buffer. Total  $T_4$  in the specimen competes with HRP-labeled  $T_4$  for binding to the immobilized antibody. After washing, enzyme substrate is added. The amount of total  $T_4$  in the sample is inversely proportional to the enzyme activity. The reaction is terminated by adding the stop solution. Absorbance is measured on a plate reader.

**Reagents:**

**- Micro-well plate:**

12 strips x 8 breakable wells coated with mouse monoclonal  $T_4$  antibody.

**- Assay buffer**

It contains Kathon (0.09%) as preservative.

**- Standards (A, B, C, D, E, F).**

The standard values are 0, 20, 50, 100, 200 and 300 nmol/l.

**- Enzyme conjugate**

$T_4$  conjugated to horseradish Peroxidase.

**- Wash solution.**

**- TMB substrate.**

**- Stop solution**

It contains 0.3 M  $H_2SO_4$ .

**Equipment:**

**- Pipette with disposable plastic tips**

50  $\mu$ l (standards samples).

**- Multi-channel pipette with disposable plastic tips**

50 – 100  $\mu$ l (conjugate substrate, stop solution).

**- Timer.**

**- Stat Fax 2100 micro-plate reader.**

- Aspiration device.

**Test procedure:**

- All reagents were allowed to reach room temperature before use.
- The wells to be used on the plate were marked.
- 50  $\mu$ l of the standards (in duplicate) and serum samples were pipetted into appropriate wells.
- 50  $\mu$ l of diluted enzyme conjugate were dispensed into the wells.
- The plate was covered and shaken for a few seconds to mix the contents of the wells, then incubated for 60 minutes at room temperature in the dark.
- The wells were aspirated and washed 3 times with 300  $\mu$ l of washing solution.
- 100  $\mu$ l of TMB-substrate solution were added into each well (at timed intervals).
- The plate was covered and incubated for 15 minutes at room temperature in the dark.
- The reaction was stopped by adding 100  $\mu$ l of stop solution into each well at the same timed intervals above, the plate was shaken gently to mix the solutions.
- The absorbance was measured at 450 nm.

**Calculation of the results:**

- The mean absorbance for each duplicate was calculated.
- Standard curve system was plotted and the concentration of T<sub>4</sub> in the samples was calculated.



### **2.4.3 Determination of serum triiodothyronine (T<sub>3</sub>) concentration using EIA:**

#### **Principle of the test:**

Micro-plates are coated with anti-T<sub>3</sub> antibodies. Upon mixing immobilized anti-T<sub>3</sub> antibodies, enzyme-T<sub>3</sub> conjugate, and serum containing the native T<sub>3</sub> antigen, a competition reaction results between the native T<sub>3</sub> and the enzyme-T<sub>3</sub> conjugate for a limited number of insolubilized binding sites. After equilibrium was attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The activity of the enzyme present on the surface of the well is quantified by reacting with a suitable substrate to produce color. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of unknown can be ascertained. From comparison to the dose response curve, an unknown specimen's activity can be correlated with total triiodothyronine concentration.

#### **Reagents:**

- Micro-well plate: One 96-well micro plate coated with sheep anti-triiodothyronine serum.
- Enzyme conjugate: Triiodothyronine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix.
- Conjugate diluents: One bottle containing buffer, red dye. MIT (150 mg/l) and BND (150 mg/dl) preservatives, and binding protein inhibitors.
- Standard curve: Six vials at concentration of 0, 50, 100, 250, 500 and 750 ng/dl.

- Wash solution: One vial contains a phosphate buffer and a detergent (Tween 20).

TMB-substrate: One bottle containing tetramethylbenzidine (TMB) in buffer.

- Stop solution: It contains 0.3 M H<sub>2</sub>SO<sub>4</sub>.

**Equipment:**

- 50 µl pipette
- Dispensers for repetitive deliveries of 0.100 ml and 0.003 ml volumes.
- Micro-plate washer (squeeze bottle).
- Stat Fax 2100 Micro-plate Reader.
- Absorbent paper for blotting the micro-plate wells.
- Timer.

**Test Procedure:**

- All reagents, serum references and controls were brought to room temperature.
- The micro-plate wells were assigned for each serum reference, control and specimen to be assayed in duplicate.
- 50 µl of the appropriate serum reference, or specimen were put into the assigned wells.
- 100 µl of triiodothyronine-enzyme conjugate solution were added to all wells.
- The micro-plate was swirled for 20 – 30 seconds and then incubated for 60 minutes.
- The contents of the micro-plate were decanted and the plate was blotted dry with absorbent paper.
- 300 µl of wash buffer were added and then decanted (two times).

- 100 µl TMB-substrate were added to all wells and then incubated for 15 min. at room temperature.
- 100 µl of the stop solution were added to each well.
- The absorbance was read in each well at 450 nm.

**Results:**

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.

**2.4.4 Determination of serum thyroid stimulating hormone (TSH) using EIA:**

**Principle of the test:**

Antibody specific to TSH molecule is immobilized on micro-well plates and other antibodies to the TSH molecule are conjugated with biotin. TSH from the sample is bound to the plates and biotin conjugate is added. After a washing step, streptavidin horseradish peroxidase (HRP) conjugate is added. After a second washing step, substrate is added. The enzymatic reaction is proportional to the amount of TSH in the sample. The reaction is terminated by adding the stop solution, absorbance is measured on a plate reader at 450 nm.

**Equipment:**

- Pipette with disposable plastic tips 10 – 50 µl (standards samples).
- Multi-channel pipette with disposable plastic tips: 100 µl
- Aspiration device.
- Stat Fax 2100 Micro-plate Reader.

**Reagents:**

- Micro-well plate coated with anti TSH antibody.
- Assay buffer containing thimerosal 0.02% and gentamycin as preservatives.

- Standards, standards values are 0, 1, 6.5, 20 and 50 mIU/L.
- Biotin conjugate antibodies labeled with biotin.
- Streptavidin HRP conjugate.
- Wash solution.
- TMB substrate.
- Stop solution.

**Test procedure:**

- All reagents were allowed to reach room temperature before use.
- The wells to be used were marked.
- 100  $\mu$ l of biotin conjugate were pipetted into the wells.
- 50  $\mu$ l of standards in duplicate and serum samples were pipetted into appropriate wells, then incubated for 60 minutes at room temperature.
- The wells were washed 5 times with 300  $\mu$ l of washing solution.
- 100  $\mu$ l of diluted streptavidin HRP conjugate were pipetted into the wells.
- The wells were washed again 5 times with 300  $\mu$ l of washing solution.
- 100  $\mu$ l of TMB substrate solution were added at timed intervals into each well. The plate was covered and incubated for 20 minutes at room temperature.
- The reaction was stopped by adding 100  $\mu$ l of stop solution into each well at the same timed intervals as above. Then the plate was mixed.
- The absorbance was measured at 450 nm.

**Calculation of the results:**

- The mean absorbance for each duplicate was calculated.
- The absorbance value of the zero standard was subtracted from the mean absorbance values of standards, control and samples.
- Standard curve was drawn and the TSH concentrations of the samples were read.

**2.4.5 Determination of serum luteinizing hormone (LH) using EIA****Principle of the test:**

The LH assay is based on simultaneous binding of LH to two monoclonal antibodies, one immobilized on micro-well plates, the other conjugated with horseradish peroxidase (HRP). After a washing step, enzyme substrate is added. The enzymatic reaction is proportional to the amount of LH in the sample. The reaction is terminated by adding the stop solution. Then absorbance is measured on a plate reader.

**Reagents:**

- Micro-well plate: breakable wells coated with LH antibody.
- Assay buffer.
- Standard curve lyophilized. The standard values are 0, 1, 2.5, 10, 40 and 100 iu/L.
- Enzyme conjugate: LH antibody conjugated with horseradish peroxidase.
- Wash solution.
- TMB substrate.
- Stop solution 0.3 M H<sub>2</sub>SO<sub>4</sub>.

**Equipment:**

- Pipette with disposable plastic tips 25  $\mu$ l (standard samples).
- Multi-channel pipette with disposable plastic tips.
- Aspiration device.
- Timer.
- Stat Fax 2100 micro-plate Reader.

**Test procedure:**

- All reagents were allowed to reach room temperature before use and the standards were reconstituted.
- The wells to be used were marked on the plate.
- 25  $\mu$ l of the assay buffer were pipetted into the wells.
- 25  $\mu$ l of the standards and serum samples were pipetted into appropriate wells.
- 25  $\mu$ l of enzyme conjugate were pipetted into the wells, the plate was covered and incubated for 60 minutes at room temperature.
- The wells were aspirated and washed 3 times with 300  $\mu$ l of the washing solution.
- 100  $\mu$ l of TMB substrate were added to each well at timed intervals.
- The plate was covered and incubated for 15 minutes at room temperature.
- The reaction was stopped by adding 100  $\mu$ l of stop solution to each well at the same timed intervals as above.
- The absorbance was measured at 450 nm.

**Calculation of the results:**

- The mean absorbance for each duplicate was calculated.
- Standard curve was made, and the concentration of LH in the samples was calculated.

**2.5 Statistical analysis:**

Two groups compared test (t-test) was used for statistical analysis of data (Gomez and Gomez, 1984) with the aid of statistical package for social science programme (SPSS).

## **CHAPTER THREE**

### **RESULTS**

Mean concentrations of serum thyroxine ( $T_4$ ), triiodothyronine ( $T_3$ ), thyroid stimulating hormone (TSH) and luteinizing hormone (LH) in rabbits given multiple I/M injections of prednisolone (2.2 mg/kg Bwt) every alternate day, were compared with those of control animals in two separate periods, day 6 and day 12 respectively.

#### **3.1 Concentration of serum thyroxine ( $T_4$ ):**

The concentrations of  $T_4$  on day 6 and day 12 of the experiment were presented in Table (1) and Fig. (3).

On day 6 of the experiment there was non-significant difference in serum  $T_4$  levels between the treated ( $106.4 \pm 14.2$ ) and the control groups ( $105.2 \pm 5.9$ ).

On day 12 the mean value of  $T_4$  was significantly ( $P < 0.05$ ) reduced in the treated group ( $96.2 \pm 4.1$ ) compared to the control ( $129.0 \pm 5.4$ ).

#### **3.2 Concentration of serum triiodothyronine ( $T_3$ ):**

The concentrations of  $T_3$  on day 6 and day 12 of the experiment were presented in Table (1) and Fig. (4).

On day 6 the mean value of the treated group ( $283 \pm 28.4$ ) was non-significantly lower than that of the control group ( $304 \pm 34$ ).

On day 12 there was a highly significant ( $P < 0.05$ ) decrease in serum  $T_3$  values of the treated group ( $272 \pm 24.8$ ) compared to the control ( $342.6 \pm 47.3$ ).

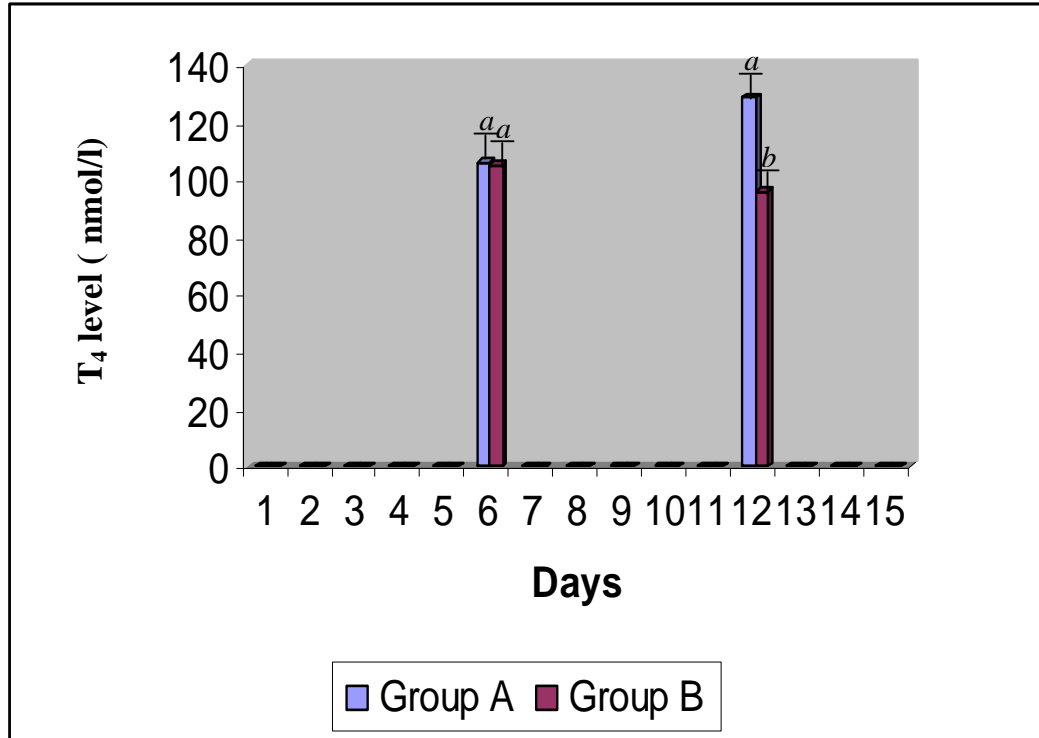


Table (1): The levels of thyroxine (T<sub>4</sub>), triiodothyronine (T<sub>3</sub>), thyroid stimulating hormone (TSH) and luteinizing hormone (LH) in rabbits treated with prednisolone (2.2 mg/kg Bwt) given I/M compared to the control on day 6 and day 12.

Hormone	Day 6		Day 12	
	Control	Treated	Control	Treated
T <sub>4</sub> nmol/l	106.4±14.2 <sup>a</sup>	105.2±5.9 <sup>a</sup>	129.0±5.4 <sup>a</sup>	96.2±4.1 <sup>b</sup>
T <sub>3</sub> ng/dl	304.0±34.0 <sup>a</sup>	283.4±28.4 <sup>a</sup>	342.6±47.3 <sup>a</sup>	272.0±24.8 <sup>b</sup>
TSH miu/l	0.24±0.1 <sup>a</sup>	0.206±0.08 <sup>a</sup>	0.17±0.05 <sup>a</sup>	0.14±0.03 <sup>a</sup>
LH miu/l	11.0±0.22 <sup>a</sup>	5.74±2.2 <sup>b</sup>	13.9±3.4 <sup>a</sup>	3.3±1.2 <sup>b</sup>

Means (± SE) having different superscript small letters within the same row are significantly different at (P< 0.05) based on (t-Test)

**Fig (1): The concentrations of T<sub>4</sub> in rabbits treated with prednisolone (2.2 mg /Kg Bwt) given I/M compared to the control on day 6 and day 12 of the experiment.**

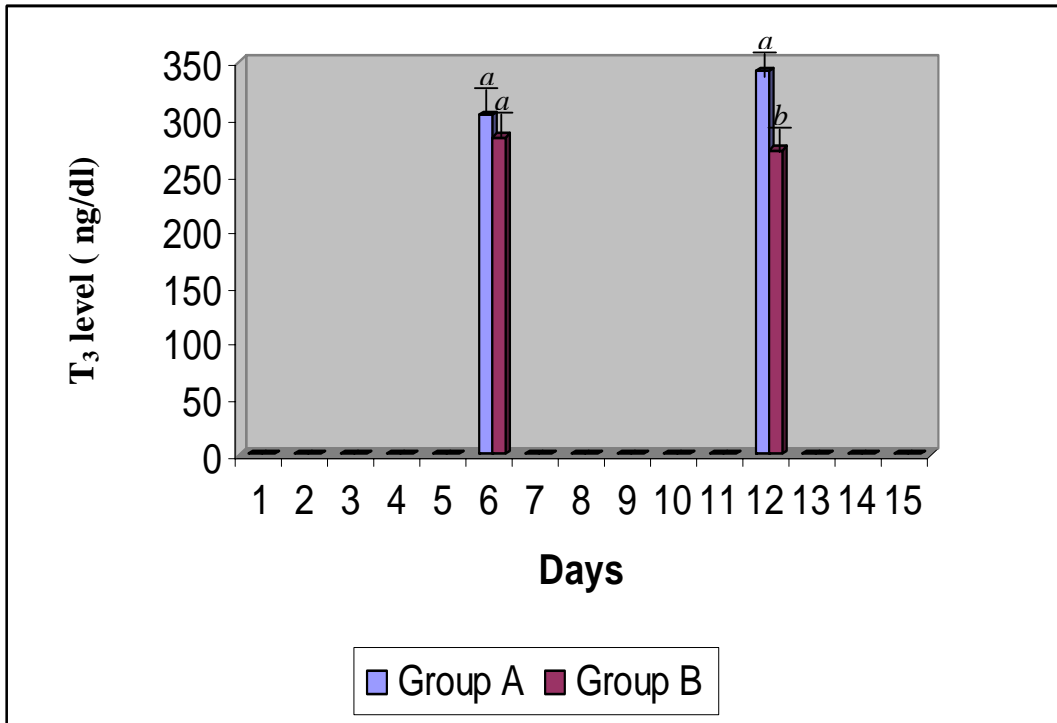


Bars having different superscript small letters are significantly different at (P< 0.05) based on (t-Test).

Group A: Control group

Group B: Treated group

**Fig (2): The concentrations of T<sub>3</sub> in rabbits treated with prednisolone (2.2 mg /Kg Bwt) given I/M compared to the control on day 6 and day 12 of the experiment.**



Bars having different superscript small letters are significantly different at ( $P < 0.05$ ) based on (t-Test).

Group A: Control group

Group B: Treated group

### **3.3 Concentration of serum thyroid stimulating hormone (TSH):**

The concentrations of TSH on day 6 and day 12 of the experiment were presented in Table (1) and Fig. (5).

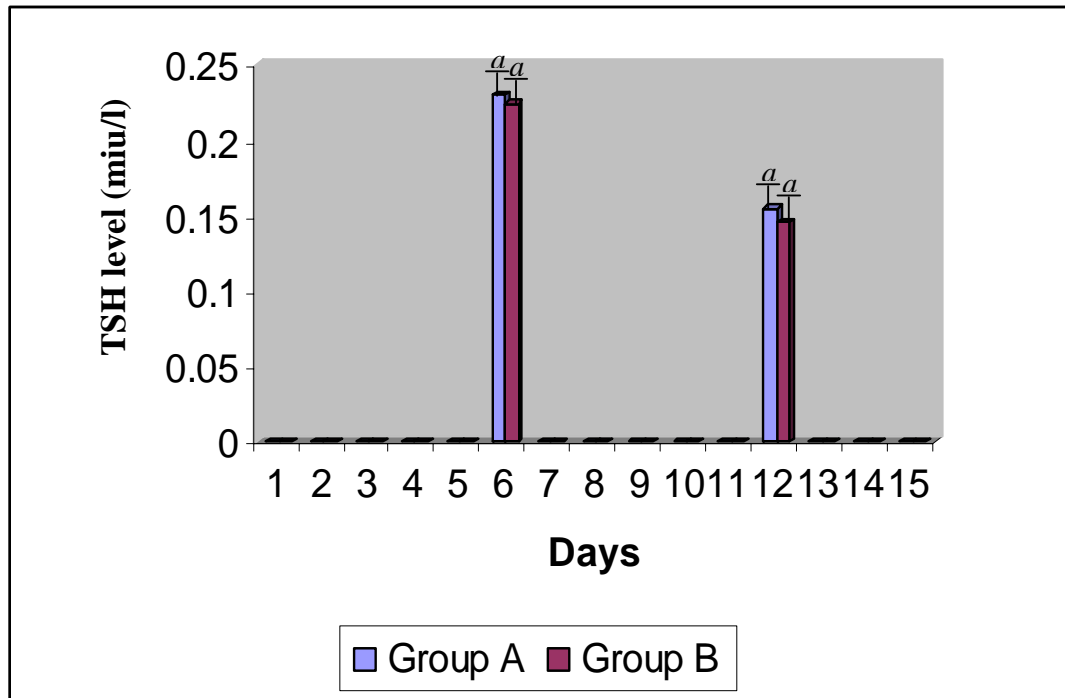
On day 6 the mean values of TSH of the treated group was non-significantly lowered ( $0.206 \pm 0.08$ ) compared to that of the control group ( $0.24 \pm 0.1$ ). Also on day 12 the mean values of TSH of the treated group ( $0.14 \pm 0.03$ ) were non-significantly decreased compared to the control group ( $0.17 \pm 0.05$ ).

### **3.4 Concentration of serum luteinizing hormone (LH):**

The concentrations of LH on day 6 and day 12 of the experiment were presented in Table (1) and Fig. (6).

On day 6 the mean values of LH of the treated group ( $5.7 \pm 2.2$ ) were significantly ( $P < 0.05$ ) reduced compared to the control group ( $11.0 \pm 0.22$ ). On day 12 the values of LH of the treated group ( $3.3 \pm 1.2$ ) were also significantly ( $P < 0.05$ ) less than that of the control group ( $13.9 \pm 3.4$ ).

**Fig (3):** The concentrations of TSH in rabbits treated with prednisolone (2.2 mg /Kg Bwt) given I/M compared to the control on day 6 and day 12 of the experiment.

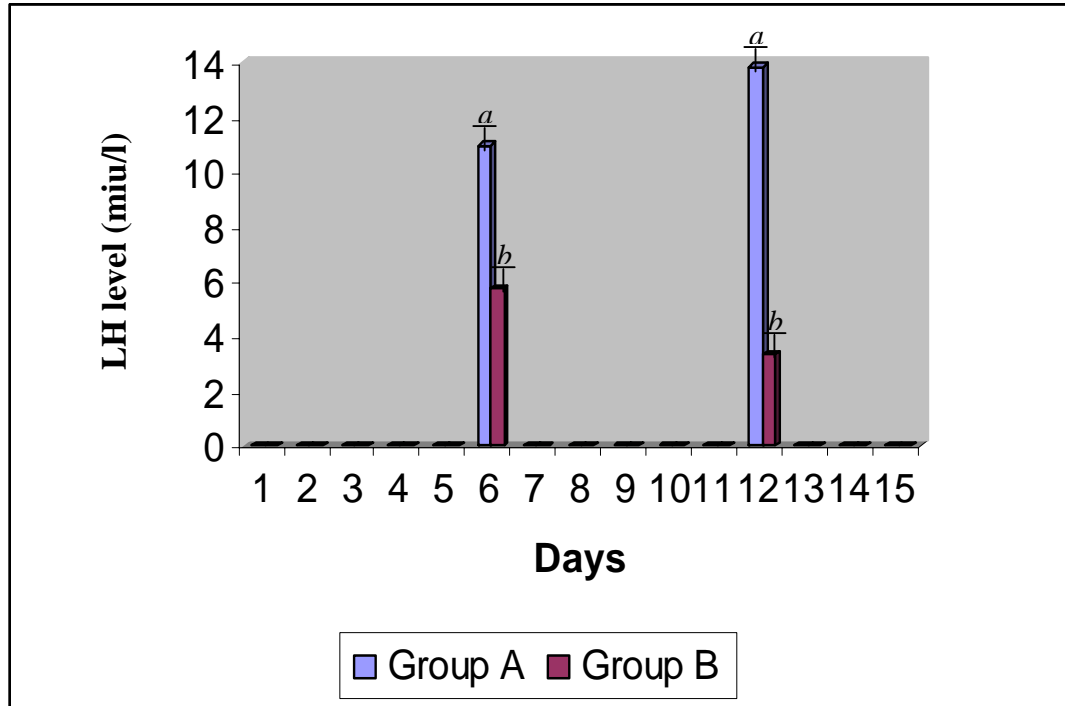


Bars having the same superscript small letters are nonsignificantly different at ( $P < 0.05$ ) based on (t-Test).

Group A: Control group

Group B: Treated group

**Fig (4):** The concentrations of LH in rabbits treated with prednisolone (2.2 mg /Kg Bwt) given I/M compared to the control on day 6 and day 12 of the experiment.



Bars having different superscript small letters are significantly different at ( $P < 0.05$ ) based on (t-Test).

Group A: Control group

Group B: Treated group

## **CHAPTER FOUR**

### **DISCUSSION**

Glucocorticoids are extensively used in bone marrow transplantation, solid organ transplantation and treatment of haematological malignancies, rheumatoid arthritis and chronic pulmonary conditions e.g. asthma (Lionakis and Kontoyiannis, 2003).

In veterinary practice huge amounts of dexamethasone and long acting dexamethasone are used in our country. Moreover prednisolone is incorporated in most of the intra-mammary syringes to treat mastitic animals.

Nowadays, there is a high occurrence of cataract and glaucoma among female university students. A Sudanese ophthalmologist mentioned in the local press and he claimed that this is due to the misuse of hydrocortisone eye drops (Personal communication). The widespread use and misuse of glucocorticoids dictates this study and still more research work on the effect of glucocorticoids on other systems of the body is needed.

#### **4.1 Serum thyroid hormones:**

In the present study, multiple administration of the synthetic glucocorticoid, prednisolone was clearly associated with substantial reduction in the serum levels of  $T_4$  and  $T_3$ . Similar reduction in the level of both hormones have been observed by Visser and Lamberts (1981) in patients with Cushing's syndrome, in healthy dogs after the third I/M injection of prednisone 2.2 mg/kg Bwt (Woltz *et al.*, 1983) and after 8 days in dogs given oral dose of prednisone 0.55 mg/kg Bwt/day (Kurtdele *et al.*, 2004).

Previous studies conducted in healthy human subjects given a single dose of betamethasone or dexamethasone failed to detect a decline in T<sub>4</sub> concentration, but did note a depression in the level of T<sub>3</sub> (Azukizawa *et al.*, 1979; Kemppainen *et al.*, 1983; Woltz *et al.*, 1983). In-contrast, in this study the levels of both T<sub>4</sub> and T<sub>3</sub> were non-significantly reduced up to the third injection (day 6) indirectly demonstrating that a single injection of prednisolone 2.2 mg/kg Bwt doesn't cause a significant reduction in the levels of T<sub>4</sub> and T<sub>3</sub> in domestic rabbits, whereas a single injection of prednisone of the same dose was able to cause a significant reduction of T<sub>3</sub> in the dog (Woltz *et al.*, 1983).

It is suggested that glucocorticoid induced suppression of T<sub>4</sub> and T<sub>3</sub> levels is a dose related phenomenon which may also be affected by the route of administration, chemical form of the agent, sex of the subject, doses, and treatment duration (Kemppainen *et al.*, 1983; Kurtdede *et al.*, 2004).

One possible explanation for the significant reduction in peripheral T<sub>4</sub> and T<sub>3</sub> levels in rabbits given prednisolone in this study is that, glucocorticoid acts directly on the thyroid gland, since lysosomal hydrolysis of colloid is a prerequisite for thyroid hormone secretion, the well-known stabilizing effect of glucocorticoids on lysosomal membranes could account for the cytoplasmic colloid accumulation, as revealed by electron microscopic examination of thyroid tissue of dogs treated with prednisone, subsequently reducing T<sub>4</sub> and T<sub>3</sub> secretion by inhibiting lysosomal hydrolysis of the colloid in the thyroid follicular cells (Kemppainen *et al.*, 1983; Woltz *et al.*, 1983; Kurtdede *et al.*, 2004).



#### **4.2 Serum TSH:**

In this study the mean values of TSH were not significantly reduced on day 6 and day 12 of the experiment. This finding does not agree with the study done by Azukizawa *et al.* (1979), who observed a profound reduction in TSH in men given a single dose of betamethasone, 8 mg for each man intravenously. The variation is possibly due to the potency of the drug, the high dose and the route of administration.

From the physiological point of view, there always exists a marked inverse relationship between plasma thyroid hormones and plasma TSH. Therefore, fall in thyroid hormones via negative feedback mechanism stimulate TSH secretion to cause rise in thyroid hormones (Jain, 2005). In-contrast, in the present study, on day12 there was a non-significant reduction of TSH while thyroid hormones were significantly reduced.

The explanation for these findings is that, low or normal TSH and low thyroid hormones test results represent a typical pattern in unwell patients with non-thyroidal illness (Lindsay and Toft, 1997; Dayan, 2001). Non-endocrinologists are frequently surprised to find TSH in the normal range in this situation, since the pituitary fails to respond adequately to low thyroid hormones concentration (Dayan, 2001). In clinical practice this is a greatest challenge in diagnosis of the condition. Glucocorticoids, dopamine and dobutamine can suppress serum TSH even in patients with overt primary hypothyroidism. Thus, thyroid function testing is best reserved for severely ill patients in whom there is a substantial clinical suspicion of hypothyroidism; otherwise abnormal results are much more likely to

represent false-positive than true-positive findings (Roberts and Ladenson, 2004).

### **4.3 Serum luteinizing hormone:**

In the present study the serum levels of LH were significantly decreased on day 6 and day 12 of the experiment. These findings are in agreement with a prior study in which administration of paramethasone acetate 5 mg daily to young women with normal menstrual cycles resulted in significantly depressed basal LH levels on the second day of administration, this suppression persisted throughout most of the menstrual cycle (Cortis-Gallegos *et al.*, 1975). Moreover, another study was conducted to determine the effect of short term administration of high doses of glucocorticoids on LH and FSH secretion and the site of this effect in women with ovulatory cycles. There was a significant reduction of the basal levels of LH and FSH and the gonadotropins response to GnRH was blunted (Sowers *et al.*, 1979).

It has been observed that patients with Cushing's disease have suppressed basal levels of LH and FSH and diminished gonadotropin responses to GnRH (Luton *et al.*, 1977).

Also this result of LH reduction due to prednisolone treatment is in agreement with the result of (Chemtarapreteeep and Thibier, 1983) who gave a single dose of 20 mg dexamethasone for each treated bull.

In an attempt to elucidate the mechanism of suppressive action of glucocorticoids on the hypothalamo-pituitary-ovarian axis, the effect of short-term high dose of dexamethasone on the LH response to GnRH was studied in healthy women with normal menstrual cycles,

they received 100 µg of GnRH intravenously on day 6 of two consecutive menstrual cycles, once with and once without pre-treatment with dexamethasone 2 mg/woman orally every 6 hrs, on days 2 through 5 of the menstrual cycle. The administration of dexamethasone suppressed baseline serum level of LH and blunted LH response to GnRH. This result indicated that pharmacological doses of glucocorticoids suppress the secretion of LH by direct effect on the pituitary and possibly by an effect at the hypothalamic level with inhibition of the release of GnRH (Sowers *et al.*, 1979). Similar studies were done by Chantaraprateep and Thibier (1978) in bulls and by Kemppainen *et al.* (1983) in dogs, who produced the same explanation.

Therefore, in the present study, it is suggested that the inhibition of LH secretion in domestic rabbits treated with prednisolone was at the hypothalamic and the pituitary level.

## **CONCLUSION AND RECOMMENDATION**

It was obvious that glucocorticoids affect thyroid and gonadotrophic hormones secretion, causing a significant reduction in thyroid hormones and luteinizing hormone leading to a great challenge in diagnosis of thyroid diseases and interpretation of fertility tests.

Thus it is recommended that:

1. Veterinarians should not use glucocorticoids during estrous cycle, in synchronized animals and in males at the time of breeding.
2. People should be informed of the potential side effects of glucocorticoids, and the information of patients receiving such drugs should be documented in the clinical record.

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