Chapter one

Introduction

1-1-Introduction:-

Infertility is an important medical and social problem in the world. In about 60 per cent of all couples experiencing infertility, male factor is responsible in about 40 per cent of the couples1. It is now generally accepted that the treatment of malefactor infertility is equally as important as thetreatment of the female factor(Emokpae et al,2005)

Inferyility **:**Is the inability to conceive after one year of un protected intercourse it is estimated that 25% of couples will experience an episode of infertility during their reproductive life. (Shannon etal .,2006) Primary Infertilityrefer to couple or patient who have had no previous successful pregnancy Secondary infertility encompasses patient who have previously conceived but are currently unable to conceive (Shannon etal .,2006) The World Health Organization (WHO) estimates that 60 to 80 million couples world wide currently suffer from infertility(Agrawal etal .,2013)

According to the standard protocol, infertility evaluation usually identifies different causes, including male infertility (30%), female infertility (35%), the combination of both (20%), and finally unexplained or “idiopathic” infertility (15%)(Agrawal etal .,2013)

Male infertility:Refers to a male's inability to cause pregnancy in a fertile female. In humans it accounts for 40-50% of infertility. There are many biological and other causes of infertility, including some that medical intervention can treat. (Makar etal .,2002)Estimates from 1997 suggest that worldwide "between three and seven per cent of all couples or women have an unresolved problem of infertility. Many more couples, however, experience involuntary childlessness for at least one year: estimates range from 12% to 28%.(Himmel et al.,1997) About 20-30% of infertility cases

are due to male infertility, 20-35% are due to female infertility, and 25-40% are due to combined problems in both parts.(ART fact sheet ,July 2014)In 10-20% of cases, no cause is found. The most common cause of female infertility is ovulatory problems which generally manifest themselves by sparse or absent menstrual periods.(National Health Service .,2014) Male infertility is most commonly due to deficiencies in the semen, and semen quality is used as a surrogate measure of male fecundity.(Cooper et al .,2010)

1-2-Justification:-

Infertility is a medical problem that affects more than 80 million people worldwide (Ochsendorf, 2006). Infertility is one of the indicators of lacking of reproductive health . It affects a vast proportion of the world's young population (10–15%) (Sakar et al., 2008). In sudan there few published studies that regarde the causes of male infertility and it is complication . This study was done to evaluate this problem in Sudan by determine the major causes of infertility in Sudanese males based onthe clinical and laboratory findings.

1.3.Objectives:-

1.3.1General objective:-

to observe the pattern of testosterone hormonal abnormalities in infertile Sudanese males with abnormal semen analysis

1.3.2Specific objectives:-

-To perform semen analysis in patients attending fertility clinic.

- To estimate testosterone levels in patients attending fertility clinic.

- To compare between testosterone level in types of abnormal semen analysis.

- To correlate between testosterone level and duration of infertility and age.

Chapter two

Literature review

2- Literature review:-

2-1-Male Reproductive biology;

The male reproductive anatomy include the penis , two testes ,and asystem of exocrine gland whose secretion form the seminal fluid .the exocrine glands consist of the two bulbouretheral glands ,two seminal vesicles and the prostate .the bilateral duct that connect this system and transport the sperm and seminal fluids are epididymis ,the vas deferens ,and the ejaculatory duct (Shannon et al.,2006**)**

The testes produce sperm and the hormones that regulate male sexual life; both functions are controlled by the hypothalamic-pituitary system. The pathways forhormone formation and the regulatory control of the testes are similar to those in the ovaries and the adrenal glandsTestes contain three principal cell types:

1. Germ cells, derived from primitive ectodermal cells of the inner cell mass (initially identifiable in the yolk sac).

2. Supporting cells, derived from the coelomic epithelium of the gonadal ridge that differentiate into the Sertoli cells in the testis (or granulosa cells in the ovary).

3. Stromal (interstitial) cells, derived from the mesenchyme of the gonadal ridge that differentiate into Leydig cells.(reed et al ,.2003)

The testes contain a network of tubules for the production and transport of sperm to the excretory-ejaculatory ducts and a system of interstitial or Leydig cells thatsynthesize androgens.The functional complexity of the tissue is illustrated . Spermatogenic tubules are composed of germ cells and Sertoli cells.Tight junctions between the Sertoli cells separate the

spermatogonia from the primary spermatocytes and form a diffusion barrier that divides the testis into twofunctional compartments*basal* and *adluminal*. The barrier between these two compartments has limited permeability to macromolecules, analogous to the blood-brainbarrier and other epithelial barriers. The basal compartment consists of the Leydig cells, the boundary tissue of the tubule including peritubular myoid cells, and theouter layers of the spermatogenic tubules that contain the spermatogonia. The adluminal compartment consists of the inner two thirds of the tubules, including primaryspermatocytes and cells in more advanced stages of spermatogenesis.(reed et al .,2003)

2.1.1.PHYSIOLOGY OF TESTICULAR FUNCTION

Hypothalamic-Pituitary-Testicular Axis:-

The hypothalamus, located in the brain, generates a hormonecalled gonadotropin-releasing hormone (GnRH) ina pulsatile fashion. GnRH is released into the portal hypophysialsystem that, in turn, determines the productionof LH and FSH from the pituitary gland. Impaired pulsegeneration of GnRH leads to inadequate production of LHand FSH, resulting in **hypogonadism**. The first, andrate-limiting, step in the testicular steroidogenesis is theconversion of cholesterol to pregnenolone.which catalyzes protein phosphorylation.This latter step induces testosterone synthesis. The testicularsteroidogenesis pathway is similar to the pathway inthe adrenal cortex and they share the same enzymatic systems.(reed et al .,2003)

2.2 Testostrone :-

Androgen are group of c19 steroid that cause masculization of genital tract and development and mainntaince of male secondary sex characreries testosterone is the major androgensecreted by leydig cell.(testosterone direct effect some aspect of secondary sex development such as libido ,deeping of voice and increasing of muscles italso has indirect effect oin tissue with high 5- reductase activity other androgen include dehydroepiandrosterone , dhydroepiandrosterone sulfate,androstenedione and androstenediol these steroids can be meiabolized in to testosterone in target tissue(Shannon et al., 2006)

Testosterone is the principal **androgen** hormone inthe blood. It is largely bound, with 2%–3% free. About50% of testosterone is bound to albumin and about 45% isbound to sex hormone–binding globulin (SHBG).Theconcentration of binding protein determines the level oftotal testosterone but not the free testosterone levels duringlaboratory estimation. Testosterone and **inhibin** arethe two hormones secreted by the testes that provide feedbackcontrol to the hypothalamus and pituitary(bishop etal .,2010)

2.2.1. Testostrone production:

The pathways of testosterone synthesis are illustrated in Figure 18-6 (Figure Not Available) and Figure 2-1 . As stated earlier, the precursor steroid cholesterol can either be synthesized de novo or derived from the plasma pool by receptor-mediated endocytosis of low-density lipoprotein (LDL),

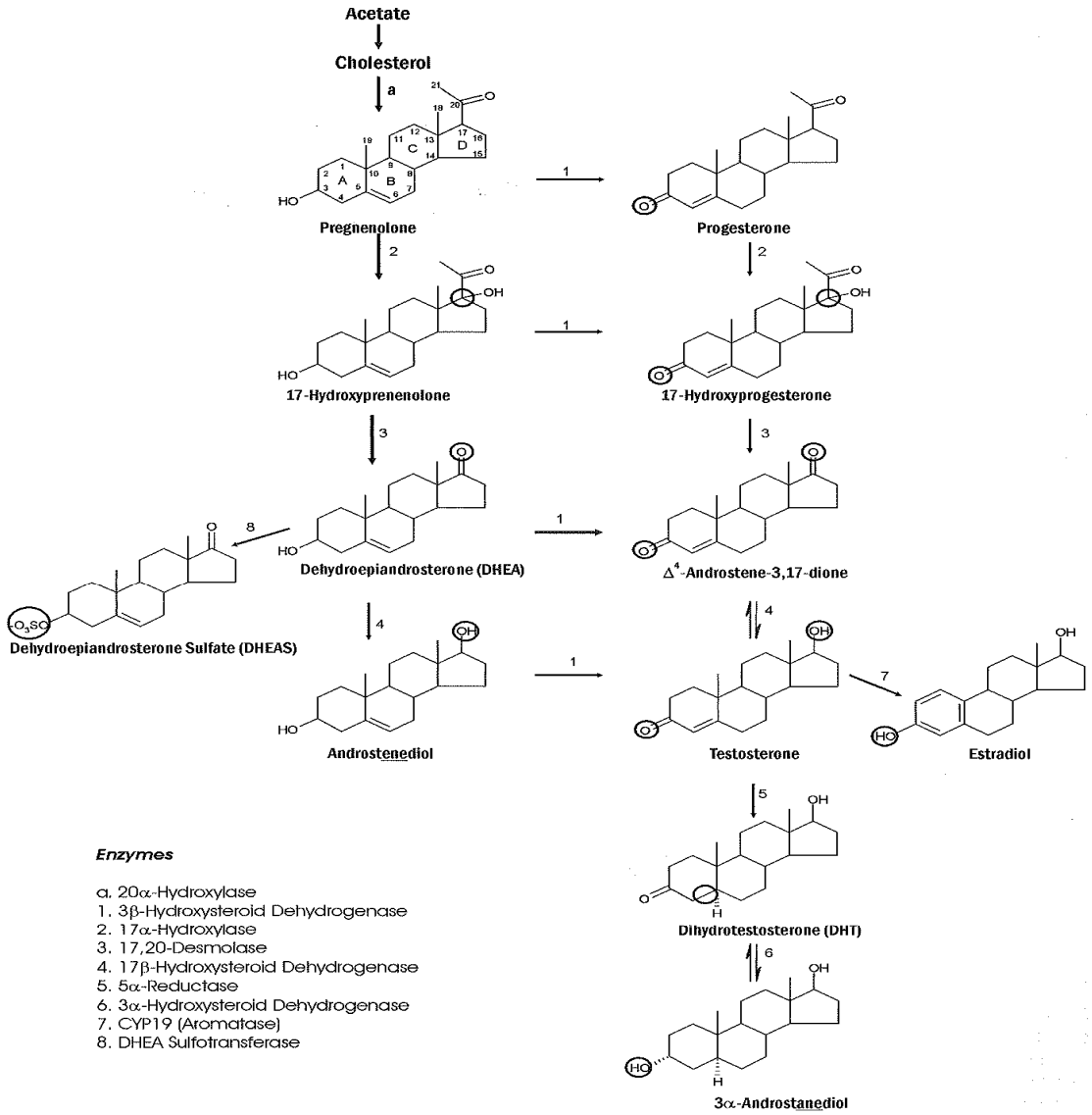


Figure 53-4 Biosynthesis of androgens (adrenal glands and testis). The *heavy arrows* indicate the preferred pathway. The *enclosed area* represents the site of chemical change.

and both sources are important in the human Leydig cell(Shannon et al .,2006)

The conversion of cholesterol to testosterone involves several enzymatic reactions. The cholesterol side chain is cleaved in two steps to reduce the size from 27 to 19carbons, and the A ring of the steroid is oxidized to the 4

,3-keto configuration. The initial reaction in the process involves the transfer of cholesterol by the StARprotein to the inner mitochondrial membrane, where it undergoes side-chain cleavage by CYP11A1 to form pregnenolone. The subsequent conversion ofpregnenolone to testosterone involves both random and ordered enzymatic reactions.For the second side-chain cleavage to take place, 17-hydroxylation and cleavage of the 17,20 bond through CYP17 must occur before the reduction of the 17-ketoneby 17-hydroxysteroid dehydrogenase III (17-HSD-III). In contrast, oxidation of the A ring by 3-HSD-II can take place at any stage in the processThe rate-limiting reaction in testosterone synthesis under most circumstances is the transport of cholesterol to the inner mitochondrial membrane. As mentionedearlier, the acute administration of LH stimulates testosterone synthesis by enhancing the delivery of cholesterol to the mitochondria through StAR protein [26] forside-chain cleavage by CYP11A1.[67] In the steady state, LH also stimulates testosterone synthesis by enhancing the formation of CYP11A1 and other enzymes in thepathway(reed et al., 2003)

2.2.2 Physiologic Actions of Testosterone:

Prenatal Development

Early in development, embryos have primordial componentsof the genital tracts of both sexes. The primitivegonads become distinguishable at about the seventhweek of embryonic stage. Both chorionic gonadotropinsand fetal LH stimulate production of testosterone by thefetal Leydig cells. Exposure of testosterone to theWolffian duct leads to differentiation of the various componentsof the male genital tract. **Sertoli cells** producemüllerian regression factor, which aids in regression ofthe female primordial genital tract. The scrotal skin isrich in 5_-reductase, which converts testosterone to

DHT. Fetal exposure to drugs that block this hormoneleads to feminization of the male fetus.(Bishop et al., 2010)

Postnatal Development

Testicular function is reactivated during puberty after aperiod of quiescence to produce testosterone that resultsin development of secondary sex hair (face, chest, axilla,and pubis), enhanced linear skeletal growth, development of internal and external genitalia, increased upper bodymusculature, and development of larynx and vocal cordswith deepening of the voice.6–8 Possible mood changesand aggression are undesired effects that may occur during puberty. The linear growth effects of testosteroneare finite, with epiphysial closure when genetically determinedheight is achieved. Hypogonadism during pubertyleads to imprecise closure of growth plates, leading to excessiveheight, long limbs, and disproportionate upper and lower body segments. Male secondary sexual characteristicscan be staged by a system of development devised byMarshall and Tanner.(Bishop et al., 2010)

Effect on Spermatogenesis

Stimulation of Leydig cells induces production of testosterone.Testosterone, acting with FSH, has paracrineeffects on the seminiferous and Sertoli cells inducingspermatogenesis. Exogenous overuse or abuse of testosterone,such as occurs with some athletes, will reduce thehigh intratesticular concentration of testosterone, leadingto reduction of sperm production.(Bishop et al., 2010)

Effect on Secondary Sexual Effects

Testosterone has growth-promoting effects on various targettissues. The secondary sex characteristics that developduring puberty are maintained into late adulthood bytestosterone.8 The prostate enlarges progressively during adulthood, while exposure of scalp hair results in regressionof the hair follicles (temporal hairline recession).Loss of secondary sexual characteristics should promptevaluation for hypogonadism because, among other effects,low testosterone levels lead to loss of bone mass anddevelopment of osteoporosis in males at any age.(Bishop et al., 2010)

2.2.3 Testosterone metabolism:-

testosterone serves as a circulating precursor or prohormone for the ormation of two types of active metabolites, which in turn mediate many androgen actions (see Fig. 18-6) (Figure Not Available) . On the one hand, testosterone can undergo irreversible 5-reduction to steroids such as dihydrotestosterone,which are responsible for many aspects of male sexual development and virilization. Dihydrotestosterone in turn can be furthermetabolized to 17-ketosteroids and polar derivatives excreted in urine. Alternatively, circulating androgens containing a 4 ,3-keto configuration can be converted toestrogens in the extraglandular tissues of both sexes. Estrogens in some instances act in concert with androgens to influence physiologic processes and in others

exert effects that are independent of androgens. Thus, the physiologic actions of testosterone are the result of the combined effects of testosterone itself plusthose of estrogen and androgen metabolites of testosterone.(Reed et al., 2006)

TOTAL TESTOSTERONE LEVELS		
SEX	**ng/dl**	**Nmol/l**
Females	6 – 86	0.5-2.4
Males	270 – 1100	9-34
Conversion factor: 1 ng/ml = 3.47 nmol/l		

2.2.4 Low testosterone:-

The National Institutes of Health includes the following as possible symptoms of low testosterone:

-Reduced sex drive

-Erectile dysfunction or impotence

-Increased breast size

-Lowered sperm count

-Hot flashes

-Depression, irritability and inability to concentrate

-Shrunken and softened testes

-Loss of muscle mass or hair

(Rachel Rettner, 2014)-Bones becoming prone to fracture

2.2.5 High testosterone:-

High testosterone levels can cause problems in women, including irregular menstrual cycles, increases in body hair and acne, and a deepening of the voice. Women with polycystic ovarian syndrome have high levels of male hormones, including testosterone, which can be a cause of infertility.(Rachel Rettner, 2014)

2.3.Semen analysis:

2.3.1Physiology:

Semen is composed of four fractions that are contributed bythe testes, ***epididymis, seminal vessels, prostate,*** and ***bulbourethralglands*** Each fraction differs in its composition,and the mixing of all four fractions during specimen The testes contain the ***seminiferous tubules.*** Germ cellsfor the production of ***spermatozoa*** are located in the epithelialcells of the seminiferous tubules. Specialized Sertoli cellsprovide support and nutrients for the germ cells as theyundergo mitosis and meiosis (spermatogenesis). When spermatogenesisis complete, the immature sperm (nonmotile)enter the epididymis. In the epididymis, the sperm matureand develop flagella. They remain stored in the epididymisuntil ejaculation. At that time they are propelled through theductus deferens (vas deferens) to the ejaculatory ducts.

The ejaculatory ducts receive both the sperm from theductus deferens and fluid from the seminal vesicles. The seminalvesicles produce the majority of the fluid present insemen (60% to 70%). The fluid contains a high concentrationof fructose. Spermatozoa metabolize the fructose for theenergy needed for the flagella to propel them through thefemale reproductive tract. In the absence of fructose, spermdo not display motility in the semen analysis .The muscular prostate gland, located just below the bladder,surrounds the upper urethra and aids in propelling thesperm through the urethra by contractions during ejaculation.Approximately 20% to 30% of the semen volume is acidicfluid produced by the prostate gland. The acidic fluid containshigh concentrations of acid phosphatase, citric acid,zinc, and

proteolytic enzymes responsible for both the coagulationand ***liquefaction*** of the semen following ejaculation.

The bulbourethral glands, located below the prostate,contribute about 5% of the fluid volume in the form of athick, alkaline mucus that helps to neutralize acidity from theprostate secretions and the vagina. It is important for sementoo be alkaline to neutralize the vaginal acidity present as aresult of normal bacterial vaginal flora. Without this neutralization,sperm motility would be diminished.(susan et al., 2008)

2.3.2 Appearance:

Normal semen has a gray-white color, appears translucent,and has a characteristic musty odor. Increased white turbidityindicates the presence of white blood cells (WBCs) and infectionwithin the reproductive tract. If required, specimen culturingis performed prior to continuing with the semen analysis. During the microscopic examination, WBCs must bedifferentiated from immature sperm ***(spermatids).*** The leukocyteesterase reagent strip test may be useful to screen for thepresence of WBCs.2 Varying amounts of red coloration areassociated with the presence of red blood cells (RBCs) and are abnormal. Yellow coloration may be caused by urine contamination,

specimen collection following prolonged abstinence,and medications. Urine is toxic to sperm, thereby affecting theevaluation of motility.(susan et al., 2008)

2.3.3 Liquefaction:

A fresh semen specimen is clotted and should liquefy within30 to 60 minutes after collection; therefore, recording thetime of collection is essential for evaluation of semen liquefaction.Analysis of the specimen

cannot begin until after liquefactionhas occurred. If after 2 hours the specimen has notliquified, proteolytic enzymes such as alpha-chymotrypsin may be added to allow the rest of the analysis to be performed.

Failure of liquefaction to occur may be caused by adeficiency in prostatic enzymes and should be reported.(susan et al., 2008)

2.3.4 Volume:

Normal semen volume ranges between 2 and 5 mL. It can bemeasured by pouring the specimen into a clean graduatedcylinder calibrated in 0.1-mL increments. Increased volumemay be seen following periods of extended abstinence.Decreased volume is more frequently associated with ***infertil-***

ViscositySpecimen viscosity refers to the consistency of the fluid and may be related to specimen liquefaction. Incompletely liquefiedspecimens are clumped and highly viscous. The normalsemen specimen should be easily drawn into a pipette andform droplets that do not appear clumped or stringy whendischarged from the pipette. Normal droplets form a thin thread when released from the pipette. Droplets with threadslonger that 2 centimeters are considered highly viscous. Ratingsof 0 (watery) to 4 (gel-like) can be assigned to the viscosityreport.3 Viscosity can also be reported as low, normal,and high. Increased viscosity and incomplete liquefaction impede sperm motility.(susan et al., 2008)

2.3.5 pH:

The normal pH of semen is alkaline with a range of 7.2 to 8.0 Increased pH is indicative of infection within the reproductivetract. A decreased pH is associated with increased prostaticfluid. Semen for pH testing can be applied

to the pH pad of aurinalysis reagent strip and the color compared with the manufacturer'schart. Dedicated pH testing paper also can be used.(susan et al., 2008)

2.3.6 Sperm Concentration/Count:

Even though fertilization is accomplished by one spermatozoon,the actual number of sperm present in a semen specimenis a valid measurement of fertility. Normal values forsperm concentration are commonly listed as greater than 20million sperm per milliliter, with concentrations between 10 and 20 million per milliliter considered borderline.(susan et al., 2008)

2.3.7 Sperm Motility:

The presence of sperm capable of forward, progressive movementis critical for fertility, because once presented to thecervix, the sperm must propel themselves through the cervicalmucosa to the uterus, fallopian tubes, and ovum. Traditionally,clinical laboratory reporting of sperm motility has been a subjective evaluation performed by examining anundiluted specimen and determining the percentage of motilesperm and the quality of the motilityIn recent years, instrumentation capable of performingcomputer-assisted semen analysis **(CASA)** has been developed.CASA provides objective determination of both spermvelocity and trajectory (direction of motion). Sperm concentrationand morphology are also included in the analysis.Currently, CASA instrumentation is found primarily in laboratories that specialize in andrology and perform a high volumeof semen analysis.(susan et al., 2008)

2.3.8Sperm Morphology:

Just as the presence of a normal number of sperm that are nonmotileproduces infertility, the presence of sperm that are morphologicallyincapable of fertilization also results in infertility.Sperm morphology is evaluated with respect to thestructure of the head, neckpiece, midpiece, and tail. Abnormalitiesin head morphology are associated with poor ovum

penetration, whereas neckpiece, midpiece, and tail abnormalitiesaffect motility.The normal sperm has an oval-shaped head approximately5 _m long and 3 _m wide and a long, flagellar tailapproximately 45 _m long (Fig. 11-3). Critical to ovum penetrationis the enzyme-containing ***acrosomal cap*** located atthe tip of the head..(susan et al., 2008)

2.3.9 Abnormal semen:

-Oligospermia or Oligozoospermia - decreased number of spermatozoa in semen

-Aspermia - complete lack of semen

-Hypospermia - reduced seminal volume

-Azoospermia - absence of sperm cells in semen

-Teratospermia - increase in sperm with abnormal morphology

-Asthenozoospermia - reduced sperm motility

2.4. Male Infertility:

Male infertility refers to a male's inability to cause pregnancy in a fertile female. In humans it accounts for 40-50% of infertility.(Men's Health - Male Factor Infertility, 2007) It affects approximately 7% of all men.(Lotti, F.; Maggi, M, 2014). Male infertility is commonly due to deficiencies in the

semen, and semen quality is used as a surrogate measure of male fecundity.(Cooper TG,2009)

2.4.1. Causes of Male Infertility:-

Pre-testicular causes:

-Hypogonadotropichypogonadism due to various causes.

-Obesity increases the risk of hypogonadotropichypogonadism.(Teerds et al., 2011)

-Undiagnosed and untreated coeliac disease (CD). Coeliac men may have reversible infertility.(Freeman., 2010)

-Drugs, alcohol

-Strenuous riding (bicycle riding, horseback riding).(Leibovitch etal ., 2005)

-Medications, including those that affect spermatogenesis such as chemotherapy, anabolic steroids, cimetidine, spironolactone.

-Genetic abnormalities such as a Robertsonian translocation

-Tobacco smoking.

-DNA damage.

Testicular factors:

-Testicular factors refer to conditions where the testes produce semen of low quantity and/or poor quality despite adequate hormonal support and include:

-Varicocele, that is present in 15% of normal men and in about 40% of infertile men. It probably causes up to 35% of primary infertility and 69-81% of secondary infertility.(Kupis et al ., 2015)

-Age.

-Genetic defects on the Y chromosome

-Abnormal set of chromosomes.

-Radiation therapy to a testis decreases its function, but infertility can efficiently be avoided by avoiding radiation to both testes.(Gutfeld et al., 2007)

Post testicular cause:

Post-testicular factors decrease male fertility due to conditions that affect the male genital system after testicular sperm production and include defects of the genital tract as well as problems in ejaculation:

-Vas deferens obstruction

-Lack of Vas deferens, often related to genetic markers for Cystic Fibrosis

-Infection, e.g. prostatitis

-Retrograde ejaculation

-Ejaculatory duct obstruction

-Hypospadias

-Impotence (shanon etal ., 2006)

2.4.2. Diagnosis of Male Infertility:-

. Medical history:-

The history should include prior testicular or penile insults (torsion, cryptorchidism, trauma), infections (mumps orchitis, epididymitis), environmental factors, excessive heat, radiation, medications, and drug use (anabolic steroids, alcohol, smoking).

Sexual habits, frequency and timing of intercourse, use of lubricants, and each partner's previous fertility experiences are important.

Physical examination:-

Usually, the patient disrobes completely and puts on a gown. The physician, physician assistant, or nurse practitioner will perform a thorough examination of the penis, scrotum, testicles, vas deferens, spermatic cords, ejaculatory ducts, urethra, urinary bladder, anus and rectum.

Sperm sample:-

The volume of the semen sample, approximate number of total sperm cells, sperm motility/forward progression, and % of sperm with normal morphology are measured. This is the most common type of fertility testing. (Hargreave TB et al, 1986) Semen deficiencies are often labeled as follows:

Blood sample:

Common hormonal test include determination of FSH and testosterone levels. A blood sample can reveal genetic causes of infertility, e.g. Klinefelter syndrome, a Y chromosome microdeletion, or cystic fibrosis.

Ultrasonography:

Ultrasonography of the scrotum is useful when there is a suspicion of some particular diseases. It may detect signs of testicular dysgenesis, which is often related to an impaired spermatogenesis and to a higher risk of testicular cancer. (Hargreave et al ., 1986)

ChapterThree

Material and Method

3- Material and Method :-

3-1-Study Design :

This study designed as analytical cross-sectional study .

3-2- Study Area :

This study was carried in Khartoum state

3-3- Study Population :

Atotal of 100 cases of Infertile men in Khartoum state

3-4- Study Period:

The study was carried between june- october . 2016

3-5- Sampling :

3-5-1- Inclusion Criteria:

Infertile men in Khartoum state

3-5-2-Exclusion Criteria :

- Female factor infertility, female factors-tubal factor, urogenital tract anomalies and obvious organic lesion in pelvis

- infertile male with normal semen analysis ,smokers and chronic disease. - infertile men unwilling to participate or sign the informed consent.

3-6- Collection:

venous blood collected from each participant men and placed in plain tube centrifuged for 10 min at 3500 rpm. The serum which obtained used in determination of Testostrone hormone level .

3-7- Methods:

3-7-1 Semen analysis:

All samples analyzied using CASA© for semen Analysis .The CASA was parameterized as follows: frames acquired – 100, frame rate – 60Hz, minimum contrast – 70, minimum cell size (SIZE) – 8 pixels, minimum staticcontrast – 30, straightness threshold – 80%, average pathway velocity (VAP) cutoff – 25 μm/s, straight line velocity (VSL) cutoff – 20 μm/s, cell intensity – 80, static elongation –11 to 80, magnification – 1.89. 25 μL of post-thaw semen was diluted into 50-100 μL of Tris (formulated for bull semen), and 5 μL of this diluted semen was loaded into a pre- warmed dual chamber slide, and then loaded into the CASA for analysis.

The CASA provided two files with results for each of the infertile male, one was asummary file (DBS) in which each of the male had overall means for each of theparameters and the second (DBT) was a file that had every live cell tracked and theparameters for each cell. The seminal parameters were similar across the two files and consist of VAP, VSL, curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), linearity (LIN), elongation, SIZE, size in pixels, and intensity. The DBS file stores other variables such as total concentration, percent alive, percent motile and percent progressively motile as well.

For this study the primary CASA outcomes of interest were percent motility.

3-7-2 Testostrone assay :

According to the manufacture, serum levels of Testosterone were measured by using TOSOH Bioscience automated immunoassay analyzer

AIA-360. Competitive immunoenzymometric assay was used, testosterone in the test sample compete with enzyme-labeled testosterone for limited number of binding sites on these hormones specific antibodies immobilized on magnetic beads, then magnetic beads were washed to remove unbound enzyme-labeled testosterone, incubated with flurogenic substrate 4-methylumbelliferyl phosphate (4 MUP). The rate of fluorescence produced by the reaction was measured fluorometricly at 365 and 445 nm. The amount of testosterone that binds to the beads is inversely proportional to the testosterone concentration in the sample

3-8- Ethical considerations :

Verbal Informed consent was obtained from all the participantsat the start of the study.

3-9- *Statistical analysis :*

Data were analyzed by computer program (SPSS)Pearson correlation test T. test were used for the calculation. P≤0.05 was considered significant

3-10-Quality Control :

Each assay includes control sample with each calibration curve and is estimated as sameas sample

Chapter four

Result

4-Results:-

This was analytical a cross-sectional study conducted in Khartoum 2016. It included 100 infertile males presenting for infertility evaluation, All of them presented for Semen analysis . After analysis among 100 infertile men 37 infertile men, were Oligosthenozoospermia (37%) while 27 infertile men were Athesnozoospermia(27%) ,22 infertile men were Azoospermia (22%), 15 infertile men were Oligozoospermia (14%). Oligosthenozoospermia was the commonest motility/morphology abnormality occurring in infertile males.

The mean serum Testostrone hormone level in infertile men withOligosthenozoospermia were (18.8±8.55nmol/L) **,**withAthesnozoospermia were (17.25±6.56 nmol/L), with Azoospermia were (16.40±6.05 nmol/L) , with Oligozoospermia (18.53±5.44 nmol/L) the Testostrone hormone level was higher in infertile men with Oligosthenozoospermia and The differences for the mean testosterone levelsbetween the infertile men with

Oligosthenozoospermia, azoospermia oligozoospermia and Athesnozoospermia were statistically highly significant (p=0.000)in all infertile men when compared with normal range (9 -37 nmol/L)the mean testosterone hormone levelsin all group were decreased.

There was weak negative significant correlation between Teststrone hormone level and age . when there were correlated in infertile Sudanese men Pearson correlation (r= -0.217) and p.value (0.03)

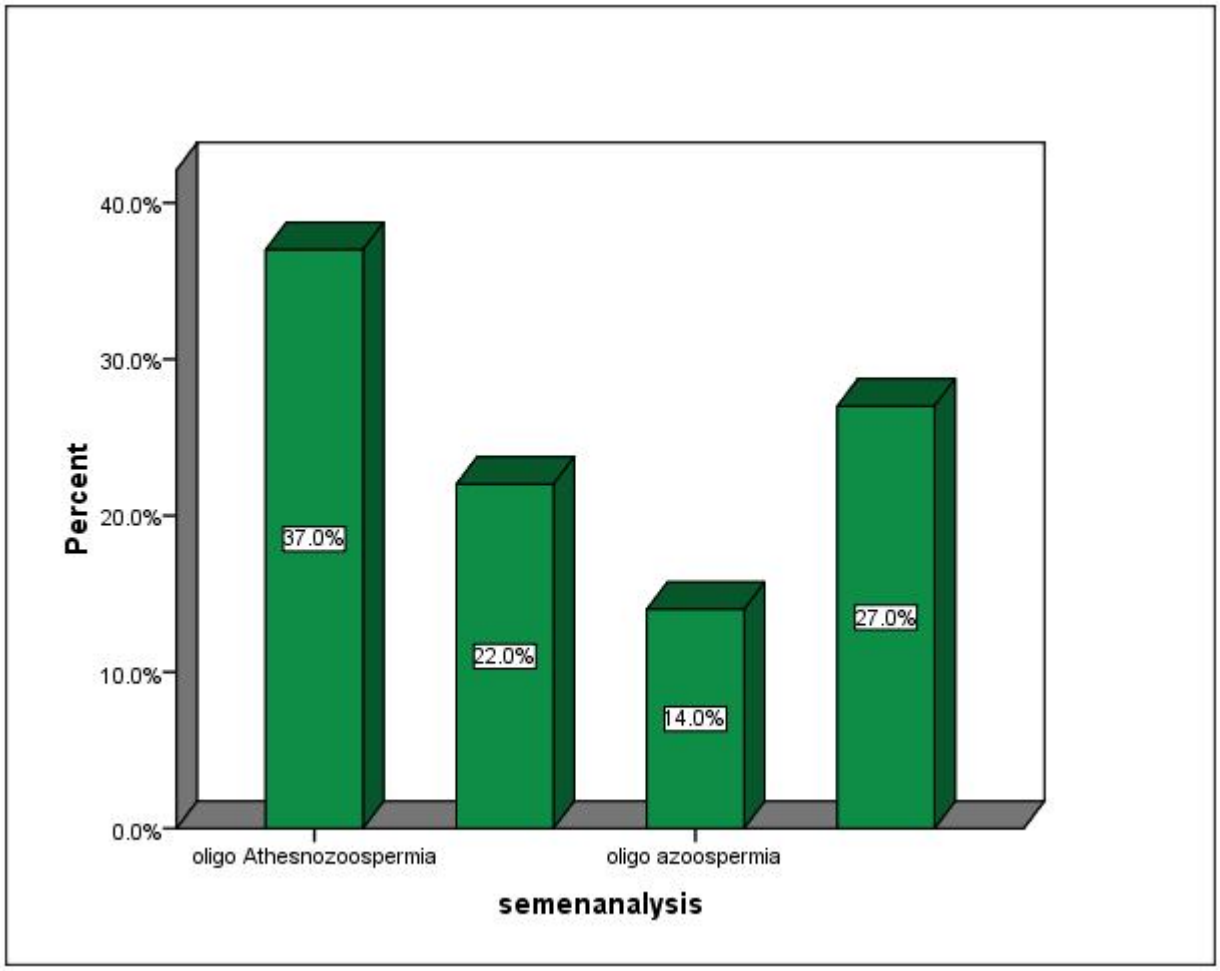
There was weak negative correlation insignificant between Teststrone hormone level and duration of infertility when there were correlated in infertile Sudanese men Pearson correlation (r=-0.098) and p.value (0. 331)

Table 4-1 Mean and SD of Ttestosterone in Infertile men with abnormal semen analysis and it comparison with normal range

Abnormal semen analysis	Mean± SD	Mean± SD	P-value
Asthenozoospermia	17.25±6.56010	23 ± 7	0.000
Oligasthenozoospermia	18.78±8.55646	23 ± 7	0.000
Azoospermia	16.40±6.05529	23 ± 7	0.000
Oligozoospermia	18.53 ± 5.4484	23 ± 7	0.000

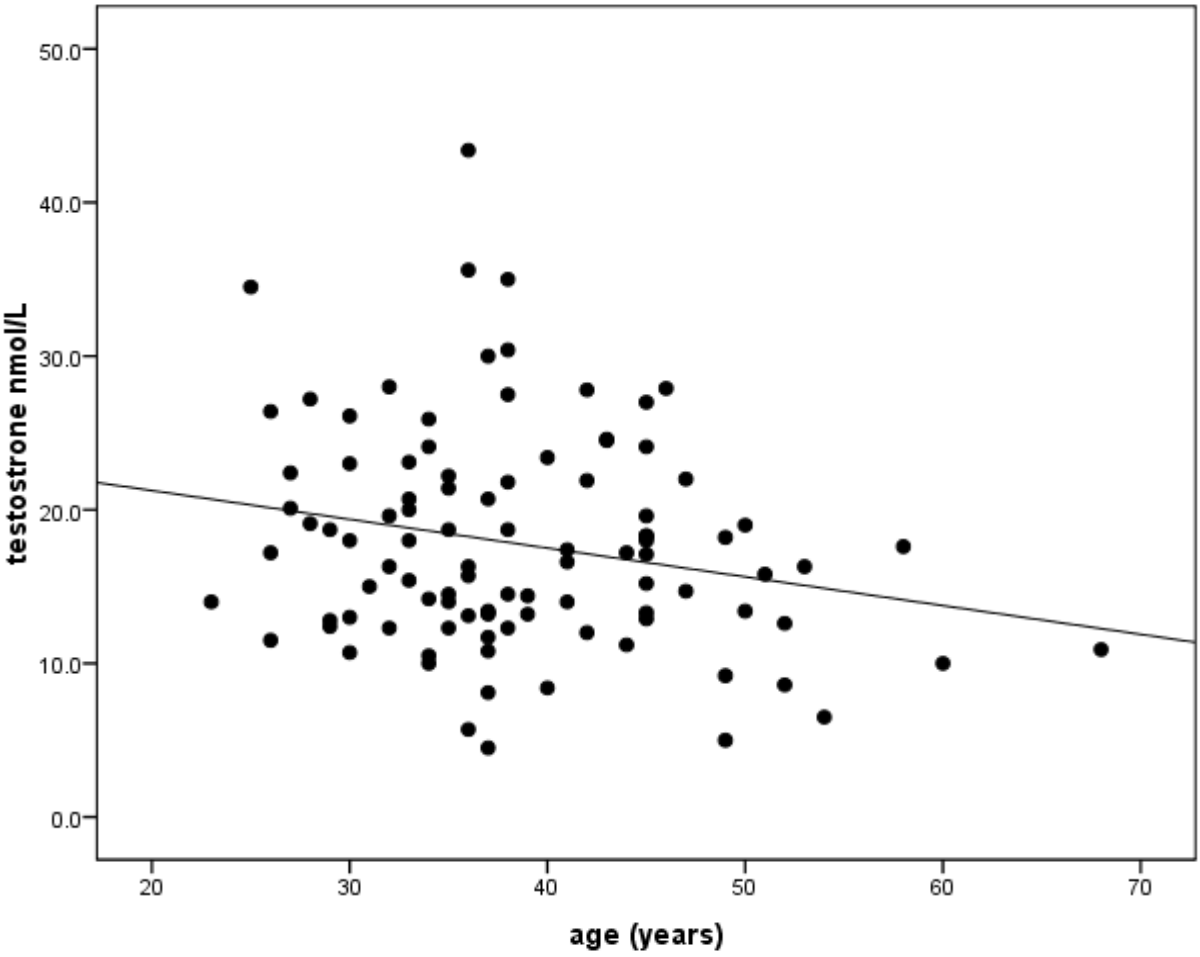
the Testostrone hormone level was higher in infertile men with Oligosthenozoospermia and was statistically highly significant (p=0.000) decresed in all infertile men when compared with normal range (23 ± 7nmol/L)

Figure 4.3(Showing percentage of abnormal Semen analysis in infertile Sudanese men)



Among 100 infertile men 37% were Oligosthenozoospermia while 27%, 22%, 14% were Athesnozoospermia, Azoospermia, Oligozoospermia and respectively

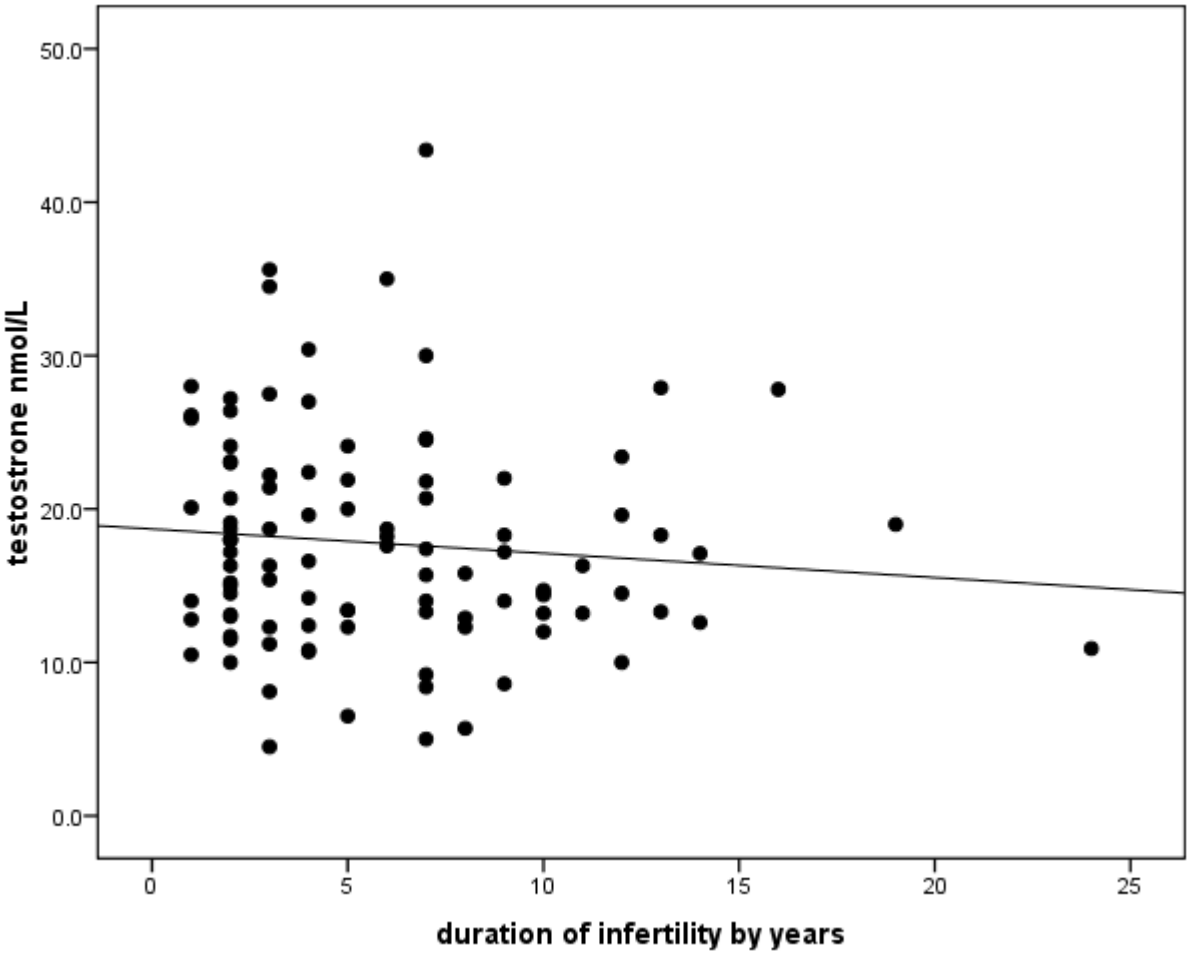
Figure.4-1 (Showing correlation between Testosterone nmol/L and age)



There was weak negative significant correlation between Teststrone hormone level and age . when there were correlated in infertile Sudanese men(r= -0.217) and p.value (0.03)

Figure.4-2 (Showing correlation between Testosterone nmol/L and duration of infertility per year

There was weak negative correlation insignificant between Teststrone



hormone level and duration of infertility when there were correlated in infertile Sudanese men(r=-0.098) and p.value (0. 331)

Chapter five

Discussion

Conclusion

Reccomrndation

5-1. discussion:

Testosterone are primeregulators of germ cell development. Thequantitative production of spermatozoa generally require the presence F SH, LH a n d testosterone

Our results are in accordance with earlier studieswhich showed the level of testosterone is decreased in all group of abnormal semen .

In the study under discussion Testosterone levels showed, as well significant decrease in Oligosthenozoospermia ,azoospermic and oligozoospermic males p.value (0.00) similar observation has been recorded in (Mohammad et al 2007) ,(Mohaned , et al, 2016),(Bassim , et al ,2010) .

In the present study In cases of asthenozoospermia,Testosterone levels showed significant decrease the p.value (0.00) similar findings has been recorded in (Mohammad et al 2007) in (Bassim , et al ,2010)it showed in significant increase Testosterone levelsIn cases of asthenozoospermia.

In this study The results showed negative weak correlation insignificant between duration of infertility and testosterone level with (R=-0.097 and *p*-value 0.338).

Many studies have now demonstrated that as men aging, their serum testosterone concentrations fall.. In this study significant negative correlation was found between serum testosterone and age of patients (R= -0.217, *p*-value 0.03) this finding was in agreement with recent studies which proposed this result due to fact that the incidence of testosterone deficiency increased with age due to decline in testosterone production .

5-2 Conclusion :-

The study has concluded that, testosterone levels are lower in Oligosthenozoospermia ,azoospermic , oligozoospermi and asthenozoospermia males in comparison with normal range, age and duration of infertility are correlated with testosterone deficiency, thus could affect spermatogenesis, which may result in infertility .

5-3 Recommendation :-

-Other studies recommended to investigate other causes of infertility in Sudanese infertile men , include a larger number of participant , conducted as prospective study,include other hormones such as FSH ,LH DEHAS and include other variable as BMI ,treatment and smooking habits .

- Based on these observations *recommended to* all patients of infertility serum testosterone should be estimated.

Chapter Sex Referencesand appendix

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