Sudan University of Science and Technology
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Assessment of Fertility Hormones among Infertile Men in Red Sea State

قياس مستوى هرمونات الخصوبة لدى الرجال المصابين بالعقم بولاية بالبحير الأحمر

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BY

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{للّه مُلِكُ السَّمَاوَاتِ وَالْأَرْضِ يَخْلُقُ ما يَشَاء لِمَن يَشَاء إِنَّهُ يَهْبُ يَهْبُ يَهْبُ مَن يَخْلُقُ وَاَلْأَرْضِ مُلْكُ ﴿لِلّهِ ﺍﻟْذُّآْوُرَ يَشَاء ﻧَٰ، إِنَّهُ ﻋَقْرِيمٌ ﻟِمَن يَشَاء إِنَاثً، ذُآْوُرً أَوْ ﻳَزْوَجُهُمْ ذَكَرًانَانْ إِنَاثًا، وَيَجْعَلُ مَن يَشَاء عَقْرِيمًا إِنَّهُ ﻋَلِيمٌ ﻗَدِيرٌ ﱡ} صدق الله العظيم

- سورة الشورى آية رقم (94-100)
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Dedication

To my father and mother who give me all hopes,

To my wife Alzeina, my son Yousef and my daughter Hebat Alla,

my support is difficult and hard.

To my brother and sister.

To my friends.

I dedicate this work
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First and last acknowledgment is to my God, who enable me to conduct this study. and to my supervisor, Dr. Badereldein Hassan Elabid, from whom I knew how to start and finish.

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Finally, I am grateful to those whom samples were taken.
Abstract

Male infertility is a common problem in Red Sea State, Sudan. In the majority the cause is previous exposure to venereal diseases, and in some cases there is no known cause of infertility. This study aimed to assess the sperm count and the plasma levels of fertility hormones among men with history of infertility in Red Sea State, Sudan.

Across-sectional study was conducted in Port Sudan Teaching Hospital, Red Sea State, Sudan, during the period from March 2012 to March 2014, 150 married men with a complain of infertility were assessed for the analysis of plasma levels of fertility hormones and the sperm count, along with 94 apparently health proven fathers as a control group. The test group and the control group were matched for age. SPSS was used for analysis of data. The data was compared using student’s t” test and Pearson's correlation was used for assessment of correlation between different variables.

This results showed that the mean of the sperm count of the test group was significantly reduced compared with that of the control group, (p = 0.000). The study showed a significant increase in the means of plasma levels of Follicle stimulating hormone, Luteinizing hormone and prolactin of the test group compared to control group, (p = 0.000). There is no significant difference between plasma levels of Testosterone of the test group and control group (p = 0.100). The results of present study showed no significant correlations between plasma levels of Follicle stimulating hormone, Luteinizing hormone and prolactin hormone with Testosterone, and also no significant correlation between plasma levels of Prolactin and Testosterone to the sperm count. A significant correlations of the Follicle stimulating hormone and Luteinizing hormone to the Sperm count.
The present data indicate that among infertile men in Red Sea State, the sperm count is significantly reduced, whereas the plasma levels of Follicle Stimulating Hormone, Luteinizing Hormone and Prolactin are significantly increased. Plasma levels of Follicle Stimulating Hormone, Luteinizing Hormone have a significant positive correlations with the sperm count.
مستخلص الدراسة

العقم عند الرجال من المشاكل الشائعة في ولاية البحر الأحمر بالسودان. في غالبية المرضى يعتقد أن السبب هو التعرض للأمراض التناسلية في فترات سابقة، وفي بعض الحالات لا يوجد سبب معروف للعقم. تهدف هذه الدراسة لتقييم عدد الحيوانات المنوية ومستويات هرمونات الخصوبة لدى الرجال.

هذه دراسة مقطعية أجريت في مستشفى بورتسودان التعليمي، ولاية البحر الأحمر بالسودان، خلال الفترة من مارس 2012 إلى مارس عام 2014، على 150 رجلا متزوجا يشكلون من العقم. تم تحليل مستويات هرمونات الخصوبة وهي تشمل الهرمون منشط الجريب (FSH)، الهرمون اللوتيني (LH)، هرمون البرولاكتين (PRL) وهرمون التستوستيرون بانطالي وجوارب البحر الاحمر والبادية. وتغطيت دراسة عدد الحيوانات المنوية TOSOH AIA360 عن طريق جهاز 60 neubauer improved champer بالطريقة اليدوية عن طريق الطريقة المتاحة في الأيام. تم استخدام برنامج كمبيوتر لتحليل البيانات. تم مقارنة البيانات باستخدام "t" وقيمة (P. value) ، ثم يستخدم ارتباط SPSS بيرسون لتقييم الارتباط بين المتغيرات المختلفة.

متوسط عدد الحيوانات المنوية في مجموعة المرضى انخفض وفقًا لدلاله إحصائيًا مقارنة مع مجموعة الضابطة حيث كان الاحتمال (0.000). أظهرت الدراسة أيضاً إرتفاعاً ذات دلاله إحصائيًا في متوسط مستويات البلازما لهرمون منشط الجريب (FSH)، الهرمون اللوتيني (LH) وهرمون البرولاكتين (PRL) في مجموعة الاختبار مقارنة مع مجموعة الضابطة حيث كان الاحتمال هو (0.000). عند مقارنة مستوى هرمون التستوستيرون في مجموعة الاختبار مع مجموعة الضابطة كان الإحتمال هو (0.10) وهي قيمة ليست ذات دلاله إحصائية.

كما أظهرت الدراسة أيضا أن العلاقة بين مستويات الهرمون المنبه للجريب (FSH)، الهرمون اللوتيني (LH) وهرمون البرولاكتين مع هرمون التستوستيرون ليست علاقات ذات دلالات إحصائية.

كما أوضحت الدراسة عدم وجود ارتباط ذو دلاله إحصائي بين مستويات الهرمون تستوستيرون وهرمون البرولاكتين مع عدد الحيوانات المنوية. كما أظهرت النتائج وجود
إرتباط ذو دلالة إحصائية بين مستويات البلازما للهرمون المنبه للجريب (FSH) والهرمون اللوتيني (LH) مع عدد الحيوانات المنوية.

تشير نتائج الدراسة عن العقم أن عدد الحيوانات المنوية يقل عند الرجال المصابين بالعقم ، في حين أن مستويات البلازما من هرمون منشط الجريب (FSH) والهرمون اللوتيني (LH) وهرمون البرولاكتين (PRL) ترتبط بعلاقة موجبة ذات دلاله إحصائيه. كما أن هرمون منشط الجريب (FSH) والهرمون اللوتيني (LH) ترتبط إرتباط بعلاقة موجبة ذات دلاله إحصائيه مع عدد الحيوانات المنوية.
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Abbreviations

FSH  Follicle stimulating hormone
LH  Luteinizing hormone
PRL  prolactin hormone
GnRH  gonadotropin-releasing hormone
GH  growth hormone
hCG  human chorionic gonadotropin
TSH  Thyroid releasing hormone
TRH  Thyroid stimulating hormone
SHBG  sex hormone binding globulin
IHH  idiopathic (isolated) hypogonadotropic hypogonadism
CHAPTER ONE

Introduction and Literature Review
Chapter One
Introduction and Literature Review

1.1. Introduction

Infertility can be defined as a failure to conceive after 12 months of unprotected sexual intercourse. (Matorras et al 1997) It is classified as primary infertility if no previous pregnancies have occurred, and secondary infertility if it occurred after one or more pregnancies (Matorras et al 1997).

Approximately 15% of couples attempting their first pregnancy meet with a failure, and another 10% face secondary infertility. (Matorras et al 1997) Data available over the past 20 years reveal that in approximately 30% of the cases of infertility, the pathology is found in the man alone, and in another 20%, the pathology of both the man and the woman are abnormal. (Matorras et al 1996) Therefore, the male factor is at least partly responsible for the infertility in about 50% of cases. (Matorras et al 1996) Male fertility depends upon an intact hypothalamopituitary-testicular axis to initiate and maintain quantitatively and qualitatively normal spermatogenesis. The initial evaluation of the male patient should be rapid, noninvasive, and cost-effective, as nearly 70% of conditions that cause infertility in men can be diagnosed with history, physical examination, and hormonal and semen analysis alone. More detailed, expensive, and invasive studies can then be ordered if necessary. A trained expert assesses the man's sperm count, their shape, movement, and other variables. Generally, a higher number of normal-shaped sperm means higher fertility. But exceptions are common. Many men with low sperm counts or abnormal semen are still fertile. And about 15% of infertile men have normal semen and plenty of normal sperm (Carlsen et al 1992).
Maintain normal secondary sex glands functions and sexual functions. (Carlsen et al 1992) Thus, it is surprising how infrequent infertile males have a recognizable endocrinopathy, even though up to 20% of male infertility can be attributable to endocrinopathy. (Matorras et al 1996) In fact, endocrine disorders which may be associated with significant medical pathology remain an important factor to consider in the etiology of male infertility because they be amenable to treatment. However, in clinical practice, endocrine evaluation is usually done only in patients with severe oligospermia or azoospermia. (Matorras et al 1996) The hormones initially evaluated include follicle stimulating hormone (FSH) luteinizing hormone (LH), testosterone and prolactin (Matorras et al 1996).

Further studies, like the evaluation of oestradiol, sexhormone binding globulin, thyroid function test amongothers, can be done, depending on the clinical scenario andthe results of the initial studies. (Jacobsen et al 1999) Based on the results of the hormonal studies, a precise endocrinological diagnosis such as hypergonadotrophic hypogonadism can be made, and the patient managed accordingly. The objective of this study was to determine the prevalence and pattern of endocrinological abnormalities in patients investigated for male infertility in environment (Jacobsen et al 1999).

Although there were much data on infertility in other African countries, no data exists about infertility in Sudan. Seven hundred and ten Sudanese couples were investigated for the infertility in Khartoum Fertility Center, Sudan: 443 (62.4%) had primary infertility and 267 (37.6%) had secondary infertility. A positive male factor alone was found in 257 (36.2%) couples and a female factor in 350 (49.3%) couples: eleven (1.5%) couples had a combination of male and female factors: and the
cause of infertility was unexplained in 92 (13.0%) couples. Oligozoospermia and asthenozoospermia were factors responsible for 16.8% and 17.5% of male infertility, respectively. Failure of ovulation (60.3%) was the most common cause of female infertility. The study revealed a high proportion of secondary infertility and a greater contribution of the female factors to infertility (Rucker et al 1998).
1.2 Literature Review

Infertility

Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse. An estimated 15% of couples meet this criterion and are considered infertile, with approximately 35% due to female factors alone, 30% due to male factors alone, 20% due to a combination of female and male factors, and 15% unexplained. Conditions of the male that affect fertility are still generally underdiagnosed and undertreated (Matorras et al 1996).

Causes of infertility in men can be explained by deficiencies in sperm formation, concentration (e.g., oligospermia [too few sperm], azoospermia [no sperm in the ejaculate]), or transportation. This general division allows an appropriate workup of potential underlying causes of infertility (Carlsen et al 1992).

The initial evaluation of the male patient should be rapid, noninvasive, and cost-effective, as nearly 70% of conditions that cause infertility in men can be diagnosed with history, physical examination, and hormonal and semen analysis alone. More detailed, expensive, and invasive studies can then be ordered if necessary (Matorras et al 1996).

Treatment options are based on the underlying etiology and range from optimizing semen production and transportation with medical therapy or surgical procedures to complex assisted reproduction techniques. Technological advancements make conceiving a child possible with as little as one viable sperm and one egg. Although the workup was traditionally delayed until a couple was unable to conceive for 12 months, evaluation may be initiated at the first visit in slightly older couples (Jacobsen et al 1999).
Normal ejaculate volume ranges from 1.5 to 5 mL and has a pH level of 7.05-7.8. The seminal vesicles provide 40-80% of the semen volume, which includes fructose for sperm nutrition, prostaglandins and other coagulating substances, and bicarbonate to buffer the acidic vaginal vault. Normal seminal fructose concentration is 120-450 mg/dL, with lower levels suggesting ejaculatory duct obstruction or absence of the seminal vesicles. The prostate gland contributes approximately 10-30% (0.5 mL) of the ejaculate. Products include enzymes and proteases to liquefy the seminal coagulum. This usually occurs within 20-25 minutes. The prostate also secretes zinc, phospholipids, phosphatase, and spermine. The testicular-epididymal component includes sperm and comprises about 5% of the ejaculate volume (Carlsen et al 1992).

In addition to the components above, semen is also composed of secretions from the bulbourethral (Cowper) glands and the (periurethral) glands of Littré, each producing 2-5% of the ejaculate volume, serving mainly to lubricate the urethra and to buffer the acidity of the residual urine. The ordered sequence of release is important for appropriate functioning (Carlsen et al 1992).

For conception, sperm must reach the cervix, penetrate the cervical mucus, migrate up the uterus to the fallopian tube, undergo capacitation and the acrosome reaction to digest the zona pellucida of the oocyte, attached to the inner membrane, and releases its genetic contents within the egg. The cervical mucus changes consistency during the ovulatory cycle, being most hospitable and easily penetrated at mid cycle. After fertilization, implantation may then take place in the uterus. Problems with any of these steps may lead to infertility (Carlsen et al 1992).

An estimated 10-15% of couples are considered infertile, defined by the World Health Organization (WHO) as the absence of conception after at
least 12 months of unprotected intercourse, poor semen quality, or both account for 90% of cases; however, studies of infertile couples without treatment reveal that 23% of these couples conceive within 2 years, and 10% more conceive within 4 years. Even patients with severe oligospermia (< 2 million sperm/mL) have a 7.6% chance of conception within 2 years (Matorras et al 1996).

Isolated conditions of the female are responsible for infertility in 35% of cases, isolated conditions of the male in 30%, conditions of both the male and female in 20%, and unexplained causes in 15%. Even if one partner has an obvious cause for the infertility, a thorough evaluation of both partners for completeness is prudent. In addition, both partners may be aided by evaluation of their sexual practices.

Patterns of male infertility vary greatly among regions and even within regions. The highest reported fertility rates are in Finland, while Great Britain has a low fertility rate. A combination of social habits, environmental conditions, and genetics is suspected to contribute to this variation.

Recent debate has occurred in the literature regarding a poorer semen quality, decreased sperm counts (113 million/mL in 1940 compared with 66 million/mL in the 1990s), and decreased fertility in men today compared with fertility 50 years ago (Carlsen et al 1992). Investigators hypothesize that environmental conditions and toxins have led to this decline; however, others argue that this is solely because of differences in counting methods, laboratory techniques, and geographic variation.

The effect of aging on fertility is unclear. As men age, their testosterone levels decrease, while estradiol and estrone levels increase (Carlsen et al 1992). Studies have shown that, as men age, their sperm density
decreases. Young men have spermatids present in 90% of seminiferous tubules, which decreases to 50% by age 50-70 years and to 10% by age 80 years. Additionally, 50% of Sertoli cells are lost by age 50 years, 50% of Leydig cells are lost by age 60 years. Despite this, aging men may achieve fertility rates similar to those in younger men, although conception often takes longer (Carlsen et al 1992).

**Causes of male infertility**

Causes of infertility can be divided into pretesticular, testicular, and posttesticular.

**Pretesticular causes of infertility**

Pretesticular causes of infertility include congenital or acquired diseases of the hypothalamus, pituitary, or peripheral organs that alter the hypothalamic-pituitary axis (Rucker et al 1998).

Disorders of the hypothalamus lead to hypogonadotropic hypogonadism. If GnRH is not secreted, the pituitary does not release LH and FSH. Ideally, patients respond to replacement with exogenous GnRH or HCG, an LH analogue, although this does not always occur (Rucker et al 1998).

**Primary testicular causes of infertility**

Primary testicular problems may be chromosomal or nonchromosomal in nature. While chromosomal failure is usually caused by abnormalities of the sex chromosomes, autosomal disorders are also observed (Rucker et al 1998).

**Chromosomal abnormalities**

An estimated 6-13% of infertile men have chromosomal abnormalities (compared with 0.6% of the general population). Patients with
azoospermia or severe oligospermia are more likely to have a chromosomal abnormality (10-15%) than infertile men with sperm density within the reference range (1%). A karyotype test and a Y chromosome test for microdeletions are indicated in patients with nonobstructive azoospermia or severe oligospermia (< 5 million sperm/mL), although indications are expanding (Rucker et al 1998).

Posttesticular causes of infertility

Posttesticular causes of infertility include problems with sperm transportation through the ductal system, either congenital or acquired. Genital duct obstruction is a potentially curable cause of infertility and is observed in 7% of infertile patients. Additionally, the sperm may be unable to cross the cervical mucus or may have ultrastructural abnormalities (Rucker et al 1998).

1.2.1. Reproductive hormones

1.2.1.1 Follicle-stimulating hormone

Follicle-stimulating hormone (FSH) is a glycoprotein gonadotropin secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH), which is released by the hypothalamus. The same pituitary cell also secretes luteinizing hormone (LH), another gonadotropin. FSH and LH are composed of alpha and beta subunits. The specific beta subunit confers the unique biological activity. FSH and LH bind to receptors in the testis and ovaries and regulate gonadal function by promoting sex steroid production and gametogenesis (Grover et al 2005).

In men, LH stimulates testosterone production from the interstitial cells of the testes (Leydig cells). FSH stimulates testicular growth and
enhances the production of an androgen-binding protein by the Sertoli cells, which are a component of the testicular tubule necessary for sustaining the maturing sperm cell. This androgen-binding protein causes high local concentrations of testosterone near the sperm, an essential factor in the development of normal spermatogenesis. Sertoli cells, under the influence of androgens, also secrete inhibin, a polypeptide, which may help to locally regulate spermatogenesis. Hence, maturation of spermatozoa requires FSH and LH (Grover et al 2005).

FSH and LH secretion are affected by a negative feedback from sex steroids. Inhibin also has a negative feedback on FSH selectively. High-dose testosterone or estrogen therapy suppresses FSH and LH. Primary gonadal failure in men and women leads to high levels of FSH and LH, except in selective destruction of testicular tubules with subsequent elevation of only FSH, as in Sertoli-cell-only syndrome. Similarly, any process leading to a low FSH level also simultaneously results in a low LH level, except in rare instances of isolated FSH deficiency or isolated LH deficiency in fertile eunuch syndrome (Grover et al 2005).

1.2.1.1.1 Causes of low FSH level (hypogonadotropic hypogonadism or secondary hypogonadism)

- (i) Congenital: Sexual differentiation is normal. In men, phallic development may be subnormal, resulting in a micropenis. Pubertal development is diminished or even absent, depending on the degree of gonadotropin deficiency.

  - Isolated idiopathic hypogonadotropic hypogonadism: This usually results from GnRH deficiency, with absence of any other abnormalities. FSH and LH levels are low. Human G protein-coupled receptor 54
GPR54) receptor model. Mutations identified in patients with idiopathic hypogonadotrophic hypogonadism are indicated (Karges and Roux, 2005).

- Kallmann syndrome: This is characterized by hypogonadotrophic hypogonadism and 1 or more nongonadal congenital abnormalities, including anosmia, red-green blindness, midline facial abnormalities (e.g., cleft palate), urogenital tract abnormalities, and neurosensory hearing loss. Hypogonadism in this syndrome is a result of deficient hypothalamic secretion of GnRH. Most cases are sporadic, but familial cases also occur. It is caused by mutations in the Kalman filter gene (Karges and Roux, 2005). This is a frequently sampled serum luteinizing hormone (LH) profile in a male patient with Kallmann syndrome (KS), compared with that in a healthy individual. A lack of LH pulsatility is seen in the former (Karges and Roux, 2005).

- Idiopathic hypogonadotrophic hypogonadism associated with mental retardation: Several syndromes (e.g., Prader-Willi syndrome) have been described in which hypogonadotrophic hypogonadism is associated with retardation and other abnormalities, including obesity.

- Combined pituitary hormone deficiency: This results from a rare mutation in the gene encoding a transcription factor (PROP1), which is necessary for the differentiation of a cell type that is a precursor to somatotroph, lactotroph, thyrotroph, and gonadotroph cells, thus resulting in deficiencies in prolactin, thyroid-stimulating hormone (TSH), growth hormone (GH), FSH, and LH (Dandona et al 2008) (Ferhi et al 2009).

- Fertile eunuch syndrome: This is thought to represent an incomplete form of GnRH deficiency in men, in which an isolated and partial LH
deficiency is present with low testosterone and normal FSH levels, resulting in preservation of spermatogenesis.

- Abnormal beta subunit of LH: This is a rare mutation in the LH beta subunit gene (Karges and Roux 2005).

- Abnormal beta subunit of FSH: This is a rare mutation in the gene for the beta subunit of FSH, resulting in a low FSH level. This condition is encountered only in women but has been studied in male mice in which the FSH beta subunit gene has been knocked out. These mice have oligospermia but are fertile (Dandona et al 2008) (Ferhi et al 2009).

(ii) Acquired: This can be caused by any disease that affects the hypothalamic-pituitary axis, impairing the secretion of GnRH, FSH, or LH.

- Mass lesions: These include pituitary adenomas, cysts, and metastatic cancer to the sella (breast in women, lung and prostate in men). These masses may cause temporary or permanent damage by extrinsic compression of pituitary cells. Hypothalamic tumors may lead to delayed puberty, hypogonadism, and obesity, originally called Fröhlich syndrome or adiposogenital dystrophy. The presence of obesity indicates that the appetite-regulating regions of the hypothalamic have been damaged.

- Hypothalamic/pituitary surgery: If sufficient normal tissue is excised inadvertently, symptomatic hypogonadism may ensue initially, followed by dysfunction of other pituitary cells.

- Hypothalamic/pituitary radiation: This may lead to multiple hormonal deficiencies, including FSH and LH.
● Infiltrative lesions: Hemochromatosis, sarcoidosis, histiocytosis, and lymphoma can cause hypogonadism by involving the hypothalamic/pituitary region.

● Infections: Meningitis, especially tuberculous, is a rare cause of hypogonadism.

● Pituitary apoplexy: Is a sudden and severe hemorrhage into the pituitary, which can result in varying degrees of hypopituitarism, excruciating headaches, visual changes, and altered mental status.

● Trauma: Head trauma of sufficient severity to fracture the skull base can the hypothalamic-pituitary stalk, preventing GnRH from reaching the pituitary, thus decreasing FSH and LH release.

● Glucocorticoid excess: Exogenous or endogenous (Cushing syndrome) glucocorticoid excess can lead to hypogonadotrophic hypogonadism. Direct inhibition of testosterone secretion may also occur at the testicular level.

● Hyperprolactinemia: This can result from a pituitary adenoma, renal or liver insufficiency, primary hypothyroidism, or some drugs (eg, neuroleptics). Hyperprolactinemia can suppress GnRH secretion through a central dopamine-related mechanism. In addition to hypogonadism, this condition can also manifest as galactorrhea and as gynecomastia in men.

● Primary hypothyroidism: This can lead to hypogonadism through hyperprolactinemia. A low thyroxine (T4) level results in a high thyrotropin-releasing hormone (TRH) level, which stimulates prolactin secretion(Karges and Roux 2005).
• Critical illness: Surgery, myocardial infarction, or other illness can cause transient hypogonadotropic hypogonadism, with resolution upon recovery (Karges and Roux, 2005).

• Excessive exercise: This can cause a functional hypothalamic hypogonadism in men, analogous to women with functional hypothalamic amenorrhea.

• Sex steroid–secreting tumors: These may be adrenal, testicular, or ovarian in origin, or, they may result from adrenal rest tumors. The excessive amount of testosterone or estradiol can inhibit FSH and LH secretion.

• Intentional (iatrogenic) secondary hypogonadism: Prolonged administration of high doses of anabolic steroids (by athletes) or GnRH analogs (for prostate cancer) can cause low FSH or LH levels. Recovery may take many months or years after cessation of the drug. Also, women who discontinue oral contraceptives may have post-pill amenorrhea; recovery of the gonadotropin axis may take up to one year.

• Pituitary infarction: This condition rarely occurs in males; but, when present, it primarily manifests in older patients with vascular insufficiency during coronary artery bypass surgery. In women, it can occur postpartum as Sheehan syndrome, usually after substantial blood loss during childbirth. This condition manifests as partial or complete hypopituitarism, depending on the hormonal deficiencies; a low FSH or LH level causing amenorrhea is the most frequent cause.

• Chronic systemic diseases: Cirrhosis, chronic renal failure, and AIDS may lead to hypogonadism, which has a dual mechanism, ie, primary and secondary (Karges and Roux 2005).
• Anorexia nervosa: In women, significant weight loss, up to 10% below the ideal body weight, may lead to functional hypothalamic amenorrhea.

• Acute alcohol ingestion: This may lead to primary or secondary hypogonadism (Karges and Roux 2005).

• Idiopathic: No cause is identified in some men and women with acquired secondary hypogonadism. The cause may be autoimmune in origin.

• Type 2 diabetes: Research has indicated that low concentrations of testosterone, LH, and FSH are prevalent in patients with type 2 diabetes who are obese. (Dandona et al 2008) Evidence suggests that inflammation may play an important part in this phenomenon.

1.2.1.1.2 Causes of high FSH level

Primary hypogonadism: Can be congenital or acquired

(i) Congenital:-

Sexual differentiation in men may vary from pseudohermaphroditism to a male with only a micropenis and lack of full pubertal development. In women, sexual differentiation is normal but puberty is delayed or absent (Ferhi et al 2009).

• Klinefelter syndrome: This syndrome is the most common congenital abnormality causing primary hypogonadism in men. The typical genotype is 47,XXY. The clinical presentation includes infertility, small and firm testes, and low testosterone with high FSH and LH levels. Males with Klinefelter syndrome usually present in their prepubertal years (Ferhi et al 2009)(Wikstrom and Dunkel 2008). Adolescent male with Klinefelter
syndrome who has female-type distribution of pubic hair, as well as testicular dysgenesis.

- Other chromosomal abnormalities: These result in testicular hypofunction; they include the 46,XY/XO and the 47,XYY karyotypes.

- Mutation in the FSH receptor gene in men: This mutation is rare and results in low sperm count with a high FSH level. Inactivating mutations of the LH receptor in females have been identified. These patients present with a milder phenotype compared to males (Peroff and Fritz 2005).

- Cryptorchidism: This refers to undescended testes, The clinical consequences depend on whether 1 or both testes are cryptorchid. If only 1 testis is affected, the sperm count is subnormal in almost 30% of patients and the FSH level is slightly elevated. If both testes remain undescended, the sperm count is usually severely subnormal with a high FSH level and low serum testosterone. Hypoplastic right hemiscrotum in a patient with an undescended right testis (Sharma et al 2007).

- Disorders of androgen biosynthesis: This involves mutations of the genes that encode the enzymes necessary for testosterone biosynthesis. They result in incomplete virilization, low sperm count, low testosterone level, and high LH and FSH levels.

- Sertoli-cell-only syndrome: The characteristic features are complete, or almost complete, absence of germ cells in all seminiferous tubules. Leydig cells are only mildly impaired. These men have azoospermia with high FSH levels. LH and testosterone levels are normal. The cause has not been identified, but it is thought to be a congenital absence or early neonatal loss of the germ cells (Sharma et al 2007) (Loret et al 2007).
- End organ resistance to androgens: This is due to androgen receptor defects. In its complete form, it is called testicular feminization. Affected individuals are genetically males but phenotypically females. The testes are located in the labia, the inguinal canal, or the abdomen. Testosterone and LH levels are high. The FSH level is normal or slightly increased (Loret et al 2007).

(ii) Acquired

- Infections: The most common is mumps orchitis. The seminiferous tubules are almost always severely affected, often resulting in infertility, especially with bilateral testicular involvement. The Leydig cells may also be damaged, resulting in decreased testosterone production with high LH levels.

- Radiation: This mostly damages the seminiferous tubules or the ovaries. The degree of damage is proportionate to the level of radiation exposure.

- Antineoplastic agents: As with cyclophosphamide, chlorambucil, cisplatin, and carboplatin may decrease the sperm count by destruction of the seminiferous tubules. Less commonly, testosterone secretion also declines. Recovery may occur over the long-term. Similarly, in women, chemotherapy may lead to ovarian failure.

- Chemicals: Chemicals such as dibromodichloropropane which was used as a soil fumigant and nematocide on over 40 different crops in the United States can decrease spermatogenesis.

- Glucocorticoids: These can lead to hypogonadism via inhibition of the pituitary and testes.
● Ketoconazole: This is an antifungal drug that inhibits testosterone biosynthesis.

● Suramin: This is an antiparasitic drug that can block testosterone synthesis by the Leydig cells.

● Trauma: Injuries can be sufficiently severe to damage both seminiferous tubules and Leydig cells.

● Testicular torsion: Torsion of more than 8 hours duration may lead to a low sperm count. Even if the torsion involves only 1 testis, both testes may be damaged; the mechanism is not known (Loret et al 2007).

● Chronic systemic diseases: Cirrhosis, chronic renal failure, and AIDS may lead to hypogonadism, both primary and secondary. Bilateral aortofemoral anastomosis in men may lead to decreased blood supply to the testes, predominantly affecting the seminiferous tubules.

● Autoimmune damage: This is due to antisperm antibodies. It may be part of an autoimmune polyglandular syndrome.

● Idiopathic: Many men and women with primary hypogonadism have idiopathic disease, and the cause is never identified. The cause may be autoimmune in origin (Peroff and Fritz 2005).

1.2.1.2. Luteinizing hormone

LH is a glycoprotein dimer composed of 2 glycosylated noncovalently-linked subunits designated alpha and beta. The alpha subunit is composed of 92 amino acids and is encoded on the long arm of chromosome 6. The beta subunit is 121 amino acids and is encoded on the long arm of chromosome 19.
The alpha subunit of LH is biologically identical to 3 other hormones: FSH, thyroid-stimulating hormone (TSH), and human chorionic gonadotropin (hCG). The beta subunit is unique and determines LH immunologic and biologic activity. The half-life of LH is 20 minutes (Peroff and Fritz 2005).

**Hypothalamic-Pituitary-Gonadal Function**

Axis gonadotropin-releasing hormone (GnRH) is secreted by neurons in the arcuate nucleus of the hypothalamus and released into the pituitary portal circulation. LH and FSH are produced by gonadotrope cells located in the anterior pituitary gland. The gonadotrope cells release LH and FSH in a pulsatile fashion approximately every hour when stimulated by GnRH. Once released into the systemic circulation, both LH and FSH stimulate the gonads of females and males to release steroid hormones (Speroff *et al* 2005).

In the male, both LH and FSH are required for spermatogenesis. LH stimulates Leydig cells to convert cholesterol to testosterone. Testosterone and FSH, in turn, modulate Sertoli cells, which serve as "nurse" cells for spermatogenesis within the lumen of the seminiferous tubules. Clinically, only FSH is used as a marker of testicular dysfunction (Sharma *et al* 2007).

Luteinizing hormone (LH) deficiency is an uncommon condition that almost always occurs in conjunction with follicle-stimulating hormone (FSH) deficiency because LH and FSH are secreted by the same pituitary gonadotrope cells. LH deficiency can manifest in females or males as delayed puberty, hypogonadism at any age, or reproductive abnormalities that can be dramatic or subtle. LH and FSH play central roles in the hypothalamic-pituitary-gonadal axis, and, thus, conditions related to LH...
and FSH deficiency can be caused by pathology of either the hypothalamus or pituitary. Careful analysis of the presenting problem, the patient’s overall health, and the hormonal profile is often necessary to determine the cause of LH deficiency and, thus, the most appropriate treatment (Lofrano et al 2007).

1.2.1.2.1 Path

1.2.1.2.1.1 Hypothalamic causes of LH deficiency

(i) Kallmann syndrome

○ Kallmann syndrome was first described by Franz Hosef Kallmann in 1944 and refers to congenital secondary hypogonadism (hypogonadotrophic hypogonadism) associated with lack of sense of smell (anosmia). The condition occurs sporadically in 60% of patients, but can be genetically transmitted as an X-linked, autosomal dominant or autosomal recessive condition.

○ This condition affects both females and males who usually present with anosmia and delayed puberty. Laboratory evaluation reveals low LH and FSH levels and normal karyotypes.

○ Females present with primary amenorrhea, and some males present with micropenis. Kallmann syndrome results from the congenital absence of GnRH-producing neurons in the hypothalamus. During embryogenesis, olfactory axonal and GnRH neurons from the olfactory placode fail to migrate to the hypothalamus. In the absence of GnRH, the pituitary gonadotrope cells are not signaled to produce LH and FSH, ultimately leading to lack of sex hormone production by the gonads. (Loret et al 2007).
Hormone replacement therapy (estrogen for females and testosterone for males) is used to induce sexual maturation and minimize the long-term risk of osteoporosis. When fertility is desired, the treatment consists of either GnRH, given by a subcutaneous pump, or exogenous gonadotropins given by injection. Women with Kallmann syndrome do not ovulate when given clomiphene citrate, which relies on an intact hypothalamic-pituitary-gonadal axis. Likewise, maintenance therapy with clomiphene citrate does not appear to increase testosterone secretion or sperm production in men with Kallmann syndrome.


(iii) Idiopathic hypogonadotropic hypogonadism

Adult onset idiopathic (isolated) hypogonadotropic hypogonadism (IHH) refers to complete or partial absence of GnRH-induced release of LH and FSH in the setting of otherwise normal anterior pituitary anatomy and function. This relatively rare condition can occur in both men and women.

In men, estrogen (produced by aromatization of testosterone) has a negative feedback effect on hypothalamic secretion of GnRH and thus inhibits pituitary gonadotropin secretion. It has been hypothesized that some cases of IHH result from an acquired defect of enhanced hypothalamic sensitivity to estrogen-mediated negative feedback since maintenance clomiphene citrate therapy can result in complete normalization of pulsatile gonadotropin secretion, serum testosterone
level, and sexual function in men with idiopathic (isolated) hypogonadotrophic hypogonadism IHH.

(iv) Stress- hypogonadotrophic related hypogonadism

○ Hypothalamic suppression can occur in women under physical or metabolic stress. Stress-related hypothalamic suppression is most commonly related to prolonged strenuous physical exercise and extreme weight loss, particularly in the context of eating disorders, such as anorexia nervosa and bulimia. (Kalantaridou et al 2004) These conditions cause an elevation of corticotropin-releasing hormone (CRH), inhibiting pulsatile GnRH release from the hypothalamus. Suppression of GnRH release in women results in decreased secretion of LH and FSH (i.e., hypogonadotrophic hypogonadism), manifesting as amenorrhea and hypoestrogenemia. (Hergenroeder 1995) Ongoing hypothalamic suppression can lead to serious consequences such as irreversible osteoporosis and bone fractures in these women (Sehu et al 2007).

1.2.1.2.1.2 Pituitary dysfunction and LH deficiency

The anterior pituitary produces a number of important peptide hormones, including LH, FSH, TSH, adrenocorticotropic hormone (ACTH), prolactin (PRL), and growth hormone (GH). LH deficiency can result from a myriad of anterior pituitary dysfunctions including pituitary tumors, inflammation, vascular accidents, and pregnancy-related hemorrhagic shock (Sheehan syndrome).

Hyperprolactinemia is a common hormonal abnormality associated with anterior pituitary dysfunction. Women with high levels of serum PRL (>20–25 ng/mL) often develop galactorrhea, and some develop amenorrhea and hypoestrogenemia. The amenorrhea related to
hyperprolactinemia is caused by alterations in the normal release and pulsatility of GnRH as well as subsequent alterations in LH/FSH secretion and the LH surge (Sehu et al 2007).

Causes of hyperprolactinemia include pituitary adenomas, hypothyroidism, hypothalamic dysfunction, and chronic renal insufficiency. Medications such as antipsychotics, estrogen, antihypertensives, metoclopramide, and cimetidine can also cause hyperprolactinemia.

1.2.1.2.2. Frequency of Hypogonadotropic Hypogonadism

- Hypogonadotropic hypogonadism has an overall incidence of approximately 1:10,000 to 1:86,000 men and women respectively. This is associated with anosmia (ie, Kallmann syndrome).

- Stress-related hypogonadotropic hypogonadism accounts for more than 30% of secondary amenorrhea in reproductive-aged women. (Loret et al 2007)

1.2.1.2.3. Causes of LH Pathophysiology

- Kallmann syndrome
  - Genetic
- Hypogonadotropic hypogonadism
  - Genetic
  - Idiopathic
  - Prolonged Strenuous Exercise
  - Anorexia Nervosa/Bulimia
  - Starvation
- Pituitary dysfunction
• Pituitary tumors
• Pituitary Infarction
• Luteal phase deficiency
  • Idiopathic

1.2.1.3. Prolactin Hormone

Prolactin is a peptide hormone discovered by Henry Friesen. that is secreted by both men and women. It is released by pituitary gland, an organ responsible for regulating many of body’s functions, including reproduction. The pituitary gland is a small, bean-shaped organ located in the middle of the brain. The pituitary gland releases prolactin on a daily basis, both in the morning and throughout the night. Certain activities like sexual intercourse and exercise also cause pituitary gland to release prolactin (Bole et al 1998).

Prolactin also acts in a cytokine-like manner and as an important regulator of the immune system. Prolactin has important cell cycle related functions as a growth-, differentiating- and anti-apoptotic factor. As a growth factor binding to cytokine like receptors it has also profound influence on hematopoiesis, angiogenesis and is involved in the regulation of blood clotting through several pathways. In summary, "more than 300 separate actions of PRL have been reported in various vertebrates, including effects on water and salt balance, growth and development, endocrinology and metabolism, brain and behavior, reproduction, and immune regulation and protection". Prolactin acts in endocrine, autocrine, and paracrine manner through the prolactin receptor and a large number of cytokine receptors (Bole et al 1998).
In breastfeeding, the act of an infant suckling the nipple stimulates the production of oxytocin, which stimulates the "milk let-down" reflex, (Bartholomew et al 2007) which fills the breast with milk via a process called lactogenesis, in preparation for the next feed.

Pituitary prolactin secretion is regulated by endocrine neurons in the hypothalamus, the most important ones being the neurosecretory tuberoinfundibulum (TIDA) neurons of the arcuate nucleus, which secrete dopamine to act on the dopamine-2 receptors of lactotrophs, causing inhibition of prolactin secretion. Thyrotropin-releasing factor (thyrotropin-releasing hormone) has a stimulatory effect on prolactin release (Bartholomew et al 2007).

Vasoactive intestinal peptide and peptide histidine isoleucine help to regulate prolactin secretion in humans (Kulick et al 2005).

Prolactin is sometimes classified as a gonadotropin (Marieb et al 2006) although in humans it has only a weak luteotropic effect while the effect of suppressing classical gonadotropic hormones is more important.

### 1.2.1.3.1. Function of Prolactin

Prolactin has many effects including regulating lactation and stimulating proliferation of oligodendrocyte precursor cells.

It stimulates the mammary glands to produce milk (lactation): Increased serum concentrations of prolactin during pregnancy causes enlargement of the mammary glands of the breasts and prepare for the production of milk. However, the high levels of progesterone during pregnancy suppress the production of milk. Milk production normally starts when the levels of progesterone fall by the end of pregnancy and a suckling stimulus is present. Sometimes, newborn babies (males as well as
females) secrete a milky substance from their nipples known as witch's milk, this is in part caused by maternal prolactin and other hormones (Gregg et al 2007).

Prolactin provides the body with sexual gratification after sexual acts: The hormone counteracts the effect of dopamine, which is responsible for sexual arousal. This is thought to cause the sexual refractory period. The amount of prolactin can be an indicator for the amount of sexual satisfaction and relaxation. Unusually high amounts are suspected to be responsible for impotence and loss of libido.

Prolactin also stimulates proliferation of oligodendrocyte precursor cells. These cells differentiate into oligodendrocytes, the cells responsible for the formation of myelin coatings on axons in the central nervous system (Gregg et al 2007).

Prolactin also has a number of other effects including contributing to surfactant synthesis of the fetal lungs at the end of the pregnancy and immune tolerance of the fetus by the maternal organism during pregnancy; it also decreases normal levels of sex hormones — estrogen in women and testosterone in men. It is this inhibition of sex steroids that is responsible for loss of the menstrual cycle in lactating women as well as lactation-associated osteoporosis. Prolactin also enhances luteinizing hormone-receptors in Leydig cells, resulting in testosterone secretion, which leads to spermatogenesis (Craven et al 2006) It has been shown that Prolactin promotes neurogenesis.
1.2.1.3.2 Production and regulation of Prolactin

In humans, prolactin is produced at least in the pituitary, decidua, myometrium, breast, lymphocytes, leukocytes and prostate (Ben et al 1996) (Gerlo et al 2006).

Pituitary PRL is controlled by the Pit-1 transcription factor and ultimately dopamine, extrapituitary PRL is controlled by a superdistal promoter and apparently unaffected by dopamine (Gerlo et al 2006).

Extrapituitary production of prolactin is thought to be special to humans and primates and may serve mostly tissue specific paracrine and autocrine purposes. It has been hypothesized that in other vertebrates such as mice a similar tissue specific effect is achieved by a large family of prolactin like proteins controlled by at least 26 paralogous PRL genes not present in primates (Gerlo et al 2006).

- dopamine (which blocks prolactin)
- serotonin (which triggers prolactin release)
- thyroid-producing hormone (which also triggers prolactin)

1.2.1.3.3. Variance in Prolactin levels

There is a diurnal as well as an ovulatory cycle in prolactin secretion. In many mammals, there is also a seasonal change in prolactin release.

During pregnancy, high circulating concentrations of estrogen and progesterone inhibit the action of prolactin on milk production. Following delivery, reduced estrogen and progesterone production allows prolactin to induce lactation (Melmed and Jameson 2005).
After childbirth, prolactin levels fall as the internal stimulus for them is removed. Sucking by the baby on the nipple then promotes further prolactin release, maintaining the ability to lactate. The sucking activates mechanoreceptors in and around the nipple. These signals are carried by nerve fibers through the spinal cord to the hypothalamus, where changes in the electrical activity of neurons that regulate the pituitary gland cause increased prolactin secretion. The suckling stimulus also triggers the release of oxytocin from the posterior pituitary gland, which triggers milk let-down: Prolactin controls milk production (lactogenesis) but not the milk-ejection reflex; the rise in prolactin fills the breast with milk in preparation for the next feed.

In usual circumstances, in the absence of galactorrhea, lactation will cease within one or two weeks of the end of demand breastfeeding.

It has also been found that compared to un-mated males, fathers and expectant fathers have increased prolactin concentrations.

High prolactin levels also tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating hormone (FSH) and gonadotropin-releasing hormone (GnRH). High prolactin levels can also contribute to mental health issues.

Prolactin levels peak during REM sleep, and in the early morning. Levels can rise after exercise, meals, sexual intercourse, minor surgical procedures, (Melmed and Jameson 2005) or following epileptic seizures (Mellors 2005).

Hypersecretion of prolactin is more common than hyposcretion. Hyperprolactinemia is the most frequent abnormality of the anterior
pituitary tumors. Clinical signs include inappropriate lactation, lack of menses, infertility in females, and impotence in males (Mellors 2005).

1.2.1.3.4. Prolactin receptors

Prolactin receptors are present in the mamillary glands, ovaries, pituitary glands, heart, lung, thymus, spleen, liver, pancreas, kidney, adrenal gland, uterus, skeletal muscle, skin, and areas of the central nervous system. (Mancini et al 2008)

1.2.1.3.5. Diagnostic use of Prolactin

Prolactin levels may be checked as part of a sex hormone workup, as elevated prolactin secretion can suppress the secretion of FSH and GnRH, leading to hypogonadism, and sometimes causing erectile dysfunction in men.

Prolactin levels may be of some use in distinguishing epileptic seizures from psychogenic non-epileptic seizures. The serum prolactin level usually rises following an epileptic seizure (Banerjee et al 2004).

1.2.1.3.6. Conditions associated with elevated prolactin secretion

Hyperprolactinaemia, or excess serum prolactin, is associated with hypoestrogenism, anovulatory infertility, oligomenorrhoa, amenorrhoa, unexpected lactation, and loss of libido in women, and erectile dysfunction and loss of libido in men.

Hyperprolactinemia can result from:

- Prolactinoma
• Excess thyrotropin-releasing hormone (TRH), usually in primary hypothyroidism
• All SSRI (selective serotonin re-uptake inhibitors) and SNRI (Serotonin–norepinephrine reuptake inhibitors) medications
• Many antipsychotic medications
• Emotional stress
• Pregnancy and lactation
• Some sexual disorders (Mancini et al 2008)

1.2.1.3.7. Conditions associated with decreased prolactin

Hypoprolactinemia, or serum prolactin deficiency, is associated with ovarian dysfunction in women, (Kauppila et al 1988)(Schwärzler et al 1997) and metabolic syndrome, anxiety, arteriogenic erectile dysfunction, premature ejaculation, (Corona et al 2009) oligozoospermia, asthenospermia, hypofunction of seminal vesicles, and hypoandrogenism (Gonzales et al 1989) in men. In one study, normal sperm characteristics were restored when prolactin levels were brought up to normal values in hypoprolactinemic men (Ufearo and Orisakwe 1995).

Hypoprolactinemia can result from:

• Bulimia
• Excess dopamine (Ufearo and Orisakwe 1995).

1.2.1.4 Testosterone Hormone

Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles, (Cox and John 2005) birds, (Reed et al 2006) and other vertebrates. In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are
also secreted by the adrenal glands. It is the principal male sex hormone and anabolic.
In men, testosterone plays a key role in the development of male reproductive tissues such as the testis and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass, and the growth of body hair. (Mooradian et al 1987) In addition, testosterone is essential for health and well-being (Bassil et al 2009) as well as the prevention of osteoporosis (Tuck and Francis 2009).

On average, an adult human male body produces about ten times more testosterone than an adult human female body, but females are more sensitive to the hormone. (Dabbs and Dabbs 2000) Testosterone is observed in most vertebrates.

1.2.1.4.1 Physiological effects of Testosterone

In general, androgens promote protein synthesis and growth of those tissues with androgen receptors. Testosterone effects can be classified as virilizing and anabolic, though the distinction is somewhat artificial, as many of the effects can be considered both. Testosterone is anabolic, meaning it builds up bone and muscle mass.

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

Androgenic effects include maturation of the sex organs, particularly the penis and the formation of the scrotum in the fetus, and after birth (usually at puberty) a deepening of the voice, growth of the beard and axillary hair. Many of these fall into the category of male secondary sex characteristics (Dabbs and Dabbs 2000).
Testosterone effects can also be classified by the age of usual occurrence. For postnatal effects in both males and females, these are mostly dependent on the levels and duration of circulating free testosterone.

1.2.1.4.2. Prenatal effects of Testosterone

The prenatal androgen effects occur during two different stages. Between 4 and 6 weeks of the gestation.

• Genital virilization (midline fusion, phallic urethra, scrotal thinning and rugation, phallic enlargement); although the role of testosterone is far smaller than that of Dihydrotestosterone.

• Development of prostate and seminal vesicles. During the 2nd trimester androgen level is associated with Gender identity (Swaab and Garcia 2009). This period affects the feminization or masculinization of the fetus and can be a better predictor of feminine or masculine behaviours such as sex typed behaviour than an adult's own levels. A mother's own testosterone level influences behavior more than the daughter's testosterone level during pregnancy (Browne 2000).

1.2.1.4.3. Early infancy effects of Testosterone

Early infancy androgen effects are the least understood. In the first weeks of life for male infants, testosterone levels rise. The levels remain in a pubertal range for a few months, but usually reach the barely detectable levels of childhood by 4–6 months of age (Forest et al 1973) (Corbier et al 1992). The function of this rise in humans is unknown. It has been speculated that "brain masculinization" is occurring since no significant changes have been identified in other parts of the body. (Dakin et al 2008) Surprisingly, the male brain is masculinized by testosterone being aromatized into estrogen, which crosses the blood-brain barrier and enters the male brain, whereas female fetuses have alpha-fetoprotein.
which binds up the estrogen so that female brains are not affected (Dakin et al. 2008).

### 1.2.1.4.4. Pre-peripubertal effects of Testosterone

Pre-peripubertal effects are the first observable effects of rising androgen levels at the end of childhood, occurring in both boys and girls.

- Adult-type body odour
- Increased oiliness of skin and hair, acne
- Pubarche (appearance of pubic hair)
- Axillary hair
- Growth spurt, accelerated bone maturation
- Hair on upper lip and sideburns (Dakin et al. 2008).

### 1.2.1.4.5. Pubertal effects of Testosterone

Pubertal effects begin to occur when androgen has been higher than normal adult female levels for months or years. In males, these are usual late pubertal effects, and occur in women after prolonged periods of heightened levels of free testosterone in the blood.

- Enlargement of sebaceous glands. This might cause acne.
- Phallic enlargement or clitoromegaly
- Increased libido and frequency of erection or clitoral engorgement
- Pubic hair extends to thighs and up toward umbilicus
- Facial hair (sideburns, beard, moustache)
- Loss of scalp hair (Androgenetic alopecia)
- Chest hair, periareolar hair, perianal hair
- Leg hair
- Axillary hair
Subcutaneous fat in face decreases
Increased muscle strength and mass (Bhasin et al 1996).
Deepening of voice
Growth of the Adam's apple
Growth of spermatogenic tissue in testicles, male fertility
Growth of jaw, brow, chin, nose, and remodeling of facial bone contours
Shoulders become broader and rib cage expands
Completion of bone maturation and termination of growth. This occurs indirectly via estradiol metabolites and hence more gradually in men than women (Bhasin et al 1996).

1.2.1.4.6. Role of Testosterone on adults

Adult testosterone effects are more clearly demonstrable in males than in females, but are likely important to both sexes. Some of these effects may decline as testosterone levels decrease in the later decades of adult life.

- Testosterone is necessary for normal sperm development. It activates genes in Sertoli cells, which promote differentiation of spermatogonia.
- Regulates acute HPA (Hypothalamic–pituitary–adrenal axis) response under dominance challenge (Mehta et al 2008).
- Mental and physical energy
- Maintenance of muscle trophism
- Testosterone regulates the population of thromboxane A2 receptors on megakaryocytes and platelets and hence platelet aggregation in humans (Ajayi and Halushka 2005)(Ajayi et al 1995).
- Testosterone does not cause or produce deleterious effects on prostate cancer. In people who have undergone testosterone deprivation
therapy, testosterone increases beyond the castrate level have been shown to increase the rate of spread of an existing prostate cancer. (Morgenthaler and Traish 2009) (Rhoden et al 2008).

Recent studies have shown conflicting results concerning the importance of testosterone in maintaining cardiovascular health. (Haddad et al 2007) (Jones and Saad 2009) Nevertheless, maintaining normal testosterone levels in elderly men has been shown to improve many parameters which are thought to reduce cardiovascular disease risk, such as increased lean body mass, decreased visceral fat mass, decreased total cholesterol, and glycemic control. (Stanworth and Jones 2008).

- Under dominance challenge, may play a role in the regulation of the fight-or-flight response (Mehta and Josephs 2006).
- Falling in love decreases men's testosterone levels while increasing women's testosterone levels. It is speculated that these changes in testosterone result in the temporary reduction of differences in behavior between the sexes. (Marazziti and Canale 2004) It has been found that when the testosterone and endorphins in the ejaculated semen meet the cervical wall after sexual intercourse, females receive a spike in testosterone, endorphin, and oxytocin levels, and males after orgasm during copulation experience an increase in endorphins and a marked increase in oxytocin levels. This adds to the hospitable physiological environment in the female internal reproductive tract for conceiving, and later for nurturing the conceptus in the pre-embryonic stages, and stimulates feelings of love, desire, and paternal care in the male (this is the only time male oxytocin levels rival a female's (Marazziti and Canale 2004).
Recent studies suggest that testosterone levels play a major role in risk-taking during financial decisions. (Sapienza et al 2009) (Apicella et al 2008).

The administration of testosterone makes men selfish and more likely to punish others for being selfish towards them (Zak et al 2009).

Fatherhood also decreases testosterone levels in men, suggesting that the resulting emotional and behavioral changes promote paternal care. (Berg and Wynne 2001).

Men whose testosterone levels are slightly above average are less likely to have high blood pressure, less likely to experience a heart attack, less likely to be obese, and less likely to rate their own health as fair or poor. However, high testosterone men are more likely to report one or more injuries, more likely to consume five or more alcoholic drinks in a day, more likely to have had a sexually transmitted infection, and more likely to smoke. (Booth et al 1999).

1.2.1.4.7. Role of Testosterone on Brain

As testosterone affects the entire body (often by enlarging; males have bigger hearts, lungs, liver, etc.), the brain is also affected by this "sexual" differentiation; (Swaab and Garcia 2009) the enzyme aromatase converts testosterone into estradiol that is responsible for masculinization of the brain in male mice. In humans, masculinization of the fetal brain appears, by observation of gender preference in patients with congenital diseases of androgen formation or androgen receptor function, to be associated with functional androgen receptors. (Wilson 2001).
A study conducted in 1996 found no immediate short term effects on mood or behavior from the administration of supraphysiologic doses of testosterone for 10 weeks on 43 healthy men. (Bhasin et al 1996) Another study (Reed et al 2006) found a correlation between testosterone and risk tolerance in career choice among women.

Literature suggests that attention, memory, and spatial ability are key cognitive functions affected by testosterone in humans. Preliminary evidence suggests that low testosterone levels may be a risk factor for cognitive decline and possibly for dementia of the Alzheimer’s type, a key argument in life extension medicine for the use of testosterone in anti-aging therapies. Much of the literature, however, suggests a curvilinear or even quadratic relationship between spatial performance and circulating testosterone, (Moffat and Hampson 1996) where both hypo- and hypersecretion (deficient- and excessive-secretion) of circulating androgens have negative effects on cognition and cognitively modulated aggressivity, as detailed above. (Moffat and Hampson 1996)

Contrary to what has been postulated in outdated studies and by certain sections of the media, aggressive behaviour is not typically seen in hypogonadal men who have their testosterone replaced adequately to the eugonadal/normal range. In fact, aggressive behaviour has been associated with hypogonadism and low testosterone levels and it would seem as though supraphysiological and low levels of testosterone and hypogonadism cause mood disorders and aggressive behaviour, with eugonderal/normal testosterone levels being important for mental well-being. Testosterone depletion is a normal consequence of aging in men. One possible consequence of this could be an increased risk for the development of Alzheimer’s disease. (Pike et al 2006) (Rosario et al 2004).
1.2.1.4.8. Aggression effect of Testosterone

The positive correlation between testosterone levels and aggression in humans has been demonstrated in many studies, but about half of studies fail to find a link. (Wright et al 2009) While testosterone itself is not shown to be the direct cause of aggression in males, the testosterone derivative estradiol is known to correlate with aggression in male mice. (Soma et al 2008).

1.2.1.4.9. Ethnic differences of Testosterone

Different ethnic groups have different incidences of prostate cancer. Differences in sex hormones including testosterone have been suggested as an explanation for these differences. A study find ethnical differences between blacks and whites in the testosterone to sex hormone binding globulin ratio in blood from the umbilical cord in infants (Rohrmann et al 2009)(McIntosh 1997)(Calistro 2010).

1.2.1.4.10. Biosynthesis effect of Testosterone

Like other steroid hormones, testosterone is derived from cholesterol. The largest amounts of testosterone (>95%) are produced by the testes in men. (Mooradian et al 1987) It is also synthesized in far smaller quantities in women by the thecal cells of the ovaries, by the placenta, as well as by the zona reticularis of the adrenal cortex and even skin in both sexes. In the testes, testosterone is produced by the Leydig cells. (Brooks 1975) The male generative glands also contain Sertoli cells which require testosterone for spermatogenesis. Like most hormones, testosterone is supplied to target tissues in the blood where much of it is transported bound to a specific plasma protein, sex hormone binding globulin (SHBG).
1.2.1.4.11. Insufficiency effect of Testosterone

Testosterone insufficiency (also termed hypotestosteronism or hypotestosteronemia) is an abnormally low testosterone production. It may occur because of testicular dysfunction (primary hypogonadism) or hypothalamic-pituitary dysfunction (secondary hypogonadism) and may be congenital or acquired. (Gould and Petty 2000) An acquired form of hypotestosteronism is a decline in testosterone levels that occurs by aging, sometimes being called "andropause" in men, as a comparison to the decline in estrogen that comes with menopause in women. Recent analysis shows average testosterone levels receding in men of all ages. (Travison et al 2007) (Dindyal 2007) Several theories from increases in obesity to exposure to endocrine disruptors have been proposed as an explanation for this reduction. (Bhasin 2007).
1.3 Objectives:

i - General Objective:
To assess the fertility hormones of infertile males in Red Sea State.

ii- Specific Objectives :
1- To compare sperm count of infertile men and healthy fertile volunteers.
2- To measure plasma levels of follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL) and Testosterone in infertile males and compare them with hormonal levels of healthy fertile males.
3- To assess the relationship between the plasma levels of FSH, LH, PRL and Testosterone and the sperm count.
1.4 Rationale

The number of infertile men in Red Sea State visit to Port Sudan Teaching Hospital and clinics in Red State is high, the exact cause is unknown for the majority of these cases.

As far as few data was available in the assessment of infertility hormones and the correlation to infertility among patients who are diagnosed as infertile men in Red Sea State.

This study is to try to answer the question" Are there any significant of A low or high level of these hormones is commonly suspected to be the cause of this infertility, this study is designed to prove or exclude this possibilities and in treatment of this problem.
CHAPTER TWO

MATERIALS and METHODS
Chapter Two

2- Materials and Methods:

2.1.Materials

2.1.1 Study Design :-

This is a cross - sectional study.

2.1.2. Study area:-

The study was conducted in Port Sudan Teaching Hospital, Port Sudan is the capital of the Red Sea State, located in the eastern Sudan 815 km from Khartoum the capital of Sudan. Port Sudan Teaching Hospital and clinics serves Port Sudan City with a population of 3,500,000 inhabitants. There is also a great number of patients who came from other nearby states and towns such as Swakin and Sinkat. The study was during the period March 2012 to March 2014.

2.1.3. Camed out study population:-

One hundred and fifty men diagnosed as infertile as a test group and not treated, and 94 healthy fertile men served as a control group.

2.1.4. Ethical consideration:

- Permission of this study was obtained from the local health authorities in the area of study and the medical director of Port Sudan Teaching Hospital.
- The objectives of the study were explained to all individuals participating in the study and the blood sample was obtained after their agreement.
- A written consent was obtained from all participants.
2.1.5. Blood samples:
Five ml of venous blood from each participant was collected in heparin container for measurement of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL) and testosterone.

2.1.6. Data collection:
Interviews with the test group and control group were done. A questionnaire, (Appendix 1) was designed to obtain the personal and clinical data.

2.1.7. Blood and seminal sampling
A local antiseptic for skin (70% alcohol) was used and 5 ml of venous blood was collected from the arm in a heparin vacotainer from each participant, and centrifuged immediately to obtain plasma.

Seminal was collected after three days of abstinence of intercourse in sterile container.

2.2. Methods:
For seminal analysis samples were collected by masturbation and the sample reach the lab within half an hour, the sample allowed to liquefies at room temperature and checked for pH by pH paper, volume with graduated pesteur pepitte, consistency and color. For microscope examination samples were mixed first and determination of sperm motility microscopically, and the sperm count was done by counting champer after dilution of sample by fixative (formal carbonate wich composed of 50 g of sodium bicarbonate (NaHCO3) and 10 ml of 35% formalin in 1000 ml of purified water) (Sayed, 2002).

Full automation technique was used Tosoh's AIA-360 (Automated Immunoassay) The AIA-Pack technology utilities an original concept patented by Tosoh based on individual test cups each containing lyophilized and ready to use reagents for measurement of plasma.
hormone levels of follicle stimulating hormone, Luteinizing hormone, Prolactin hormone, Testosterone hormone. The TOSOH AIA 360 Analyzer perform all samples and reagents handling operations automatically. The tosoh AIA system analyzers was read the rate of fluorescence produced by the reaction and automatically convert the rate to test concentration in its unit, this was calculated from the curve of the target test.

Fluorescence Enzyme Immunoassays rely on the ability of an antibody to recognize and bind a specific macro molecule in what might be a complex mixture of macro molecules(Antigen antibody reaction). In immunology the particular macromolecule bound by an antibody is referred to as an antigen and the area on an antigen to which the antibody binds is called an epitope. (Smith and Osikowicz 1993).

2.2.1. **Measurement of plasma FSH:-**

**Principle**

Full automation technique is used Tosoh's AIA-360( Automated Immunoassay ) the ST AIA-PACK FSH is a two-site Fluorescence Enzyme Immunoassay assay which is performed entirely in the AIA-PACK test cup. FSH present in the sample bound with monoclonal antibody immobilized on magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-pack test cup. The magnetic beads was washed to remove unbound enzyme–labeled monoclonal antibody and then react with fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme–labeled monoclonal antibody that binds to the beads is directly proportional to the FSH concentration in the test sample (Smith and Osikowicz 1993).
**Calculations :-**

A standard curve was constructed using calibrators with known concentrations, an unknown sample concentration was calculated using this curve.

**Reagent composition and reagent preparation :-**

(Appendix 2)

**Reference Values :-**

Male 2.1 – 18.6 mIU/ml (Appendix 2)

**Linearity limit :-** Up to 200 mIU/ml (Appendix 2)

**Sensitivity limit :-**

The lowest measurable concentration in specimens is 1.0 mIU/ml (Appendix 2).

### 2.2.2 Measurement of plasma LH:-

**Principle**

Full automation technique was used Tosoh's AIA-360 (Automated Immunoassay), the ST AIA-PACK LH is a two-site Fluorescence Enzyme Immunoassay assay which is performed entirely in the AIA-PACK test cup. LH present in the sample is bound with monoclonal antibody immobilized on magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-pack test cup. The magnetic beads were washed to remove unbound enzyme–labeled monoclonal antibody and were then react with fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme–labeled monoclonal antibody that binds to the beads is directly proportional to the LH concentration in the test sample (Smith and Osikowicz 1993).
Calculations :-
A standard curve was constructed by using calibrators with known concentrations, an unknown sample concentration are calculated using this curve.

**Reagent composition and reagent preparation :**
(Appendix 3)

**Reference Values :-**
Male 1.7 – 11.2 mIU/ml (Appendix 3)

**Linearity limit :-** Up to 200 mIU/ml (Appendix 3)

**Sensitivity limit:**
The lowest measurable concentration in specimens is 0.2 mIU/ml. (Appendix 3)

**2.2.3. Measurement of plasma PRL:-**

Principle of the method

Full automation technique was used Tosoh's AIA-360( Automated Immunoassay ), the ST AIA-PACK PRL is a two-site Fluorescence Enzyme Immunoassay assay which is performed entirely in the AIA-PACK test cup. PRL present in the sample is bound with monoclonal antibody immobilized on magnetic solid phase and enzyme-labeled monoclonal antibody in the AIA-pack test cup. The magnetic beads are washed to remove unbound enzyme–labeled monoclonal antibody and are then incubated with fluorogenic substrate 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme–labeled monoclonal antibody that binds to the beads is directly proportional to the PRL concentration in the test sample (Smith and Osikowicz 1993).
Calculations :-
A standard curve is constructed by using calibrators with known concentrations, an unknown sample concentration are calculated using this curve.

Reagent composition and reagent preparation :-
(Appendix 4).

Reference Values :-
Male 3.6 – 16.3 ng/ml (Appendix 4).

Linearity limit :- Up to 200 ng/ml (Appendix 4).

Sensitivity limit:- The lowest measurable concentration in specimens is 1.0 ng/ml. (Appendix 4)

2.2.4. Measurement of plasma Testosterone :-

Principle of the method
Full automation technique is used Tosoh's AIA-360( Automated Immunoassay ), the ST AIA-PACK Testosterone is a competitive Fluorescence Enzyme Immunoassay which is performed entirely in the ST AIA-PACK Testosterone test cup. Testosterone present in the sample competes with enzyme-labeled testosterone limited number of binding sites on the testosterone specific monoclonal antibody immobilized on a magnetic solid phase. The magnetic beads are washed to remove unbound enzyme-labeled testosterone and are then incubated with a fluorogenic substrate, substrate 4-methylumbelliferyl phosphate (4MUP), the amount of enzyme-labeled testosterone that bind to the beads is inversely proportional to the testosterone concentration in the test sample. A standard curve is constructed, and unknown sample concentration are calculated using this curve (Smith and Osikowicz 1993).
Calculations of results :-

A standard curve which constructed by using calibrators with known concentrations, an unknown sample concentration are calculated using this curve.

Reagent composition and reagent preparation :-

(Appendix 5).

Reference Values :-

Male 262 – 870 ng/dl (Appendix 5).

Linearity limit :-

Up to 2000 ng/dl (Appendix 5).

Sensitivity limit: -

The lowest measurable concentration in specimens is 7.0 ng/dl. (Appendix 5)

2.2.5. Quality control:-

The precision and accuracy of all methods used in this study were checked at least once per day with commercially available control, was done at least two levels of control (normal and abnormal).

2.2.6. Statistical analysis:-

The data collected in this study were analyzed using SSPS computer program. The means and standard deviations of plasma levels of FSH, LH, PRL, Testosterone were obtained for both the test group and the control group and calculated the t-test was used for comparison (p value \( \leq 0.05 \) was considered to be significant)

Linear regression analysis was used to assess correlation between FSH, LH, PRL and Testosterone.
CHAPTER THREE

RESULTS
Chapter Three

3. Results

A total of 150 Sudanese patients from red sea state who were diagnosed as infertile males were investigated in this study as test group, age of test group ranged between 20 and 56 years, 94 healthy fertile volunteers as a control group were enrolled in this study. In this study the mean of duration of married was 6.34 years, also there was 14% of test group had a past sexual transmitted diseases and 86% were not infected, also there was 4% were diabetic and 96% non diabetic, also it found that 7.3% were infected by mumps and the other 92.7% had no previous infection, among the population of the study there were 14% had testicular pain and 86% did not complain from testicular pain, also there was 12.7% had past history of testicular surgery but 87.3% had no past history of testicular surgery. 2% of study population had a congenital defects but 98% were normal. 26% were smoker but 74% were not smokers, 29.3% snough users but 70.7% don't use it. 2% drink alcohol and 98% not use alcohol. In the current study 66.3% of were oligospermic, 36.7% were azoospermic. The mean of seminal volume of azoospermic was 2.42 ±1.4 ml, and of oligospermic was 2.4±1.4 ml. Table (3-1) showed a significant decrease of the sperm count of the test group when compared to the control group, (p = 0.000). It showed a significant increase in the mean of plasma levels of Follicle stimulating hormone, Luteinizing hormone and prolactin of the test group compared to that of the control group, (p = 0.00). No significant difference between plasma levels of testosterone hormone of the test group compared to control, (p = 0.100).
Fig. (3-1): Showed a scatter plot with no significant correlation between the plasma levels of Follicle stimulating hormone and Testosterone (r= - 0.11, p 0.088)

Fig. (3-2): Showed a scatter plot with no significant correlation between the plasma levels of Luteinizing hormone and Testosterone (r= - 0.11, p 0.165).

Fig. (3-3): Showed a scatter plot with no significant correlation between the plasma levels of prolactin hormone and Testosterone (r= - 0.03, p 0.362).

Fig. (3-4): Showed a scatter plot with no significant correlation between the plasma levels of Testosterone and the sperm count (r= 0.04, p 0.658).

Fig. (3-5): Showed a scatter plot with a significant correlation between the plasma levels of Follicle stimulating hormone and the sperm count (r= - 0.17, p 0.018).

Fig. (3-6): Showed a scatter plot with a significant week correlation between the plasma levels of Luteinizing hormone and the sperm count (r= - 0.21, p 0.004).

Fig. (3-7): Showed a scatter plot with no significant correlation between the plasma levels of prolactin hormone and the sperm count (r= - 0.03, p 0.371).
Table (3 -1): Plasma levels of FSH, LH, PRL and Testosterone of the study group compared to control groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test group (n=150)</th>
<th>Control (n=94)</th>
<th>p-value</th>
</tr>
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<tr>
<td>Sperm count (million/ml)</td>
<td>4.41 ± 0.96</td>
<td>82.13 ± 15.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Follicle Stimulating Hormone(mIU/mL)</td>
<td>14.79 ± 2.8</td>
<td>5.31 ± 2.5</td>
<td>0.000</td>
</tr>
<tr>
<td>Luteinizing Hormone(mIU/mL)</td>
<td>8.49 ± 2.0</td>
<td>5.01 ± 2.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Prolactin Hormone(ng/mL)</td>
<td>17.15 ± 0.6</td>
<td>14.04 ± 4.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Testosterone Hormone (ng/dL)</td>
<td>533.39 ±253.29</td>
<td>555.53 ± 239.4</td>
<td>0.100</td>
</tr>
</tbody>
</table>
Fig. (3-1): A scatter plot shows the correlation between the plasma levels of Follicle stimulating hormone and Testosterone ($r = -0.11$, $P = 0.088$) among the test group which is insignificant.
Fig. (3-2): A scatter plot shows the correlation between the plasma levels of Luteinizing hormone and Testosterone ($r = -0.11$, $P = 0.165$) among the test group which is insignificant.
Fig. (3-3): A scatter plot shows the correlation between the plasma levels of prolactin hormone and Testosterone ($r=-0.03$, $P=0.362$) among the test group which is insignificant.
Fig. (3-4): A scatter plot shows the correlation between the plasma levels of Testosterone and the sperm count ($r=0.04$, $P=0.658$) among the test group which is insignificant.
Fig. (3-5): A scatter plot shows the correlation between the plasma levels of Follicle stimulating hormone and the sperm count ($r = -0.17$, $P < 0.018$) among the test group which is significant.
Fig. (3-6): A scatter plot shows the correlation between the plasma levels of Luteinizing hormone and the sperm count ($r = -0.21$, $P = 0.004$) among the test group which is significant.
Fig. (3-7): A scatter plot shows the correlation between the plasma levels of prolactin hormone and the sperm count \((r = -0.03, P 0.371)\) among the test group which is insignificant.
CHAPTER FOUR

DISCUSSION, CONCLUSION

and

RECOMENDATIONS
Chapter Four

4. Discussion, Conclusion and Recommendations

4.1. Discussion

This study aimed to determine plasma levels of Follicle stimulating hormone, Luteinizing hormone, Prolactin and Testosterone of patients diagnosed as infertile males in red sea state, age of the patients ranged from 20 to 56 year with mean of 37.8 ±7.2 year, 80% had primary infertility and 20% had secondary infertility.

The results of the sperm count in this study showed a significant reduction when the mean of the sperm count of the test group when compared with that of the control group, (p = 0.000), in test group there were 36.7% of them were found azoospermia and 63.3% were oligospermia.

In this study there were 14% had a past sexual transmitted disease and 86% not infected never and the correlation between the sperm count of test group and sexually transmitted diseases (p =0. 011) which indicating significant meaning that these diseases affect the sperm production.

In the study the correlation between the sperm count of patients who are diabetic and who are not diabetic (p =0. 010) the results showed that Diabetes mellitus cause a decrease in sperm production, also the correlation between the sperm count of patients who was infected by mumps and who hadn't previous infection was (p =0. 109) which is insignificant mean that mumps doesn't affect the production of sperm in test group, in the correlation between the sperm count of patients who had Tests pain and who hadn't a pain was (p =0. 074) which is insignificant mean that the tests pain may not affect the production of
sperm, also the correlation between the sperm count of patients who had past history of tests surgery and who hadn't was (p = 0.004) which is significant and the Tests surgery may cause of low sperm count. The correlation between the sperm count of patients who had a congenital defects and who hadn't was (p = 0.462) which is insignificant because the most of this patients the defect was they had one test which can do the job.

According to social habits the correlation between the sperm count of patients that who were smoke and who weren't smoke (p = 0.350) which is insignificant. The correlation between the sperm count of patients that who were use snough and who weren't use it (p = 0.278) which is insignificant, and also the correlation between the sperm count of patients that who were drink alcohol and who weren't drink it (p = 0.911) which is insignificant.

The present study showed a significant increase in the mean value of the plasma levels of Follicle stimulating hormone (FSH) of the test group compared with control group as shown in table (4-1), (p = 0.00). These results agrees with a study done by (Geidam et al 2008) and (Burney 2002) who reported that the level of FSH usually correlates inversely with spermatogenesis. Elevation of FSH occur in men with intact hypothalamic – pituitary-adrenal axis when there is severe damage of germinal epithelium, and high FSH value are compatible with primary testicular failure. It has been known for a long time that low levels of inhibin causes raised levels of FSH, a significant positive correlation between the two variables showed in Fig. (4-5).

The current study also showed a significant increase in plasma levels of Luteinizing hormone (LH) of the test group compared to control group, (p = 0.00), this agreed with a study done by Geidam (Geidam et al
2008), and who reported that the LH in the test group is higher than normal range, in Fig. (4-6). The present study also showed a significant weak correlation between that plasma levels of LH and the sperm count. This suggested that a secondary increase of LH occur as a result of reduction in the sperm count.

The results also showed a significant increase of the levels of serum Prolactin hormone of the test group when compared to control group (p = 0.001), as (Burney 2002) study reported that PRL is elevated in patients with normal count of sperm but infertile and this study find that PRL is elevated in test group patients whom were known to have low sperm count, as the (Carter et al 1978) reported, as it may signify prolactin-secreting pituitary adenoma, which is amenable to therapy, however, as prolactin elevation may be induced by some abnormalities of the thyroid gland, further evaluation of these patients should include pituitary imaging and thyroid function test.

No significant correlation between the plasma levels of Prolactin hormone and the sperm count in the test group.

The current study also shows no significant difference between the mean of the plasma levels of testosterone hormone of the test group and to that of the control group (p = 0.100). These results agreed with the results of Geidam (Geidam et al 2008) and El- Migadi (El-Migdadi et al 2005)

No significant correlation between the plasma levels of testosterone and the sperm count in the test group.

The results showed insignificant negative correlations between FSH, LH, PRL and Testosterone.
4.2. Conclusion

1. The sperm count is significantly decreased in test group compare to control group.

2. Plasma levels of Follicle Stimulating hormone, Luteinizing hormone and Prolactin significantly increased in test group.

3. No significant difference in Testosterone plasma levels of test group compare to control.

4. No significant correlation of Follicle Stimulating Hormone, Luteinizing Hormone and Prolactin plasma levels and that of Testosterone, and also no significant correlation of Testosterone, Prolactin and the sperm count.

5. There is a significant correlation between the plasma levels of Follicle Stimulating hormone, Luteinizing hormone and the sperm count.
4.3 Recommendations:

1. Beside seminal analysis, FSH, LH, PRL and testosterone investigation is recommended this may help in infertility diagnosis.

2. Further study should be done to assess thyroid function, oestradiol, and gonadotropin releasing hormone in infertile men, since these hormones have a role in normal reproductive females.
References


86. Zak PJ, Aleman, André Ed. (2009) Testosterone Administration Decreases Generosity in the Ultimatum Game . 4 (12)
Appendix - 1

Categories

Evaluation of Fertility Hormones among Infertile Men in Red Sea State

Questionnaire no:

5.1 Personal Data:
Name: 
Age: 
Laboratory Number: 
Hospital or clinic: 
Duration of married:

5.2 Clinical Presentation:
Has child 
Past history of STD 
Diabetes mellitus 
Hypertension 
Mumps in life 
Testicular pain 
Testicular surgery 

Ys | No 
---|--- 
---|---
Congenital defect:  

| YES | NO |

5.3. Habits:

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<tr>
<th>Snough</th>
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5.4. Laboratory Findings:

A) Blood Test:

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<th>FHS Level</th>
<th>LH level</th>
<th>PRL level</th>
<th>Testosterone level</th>
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B) Seminal tests:

Seminal volume

Sperm count:

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Appendix - 2

ST AIA-PACK FSH

NAME AND INTENDED USE
ST AIA-PACK FSH is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of follicle-stimulating hormone (FSH) in serum or heparinized plasma on TOSHI AIA System Analyzers.

SUMMARY AND EXPLANATION OF TEST
Follicle-stimulating hormone (FSH) is a glycoprotein which, like LH, RCG and FSH, consists of alpha and beta chains. The total mass of beta chains is somewhat lower in FSH than in LH and RCG. The total mass of beta chains is somewhat lower in FSH than in LH and RCG.

PRINCIPLE OF THE ASSAY
The ST AIA-PACK FSH is a two-site immunoradiometric assay which is performed entirely in the AIA-PACK test cups. FSH present in the test sample is bound with iodinated antiserum immobilized on a magnetic solid phase and excess of unlabelled monovalent antibody in the AIA-PACK test cup. The magnetic beads are washed in order to remove unbound antibody and free plasma FSH is removed with a fluorographic substrate 4-mercaptobenzothiazole phosphate. The amount of bound radioactive iodine is directly proportional to the FSH concentration in the test sample. A standard curve is constructed and unknown sample concentrations are calculated using this curve.

MATERIALS PROVIDED (ST AIA-PACK FSH, Cat. No. 0032665)
Pasted test cups containing magnetic beads, substrate control, and anti-FSH (mouse monoclonal antibody) and 100 mL of 0.01 M sodium phosphate buffer (pH 7.4) are available separately from TOSHI.

Materials

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Only materials obtained from TOSHI should be used. Materials obtained elsewhere should not be used once assay performance is characterized based strictly on TOSHI materials.

FEATURES
1. The ST AIA-PACK FSH is intended for in vitro diagnostic use only.
2. The ST AIA-PACK FSH is for use with the TOSHI AIA System. Results obtained with test cups from other manufacturers may not be comparable.
3. The ST AIA-PACK FSH contains no dextran sulfate. No special precautions are necessary for disposing of test cups and other assay materials.
4. The ST AIA-PACK FSH is designed for use with the TOSHI AIA System. Other assay systems may not be compatible.
5. Do not use beyond the expiration date.

The ST AIA-PACK FSH has been designed so that the high dose "zero effect" is not present in the assay mixture. The concentrations of various species with FSH concentrations between 50 and 20,000 mIU/mL will result in 350 mIU/mL FSH. The "zero effect" phenomenon may occur in FSH concentrations >20,000 mIU/mL.

STORAGE AND STABILITY
All reagents are stable until the expiration date as labeled when stored at the specified temperature.

Materials

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<td>ST AIA-PACK FSH CALIBRATOR SET</td>
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<td>AIA-PACK WASH CONCENTRATE</td>
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<td>AIA-PACK DILUTENT CONCENTRATE</td>
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<td>AIA-PACK DETECTOR STANDARDIZATION TEST CUP</td>
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</table>

ST AIA-PACK FSH test cups may be stored at 18-25°C for up to 1 day. Calibrators and Sample Diluting Solutions should be used within 1 day and 2 days of opening, respectively. The test cups are kept tightly sealed and refrigerated at 4°C. Reconstituted reagent solutions are stable for 5 days at 18-25°C or at 7 days at 2-8°C. Working diluent and wash solutions are stable for 30 days at 18-25°C. Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING
Specimen or hormone standards for the assay. EDTA and citrated plasma should NOT BE USED.

1. If plasma, serum, a venous blood sample is collected aseptically without additives. Store at 18-25°C until the clot has formed (usually 1-4 hours) and centrifuge to obtain the specimen for aspiration.
2. If heparinized plasma, a venous blood sample is collected aseptically with heparin as an additive. Centrifuge and separate plasma from the packed cells as soon as possible.
3. Specimen types should not be used interchangeably during serial monitoring of an individual patient. Measured concentrations may vary slightly between sample types in certain patients.
4. Samples may be stored at 2-8°C for up to 24 hours prior to analysis. If the analysis cannot be performed within 24 hours, the sample should be stored frozen at -20°C or below for up to 60 minutes.
5. Repeated freeze-thaw cycles should be avoided. Tuned serum samples or samples containing particulate material should be centrifuged prior to testing. Prior to assay, bring frozen samples to 18-25°C slowly and avoid vortex.
6. The sample required for analysis is 50 mL.

PROCEDURE

1. Reagent Preparation
   a. Buffer Solution
      Bring all reagents to 18-25°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE REAGENT II (10 mL) to the hypotized AIA-PACK SUBSTRATE REAGENT I (20 mL) thoroughly to dissolve the solid reagent.
   b. Wash Solution
      Add the entire contents of the AIA-PACK WASH CONCENTRATE (10 mL) to 270 mL of CAP Class I or NICKELS Type 1 Reagent Grade water, mix well, and adjust the final volume to 2.5 L.
   c. Reagent
      Add the entire contents of the AIA-PACK DILUTENT CONCENTRATE (10 mL) to approximately 4.8 L of CAP Class I or NICKELS Type 1 Reagent Grade water, mix well, and adjust the final volume to 5.0 L.

2. Calibration Procedure
   b. Calibration Curve
      The reagents for use with the TO SHI AIA-PACK FSH have been standardized on WHO 2nd hIRP 78/549 (1980).
      The calibration curve for ST AIA-PACK FSH is scale for up to 90 days. Calibration accuracy is monitored by quality control performance and is determined on patient magnetic and TOShi AIA System performance according to the manufacturer's instructions.
      Recapitulation may be necessary more frequently if controls are out of the established range for this assay or when certain specific procedures are performed by a different technician. Using multiple reagent changes, maintain the wash probe or dilute upward adjustment to ensure the correct dilution. For further information regarding instrument operation, consult the TOSHI AIA.
      A sample calibration curve from AIA-1000 follows and shows the algorithm used for calculating results.

3. Calibration Procedure
   b. Calibration Procedure
      1. Refer to the appropriate TOSHI AIA System Operator's Manual for the procedural instructions.
      2. Verify that both the calibrator for and concentration numbers have been correctly entered into the software.
      3. Calibrators for ST AIA-PACK FSH are provided ready for use. TOSHI recommends that all calibrators be used in a dilution, 1:2, 1:4, and 1:8. For further information regarding instrument operation, consult the TOSHI AIA System Operator's Manual.
      5. Calibration Accuracy by Chart
      a. The linearity for the ZERO CALIBRATOR should be < 3.0% (area %). The linear relationship between concentration and rate should increase as the concentration increases.
      b. The linearity for the точки is within ± 10% range.
      c. Calibration Review and Acceptance
      1. Review the calibration curve. Accept the test results over the selected range. Where above.
      2. Edit the calibration if necessary, then accept the calibration.

For further information regarding calibration, consult the TOSHI AIA System Operator's Manual.
**Diabetes Mellitus**

**Diabetes Mellitus** is a chronic condition that involves high levels of glucose in the blood. It occurs when the body cannot effectively use the hormone insulin to convert food into energy, or when the body does not produce enough insulin. There are two main types of diabetes: Type 1 and Type 2.

**Type 1 Diabetes** is an autoimmune disease in which the body's immune system attacks and destroys the insulin-producing cells in the pancreas. This type affects about 5% of people with diabetes and is usually diagnosed in children and young adults. Treatment involves daily insulin injections or an insulin pump to manage blood sugar levels.

**Type 2 Diabetes** is more common and usually develops later in life. It occurs when the body becomes less sensitive to insulin or the pancreas is unable to produce enough insulin to keep blood sugar levels in check. Type 2 diabetes affects about 90% of people with diabetes and is typically managed through a combination of lifestyle changes, medication, and sometimes insulin injections.

Diabetic individuals face various health risks including heart disease, high blood pressure, kidney disease, blindness, nerve damage, and foot problems. Regular monitoring and treatment are crucial to prevent complications. Treatment may include medication, diet, exercise, and in some cases, insulin therapy.

**Diabetic Foot Care** is essential to prevent complications. Regularly inspecting the feet for any signs of injury, maintaining good foot hygiene, and ensuring appropriate footwear can help prevent diabetic foot problems.
Appendix - 3

ST AIA-PACK LH II

For Quantitative Measurement of Stimulating hormone (LH) in Serum or Hypophysectomized Plasma.

NAME AND INTENDED USE

ST AIA-PACK LH II is designed for use in vitro diagnostic use only (in the qualitative measurement of stimulating hormone (LH) in human serum or hypophysectomized plasma on TOYOBO Analyzer Systems).

SUMMARY AND EXPLANATION OF TEST

Human stimulating hormone (LH) is a gonadotrophin which, like FSH, HCG and TSH, consists of alpha and beta chains. The alpha chain is virtually identical in all four hormones, whereas the beta chains are different and determine both the specific biological and immunological characteristics of each hormone. (22)

Human stimulating hormone (LH) is a glycoprotein hormone which has a molecular weight of approximately 20,000 daltons (24). LH binds to receptors and stimulates the ovary, uterus and other estrogen producing organs including the pituitary gland. (25)

LH is legally defined as (26): Biological activity of both LH and FSH is measured by the ovulatory response of the intact rabbit and Sertoli tissue test for the measurement of gonadotrophins, respectively. (27)

PRINCIPLES OF THIS ASSAY

The ST AIA-PACK LH II is a one-step immunoresistance test which is performed similarly to the ST AIA-PACK test kit. The kit contains the reagents necessary for the specific measurement of LH in the serum or plasma of human subjects using specific antibodies. The assay is performed in a single tube in the presence of specific antibodies and is done with two rolls of the plastic tube to mix the reagents in order to obtain a homogeneous mixture. The assay of LH is performed using the ST AIA-PACK LH II assay as described below:

MATERIALS PROVIDED (ST AIA-PACK LH II, Cat. No. 0023520)

The following materials are provided for the performance of the assay:

- ST AIA-PACK LH II assay kit
- Buffer solution for the assay
- ST AIA-PACK LH II assay buffer
- ST AIA-PACK LH II assay saline
- ST AIA-PACK LH II assay reagent
- ST AIA-PACK LH II assay control
- ST AIA-PACK LH II assay standard
- ST AIA-PACK LH II assay diluent
- ST AIA-PACK LH II assay control

MATERIALS REQUIRED BUT NOT PROVIDED

The following materials are not provided but are required for the performance of the assay:

- Buffer solution for the assay
- ST AIA-PACK LH II assay buffer
- ST AIA-PACK LH II assay saline
- ST AIA-PACK LH II assay reagent
- ST AIA-PACK LH II assay control
- ST AIA-PACK LH II assay standard
- ST AIA-PACK LH II assay diluent
- ST AIA-PACK LH II assay control

PROCEDURE

1. Preparation of Buffers
2. Preparation of Reagents
3. Assay Procedure

1. Preparation of Buffers

Prepare all reagents at 10% neutral buffered saline solution and store at 4°C. The reagents should be used within 2 weeks after opening and at 4°C.

2. Preparation of Reagents

Prepare all reagents at 10% neutral buffered saline solution and store at 4°C. The reagents should be used within 2 weeks after opening and at 4°C.

3. Assay Procedure

Perform the assay according to the following procedure:

- **Buffer solution for the assay**
- **ST AIA-PACK LH II assay buffer**
- **ST AIA-PACK LH II assay saline**
- **ST AIA-PACK LH II assay reagent**
- **ST AIA-PACK LH II assay control**
- **ST AIA-PACK LH II assay standard**
- **ST AIA-PACK LH II assay diluent**
- **ST AIA-PACK LH II assay control**

DO NOT USE IN COMBINATION WITH ST AIA-PACK LH CALIBRATION SET (CAT. NO. 0023590)

ASSAY PREPARATION

1. The ST AIA-PACK LH II assay kit is intended for in vitro diagnostic use only. REFER TO THE MANUFACTURER’S INSTRUCTIONS MANUAL.

2. Bring all test samples and controls to 18-28°C, mix well.

WARNINGS AND PRECAUTIONS

1. The ST AIA-PACK LH II assay kit is intended for in vitro diagnostic use only. Mix all test samples and controls to 18-28°C, mix well.

2. The ST AIA-PACK LH II assay kit is intended for in vitro diagnostic use only. Mix all test samples and controls to 18-28°C, mix well.

3. The ST AIA-PACK LH II assay kit is intended for in vitro diagnostic use only. Mix all test samples and controls to 18-28°C, mix well.

4. Human serum is not used in the preparation of the product however, since human specimens are to be used for controls and other quality control products in the kit may be brought from human sources, for use in standardized laboratory safety procedures in handling all specimens and controls.

5. Do not use for the treatment of patients.

6. The ST AIA-PACK LH II assay kit is intended for in vitro diagnostic use only. Mix all test samples and controls to 18-28°C, mix well.

7. TOYOBO AIA-200 Crim mononucleosome Assays are NOT to be used in the preparation of the ST AIA-PACK LH II assay kit.

STORAGE AND STABILITY

All standard solutions are stable until the expiration date on the label when stored at the specified temperature.

MATERIALS

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Example:

- **ST AIA-PACK LH II assay kit**
- **ST AIA-PACK LH II assay buffer**
- **ST AIA-PACK LH II assay saline**
- **ST AIA-PACK LH II assay reagent**
- **ST AIA-PACK LH II assay control**
- **ST AIA-PACK LH II assay standard**
- **ST AIA-PACK LH II assay diluent**
- **ST AIA-PACK LH II assay control**
Appendix - 3

ST AIA-PACK LH II

For Quantitative Measurement of luteinizing hormone (LH) in Serum or Heparinized Plasma

NAME AND INTENDED USE
ST AIA-PACK LH II is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of luteinizing hormone (LH) in human serum or heparinized plasma on TOYOLO AIA Systems.

SUMMARY AND EXPLANATION OF TEST
Human luteinizing hormone (LH) is a glycoprotein which, like FSH, HCG and TSH, consists of alpha and beta chains. The alpha chain is virtually identical in all four hormones, whereas the beta chains are different and determine both the specific biological activity and immunological characterization of each hormone (2.2).

The LH molecule contains 15-16% carbohydrate and has a molecular weight of approximately 29,000 daltons (2.4). LH stimulates ovulation and thereafter maintains the corpus luteum and progesterone production in women (2.3). In men, LH stimulates testosterone production by the Leydig cells (2.5,6).

Hyperreactivity of both LH and FSH secretions by the anterior pituitary appears to be a common finding in hyperthyroidism, hypothyroidism (2.7) or hyperpituitarism (2.8). In addition, there may be an increased LH response to the hypoglycemic level by glucose in females and lactation in males.

Through the use of immunological antibody technique which provides the necessary specificity and sensitivity, determination of LH concentration is critically important in assessment and monitoring of patients with suspected infertility (11,12). Elevated levels of LH and FSH ratio is frequently found in polyovulatory syndrome (PODS), a common cause of infertility in women (12,13) and other physiological disorders associated with abnormal ovarian function (12,14).

PRINCIPLE OF THE ASSAY
The ST AIA-PACK LH II is a two-site immunometric assay which is performed entirely in the AIA-PACK test cups. LH present in the test sample is bound to monoclonal antibody immobilized on a magnetic solid phase and antibody labeled monoclonal antibody in the AIA-PACK test cups. The magnetic beads are removed from the antibody labeled monoclonal antibody and are then resuspended with a fluorescein substrate 4-phenyl-1,2,3-benzotriazolyl phosphoric acid. The reaction mixture is incubated for 40 minutes. The unbound magnetic beads that bind to the beads in the presence of anti-LH antibody are removed by magnetic separation. The concentration of LH in the test sample is determined by comparing the absorbance of the test sample to that of the standard curve.

MATERIAL PROVIDED (ST AIA-PACK LH II, Cat. No. 0025236)
Plastic test cups containing labeled positive magnetic beads coated with mouse anti-LH monoclonal antibody and 100 µL of mouse anti-LH monoclonal antibody (to human LH) conjugated to bovine alkaline phosphatase with a nucleotide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED
The following materials are not provided but are required to perform immunometric hormone analysis using the ST AIA-PACK LH II (Cat. No. 0025236) on the TOYOLO AIA System. They are available separately from TOYOLO.

Materials

<table>
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The following materials are not provided but are required to perform quantitative hormone analysis using the ST AIA-PACK LH II (Cat. No. 0025236) on the TOYOLO AIA System. They are available separately from TOYOLO.

DO NOT USE IN COMBINATION WITH AIA-PACK LH II CALIBRATOR SET (Cat. No. 002936)

ASSAY PREPARATION
1. See the TOYOLO AIA System Analyzer for immunometric hormone analysis. REFER TO THE APPROPRIATE TOYOLO AIA SYSTEM ANALYZERS OPERATOR'S MANUAL.
2. Bring all test samples and control to 18-22°C, and use within 4 hours of opening.

WARRIORS AND PRECAUTIONS
1. The ST AIA-PACK LH II is intended for in vitro diagnostic use only.
2. This assay is performed on two different instruments, and the results of each instrument may differ. When using more than one instrument, always flush with large volume of water to prevent adsorb build-up.
3. Heparin-treated plasma should not be used for the preparation of this product because trace amounts of heparin may interact with the antibodies, thereby leading to false positive test results.
4. Some ivermectinem will be used for the samples and other quality control products in the tube lab may be derived from human serum, please use standard laboratory safety procedures in handling all specimens and controls.
5. Do not use beyond the expiration date.

STORAGE AND STABILITY
All reagents are stable until the expiration date on the label where stored at the specified temperature.

<table>
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ST AIA-PACK LH II test kits may be stored at 18-22°C for up to 1 year. Calibrator and Sample Diluting Solution should be used within 1 day and 7 days of opening or reconstituting, respectively, provided the vials are kept tightly sealed and refrigerated at 2-8°C. Reconstituted solutions are stable for 7 days at 18-22°C or 3 days at 2-8°C. Working diluent and wash solutions are stable for 20 days at 18-22°C. Reagents should not be used if they appear cloudy or discolored.

SPECIMEN COLLECTION AND HANDLING
1. Blood is obtained from the patient for the assay. EDTA and citrated plasma SHOULD NOT BE USED.
2. If using serum, a venous blood sample is collected saccarose without additives. Store at 18-22°C until a clot has formed (usually 15-45 minutes), then centrifuge to obtain the serum specimen for assay.
3. The blood sample is placed in a vial with a cap that will be used to mix with the reagents (12.5%). The sample will be placed into a solution at 2-8°C and mixing will be done prior to analysis. If the solution cannot be done within 24 hours, the sample should be stored frozen at -20°C or below for up to 60 days.
4. Repeated freeze-thaw cycles could be avoided. Partial serum specimens or specimens containing particulate matter should be centrifuged prior to testing. Prior to testing, bring frozen specimens to room temperature and mix gently.
5. Diluted sera or hemolyzed specimens should be used within 24 hours and should not be stored.
6. The sample is required for 40 µL.

PROCEDURE
For the AIA Nex/Car AIA-21, AIA-500 B, AIA-2000, AIA-5000 and AIA-100, please refer to their Operator’s Manual for detailed instructions.

1. Reagent Preparation
   a. Substrate Solution
      Bring all reagents to 18-22°C before preparing the working reagent. Add the entire contents of the AIA-PACK SUBSTRATE REAGENT I (100 µL) to the AIA-PACK SUBSTRATE REAGENT II, mix thoroughly to dissolve the solid material.
   b. Wash Solution
      Add the entire contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 2 L of CAP Class I or NCCS Type I Reagent Grade water, mix well, and adjust the final volume to 5 L.

2. Calibration Procedure
   a. Calibrator Curve
      The calibrator curve for the ST AIA-PACK LH II is stable for up to 90 days. Calibration stability is maintained by quality control performance and is dependent on proper reagent handling and TOYOLO AIA System maintenance according to the manufacturer’s instructions. Recalibration may be necessary more frequently if controls are out of the established range for this assay within certain service procedures are performed (e.g., temperature adjustment, maintenance of the wash probe or detector lamp adjustments or changes). For further information regarding instrument operation, consult the TOYOLO AIA System Operator’s Manual. A sample calibration curve from AIA-100 follows and shows the algorithm used for calculating results.
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ST AIA-PACK PRL

For Quantitative Measurement of proteins (PRL) in serum or heparinized plasma on TOSOH AIA System

NAME AND INTENDED USE
ST AIA-PACK PRL is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of prolactin (PRL) in serum or heparinized plasma on TOSOH AIA System.

SUMMARY AND EXPLANATION OF TEST
Prolactin (PRL) is a protein of 191 amino acids with a molecular weight of approximately 21,000. PRL is secreted from the anterior pituitary gland in response to the release of hypothalamic hormones, particularly dopamine. Deficiency of dopamine leads to increased levels of PRL. PRL concentrations are raised during pregnancy and lactation and suppressed during late pregnancy.

MATERIAL PROVIDED (ST AIA-PACK PRL, Cat. No. 60022255)
- All materials and reagents for the immunoassay are provided in this kit. Only water is needed to perform the assay.

MATERIALS REQUIRED BUT NOT PROVIDED
- Candidates with no prior knowledge of the assay should be supervised by a qualified technician.

PROCEDURE
1. Prepare all reagents and materials as instructed in the assay manual.
2. Perform the assay as directed in the manual to ensure accurate results.

STORAGE AND STABILITY
All unpacked materials are stable until the expiration date or until stored at the specified temperature.

The sample is required for analysis in 150 μL.

PROCEDURE
A) Calibration Preparation
- All standards should be prepared freshly. Calibration should be performed at least twice during the day.

B) Sample Preparation
- Samples should be prepared immediately before the assay. Samples should not be frozen.

C) Assay Procedure
- Add the appropriate volume of reagents to each well of the assay plate, and incubate for the specified time.

D) Calculation
- Calculate the results according to the calibration curve.

Appendix - 4
EXPECTED VALUES

Each laboratory should determine a reference interval which characterizes the distribution of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to each patient's clinical management and to the purpose of the test.

Reference Range

The interval given below was determined in 158 serum samples from apparently healthy Asian individuals.

- D. (134) 3.6 - 16.5 mg/dL
- D. (24) 4.1 - 28.9 mg/dL

Conversion Factors

Concentrations in this application are in units of mg/dL. Conversion to SI units of µmol/L and mmol/L may be made using the following equation:

\( \text{µmol/L} = \frac{\text{mg/dL} \times 58.0}{0.57} \)

\( \text{mmol/L} = \frac{\text{mg/dL} \times 58.0}{0.57} \)

PERFORMANCE CHARACTERISTICS

Accuracy

a. Recovery: Three serum pools were spiked with three different levels of protein and assayed before and after spiking.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Value (mg/dL)</th>
<th>Added Value (mg/dL)</th>
<th>Expected Value (mg/dL)</th>
<th>Measured Value (mg/dL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>4.1</td>
<td>4.1</td>
<td>8.2</td>
<td>8.4</td>
<td>100.4</td>
</tr>
<tr>
<td>Serum B</td>
<td>6.2</td>
<td>6.2</td>
<td>12.4</td>
<td>12.5</td>
<td>100.8</td>
</tr>
<tr>
<td>Serum C</td>
<td>8.3</td>
<td>8.3</td>
<td>16.6</td>
<td>16.8</td>
<td>101.2</td>
</tr>
</tbody>
</table>

b. Dilution: Three serum samples containing high concentrations of protein were serially diluted to the AIA-PACK PELLE DILUTION SOLUTION and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Value (mg/dL)</th>
<th>Measured Value (mg/dL)</th>
<th>Percent Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A2</td>
<td>25.0</td>
<td>25.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Serum B2</td>
<td>50.0</td>
<td>49.5</td>
<td>99.0</td>
</tr>
<tr>
<td>Serum C2</td>
<td>75.0</td>
<td>73.5</td>
<td>98.0</td>
</tr>
</tbody>
</table>

Precision

Within-run precision was determined using three controls in a run of 20 tests. Within each run, one set of duplicates per control was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation (CV).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Value (mg/dL)</th>
<th>SD Value (mg/dL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>4.1</td>
<td>0.1</td>
<td>2.5</td>
</tr>
<tr>
<td>Serum B</td>
<td>6.2</td>
<td>0.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Serum C</td>
<td>8.3</td>
<td>0.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Correlation

The correlation between serum (a) and hepatic plasma (b) on ST-AIA PACK PELLE was carried out using 197 patient specimens.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PELLE</td>
<td>100</td>
</tr>
<tr>
<td>UF</td>
<td>100</td>
</tr>
<tr>
<td>FSH</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TSH</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>hCG</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Sensitivity

The minimal detectable concentration (MDC) of protein is estimated to be 1.0 mg/dL. The MDC is defined as the concentration of protein which corresponds to the base of the curve.

The MDC is estimated to be 1.0 mg/dL. The MDC is estimated to be 1.0 mg/dL.
INTERFERENCE

Interference is defined as the presence of substances other than those being assayed that affect the assay results. The following substances have been tested for interference:

- Hemoglobin (up to 300 mg/dL) does not interfere with the assay.
- Uric acid (up to 12 mg/dL) does not interfere with the assay.
- Ascorbic acid (up to 10 mg/dL) does not interfere with the assay.
- Bilirubin (up to 20 mg/dL) does not interfere with the assay.
- Drugs (up to 10 mg/dL) do not interfere with the assay.

REFERENCES

Appendix 5

ST AIA-PACK Testosterone
For Quantitative Measurement of Testosterone in Human or Equine Plasma

NAME AND INTENDED USE
ST AIA-PACK Testosterone is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of testosterone in human or equine plasma on TOYOSHI AIA System Analyzers. Measurement of testosterone is used to aid in the diagnosis and management of conditions involving excess of deficiency of this hormone.

SUMMARY AND EXPLANATION OF TEST
Testosterone is a naturally occurring steroid hormone, produced by the adrenals, ovaries, placenta, testes, and skin. The major metabolite is androstenedione. The major hormone in serum is testosterone, which is found in a free, albumin-bound, and sex hormone-binding globulin (SHBG)-bound form. There is no intravenous administration of testosterone to prevent the release of free testosterone, and the plasma concentration of testosterone is measured.

PRINCIPLE OF THE ASSAY
ST AIA-PACK Testosterone is a competitive enzyme immunoassay which is performed quantitatively and reproducibly in all assay. Testosterone is labeled with a highly sensitive label, which is competitive with the endogenous unlabeled testosterone. The amount of endogenous unlabeled testosterone that binds to the antibody is inversely proportional to the testosterone concentration in the test sample.

MATERIAL PROVIDED (ST AIA-PACK Testosterone Cat. No. 003325)
Plastic test cups containing polystyrene magnetic beads coated with similar anti-testosterone monoclonal antibody and RIA kit of testosterone conjugate to bovine albumin-phosphate with sodium azide as a preservative.

MATERIALS REQUIRED BUT NOT PROVIDED
The following materials are not provided, but are required to perform hormone analysis using these test kits:
- To measure the hormone levels using these test kits.

STORAGE AND STABILITY
All required materials are stable until the expiration date on the label when stored at the specified temperature.

Materials
ST AIA-PACK Testosterone (0032504)
ST AIA-PACK Testosterone CALIBRATOR 1ST (0031394)
ST AIA-PACK Testosterone CALIBRATOR 2ND (0031395)
ST AIA-PACK Testosterone SAMPLE DILUTING SOLUTION (0032504)
ST AIA-PACK WASH CONCENTRATE (0020958)
ST AIA-PACK DILUENT CONCENTRATE (0020956)
ST AIA-PACK DILUTER STANDARDIZATION TEST CUP (0020970)
ST AIA-PACK SAMPLE TREATMENT CUP (0020971)

PROCEDURE

1) Measurement

a. Preparation
- Bring all reagents to 18-25°C before preparing the reagents. Add the contents of the AIA-PACK SUBSTRATE REAGENT II (100 mL) to the stabilized AIA-PACK SUBSTRATE REAGENT I and mix thoroughly to dissolve the solid material.
- Add the contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent grade water, mix well, and adjust the final volume to 5.0 L.
- Dilute the contents of the AIA-PACK DILUTANT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent grade water, mix well, and adjust the final volume to 5.0 L.

2) Calibration Procedure
- Calibration. For calibration, use the AIA-1800 following the algorithm used for calculating results.

*Note: Use of this product for the indicated purpose is intended for in vitro diagnostic use only.

1) Measurement

a. Preparation
- Bring all reagents to 18-25°C before preparing the reagents. Add the contents of the AIA-PACK SUBSTRATE REAGENT II (100 mL) to the stabilized AIA-PACK SUBSTRATE REAGENT I and mix thoroughly to dissolve the solid material.
- Add the contents of the AIA-PACK WASH CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent grade water, mix well, and adjust the final volume to 5.0 L.
- Dilute the contents of the AIA-PACK DILUTANT CONCENTRATE (100 mL) to approximately 4.0 L of CAP Class I or NCCLS Type I Reagent grade water, mix well, and adjust the final volume to 5.0 L.

2) Calibration Procedure

a. Calibration. For calibration, use the AIA-1800 following the algorithm used for calculating results.
II. Quality Control Procedure

A. Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two levels of control, normal and abnormal, be used. Laboratory policy for this particular procedure will define the number of levels of control to be used.

B. Specimen Processing

1. AFTER SEPARATION of the specimen is completed, the specimen is sent to the Analytical Laboratory for processing. The specimen is then diluted with the appropriate diluent and assayed for the presence of serum, platelet, or red blood cell antigens.

C. Performance Characteristics

1. Recovery:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Value</th>
<th>Transformed Value</th>
<th>Expected Value</th>
<th>Measured Value</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>15.1</td>
<td>24.1</td>
<td>26.4</td>
<td>28.3</td>
<td>103</td>
</tr>
<tr>
<td>Serum B</td>
<td>15.1</td>
<td>24.1</td>
<td>26.4</td>
<td>28.3</td>
<td>103</td>
</tr>
<tr>
<td>Serum C</td>
<td>15.1</td>
<td>24.1</td>
<td>26.4</td>
<td>28.3</td>
<td>103</td>
</tr>
</tbody>
</table>

2. Dilution:

- Dilution: Three serum samples containing high concentrations of transferrin were serially diluted with the ST-AL-PAK Transferrin SAMPLE DELIGHT SOLUTION and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial Value</th>
<th>Transformed Value</th>
<th>Expected Value</th>
<th>Measured Value</th>
<th>Percent Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>15.1</td>
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<td>28.3</td>
<td>103</td>
</tr>
<tr>
<td>Serum B</td>
<td>15.1</td>
<td>24.1</td>
<td>26.4</td>
<td>28.3</td>
<td>103</td>
</tr>
<tr>
<td>Serum C</td>
<td>15.1</td>
<td>24.1</td>
<td>26.4</td>
<td>28.3</td>
<td>103</td>
</tr>
</tbody>
</table>

3. Precipitation:

The theoretical conditions of solution (within 7% precision) was evaluated in three control samples by 10 replicate measurements.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sample Number</th>
<th>Number of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td>Sample B</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td>Sample C</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

4. Precipitation:

The theoretical conditions of solution (within 7% precision) was evaluated in three control samples by 10 replicate measurements.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sample Number</th>
<th>Number of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td>Sample B</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
<tr>
<td>Sample C</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>10</td>
</tr>
</tbody>
</table>

5. Correlation:

The correlation between serum (a) and hematocrit values (b) on ST-AL-PAK Transferrin was carried out as 239 patient specimens.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum A</td>
<td>20</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Serum B</td>
<td>20</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Serum C</td>
<td>20</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
</tbody>
</table>

6. Specificity:

The following substances were tested for specificity: Cross-reactivity (%) is the percentage of the compound which will be identified as transferrin. If these compounds are present in the specimen and are correctly identified, the first value will be identical with the second percentage.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Cross-reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.0</td>
</tr>
<tr>
<td>IgG</td>
<td>0.0</td>
</tr>
<tr>
<td>IgM</td>
<td>0.0</td>
</tr>
<tr>
<td>IgA</td>
<td>0.0</td>
</tr>
<tr>
<td>IgD</td>
<td>0.0</td>
</tr>
<tr>
<td>IgE</td>
<td>0.0</td>
</tr>
</tbody>
</table>

7. Sensitivity:

The sensitivity of this method is expressed in terms of its ability to detect transferrin in the presence of other serum proteins. Sensitivity is defined as the concentration of transferrin which corresponds to the ratio of transferrin to nonspecific protein which is 0.20.

<table>
<thead>
<tr>
<th>Product</th>
<th>Sensitivity (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.0</td>
</tr>
<tr>
<td>IgG</td>
<td>0.0</td>
</tr>
<tr>
<td>IgM</td>
<td>0.0</td>
</tr>
<tr>
<td>IgA</td>
<td>0.0</td>
</tr>
<tr>
<td>IgD</td>
<td>0.0</td>
</tr>
<tr>
<td>IgE</td>
<td>0.0</td>
</tr>
</tbody>
</table>

8. Specificity:

The theoretical conditions of solution (within 7% precision) was evaluated in three control samples by 10 replicate measurements.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sample Number</th>
<th>Number of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Correlation (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Sample B</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
<tr>
<td>Sample C</td>
<td>20</td>
<td>3</td>
<td>2.0</td>
<td>0.2</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Reference Range:

- Males: 380-1000 mg/dL
- Females: 280-800 mg/dL

Conversion Factors:

- Census: 1 microgram/litre = 1 ng/ml
- Conversion to US units: 1 microgram/litre = 0.001973 ng/ml

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REFERENCES


