

1 Introduction – Rationale and Objectives

1.1 Introduction
Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year’ (Jungwirth and Diemer., 2010).
Reasons for a reduction in male infertility: congenital factors (cryptorchidism and testicular dysgenesis, congenital absence of the vas deferens); acquired urogenital abnormalities (obstructions, testicular torsion, testicular tumor, orchitis), urogenital tract infections, increased scrotal temperature (e.g. as a consequence of varicocele), Endocrine disturbances, genetic abnormalities, immunological factors, systemic Diseases, exogenous factors (medications, toxins, irradiation, lifestyle factors). Idiopathic (40-50% of cases) (Jungwirth and Diemer., 2010).
Abnormal semen was classified as follows: Oligozoospermia: Spermatozoa concentration lower than 15 million/ml, asthenozoospermia: Proportion of progressively motile spermatozoa less than 32%, Teratozoospermia: Proportion of spermatozoa with normal morphology less than <4%, Oligoasthenoteratozoospermia: Co-presence of above described three defects in terms of number, motility and morphology indicates severe male infertility. Azoospermia: Absence of spermatozoa in ejaculate, Criptozoospermia: Presence of spermatozoa after high speed centrifuge, which is absent in fresh sample, Necrozoospermia: Less vital more immotile spermatozoon in ejaculate. Leukospermia: Presence of leukocyte in ejaculate in an amount greater than reference values and Aspermia: Absence of ejaculate (Omer et al., 2012).
Spermatogenesis is primarily controlled by the gonadotropins: Follicle stimulating hormone and Luteinizing hormone. The Sertoli cells possess specific high-affinity FSH receptors. FSH is necessary for the maintenance of quantitatively normal sperm production and is
particularly important for initiating spermatogenesis in pubertal males and reinitiating spermatogenesis in men whose germinal epithelium has regressed after hypophysectomy. *qualitative* sperm production can be achieved by replacement of either FSH or LH alone. However, both FSH and LH are necessary to maintain *quantitative* normal spermatogenesis in humans (Dale and Frcs., 2005).

1.2 Rationale

Despite years of intensive research, educational efforts, male infertility remain major social and medical problem globally, which affects people both medically and psychosocially. Directly responsible for 60% of cases involving reproductive age couples with fertility issues. Although the actual fertility of a semen sample cannot be completely determined until it is known to achieve fertilization, careful and thorough analysis of all the semen’s parameters by a specialised laboratory can allow treatment options to be appropriately considered. So evaluation of male infertility and finding relations between semen quality and gonadotropins (FSH, LH) is important.

1.3 Objectives:

1.3.1 General Objectives:
To assess serum gonadotropins levels (FSH and LH) among Sudanese men with infertility.

1.3.2 Specific objectives:
1-To estimate serum levels of lutenizing hormone (LH) and follicle stimulating hormone (FSH) in study group and control group.
2-To compare between levels of gondotropins (FSH, LH) in both azoospermia and oligospermia groups and control group.
3-To correlate between serum levels of (FSH) and (LH) and sperm count
4- To correlate between sperm count, age, residence and occupation.
2-literature review

2.1 Anatomy of the Male Reproductive Tract:
The testes are located outside the body, encased by a muscular sac. Blood flow is governed by an intricate plexus of arterial and venous Blood flow that, together with contraction of the dartos muscle in the scrotal sac, regulates the temperature of the testicles to 2°C below core body temperature. This important function is vital to uninterrupted sperm production. Also encased in the muscular sheath is the spermatic cord which has the ability to retract the testicles into the inguinal canal in instances of threatened injury. The testes themselves are comprised of two anatomical units: a network of tubules, known as miniferous tubules, and an interstitium. The tubules contain germ cells and Sertoli cells and are responsible for sperm production. The sperm move sequentially through the tubuli recti, rete testes, ductuli efferentes testes; the head, body, and tail of the epididymis, and, finally, into the vas deferens. Various secretory products of the seminal vesicles and prostate mix with sperm to form the final product: semen. Seminal vesicle secretions are rich in vitamin C and fructose, important for the preservation of motility of the sperm (Bishop, 2010).

2.1.1 Physiology of the Testicles

*Spermatogenesis:* Sperm are formed from stem cells called spermatogonia. The spermatogonia undergo mitosis and meiosis, finally the haploid cells transform to form mature sperm. The mature sperm has a head, body and tail, which enables it to swim for the purpose of forming a zygote with the haploid ovum. Certain spermatogonia stagger division so that sperm production is uninterrupted and continuous. The Sertoli cells are polyfunctional cells that aid in the development and maturation of sperm (padam, 2013).
2.1.2 Hormonogenesis:
Testosterone, the predominant hormone secreted by the testes is controlled primarily by two pituitary hormones: follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Because these hormones were first described in women, they are named in reference to the menstrual cycle. Both hormones are produced by a single group of cells in the pituitary called gonadotrophs. FSH acts primarily on germinal stem cells and LH acts primarily on the Leydig cells—located in the testicular interstitium—that synthesize testosterone. (Suzan and Marjorie, 2008).

2.1.3 Cellular Mechanism of Testosterone Action
Testosterone enters the cell and converts to dihydrotosterone (DHT). DHT complexes with an intracellular receptor protein and this complex binds to the nuclear receptor, effecting protein synthesis and cell growth (Bishop, 2010).

2.1.4 Physiologic Actions of Testosterone

Prenatal Development:
Early in development embryos have primordial components of the genital tracts of both sexes. The primitive gonads become distinguishable at about the seventh week of embryonic stage. Both chorionic gonadotropins and fetal LH stimulate production of testosterone by the fetal Leydig cells. Exposure of testosterone to the Wolffian duct leads to differentiation of the various components of the male genital tract. Sertoli cells produce müllerian regression factor, which aids in regression of the female primordial genital tract. The scrotal skin is rich in 5-reductase, which converts testosterone to DHT. Fetal exposure to drugs that block this hormone leads to feminization of the male fetus (Suzan and Marjorie, 2008)
2.1.5 Postnatal Development:
Testicular function is reactivated during puberty after a period of quiescence to produce testosterone that results in development of secondary sexual hair (face, chest, axilla, and pubis), enhanced linear skeletal growth, development of internal and external genitalia, increased upper body musculature, and development of larynx and vocal cords with deepening of the voice. Possible mood changes and aggression are undesired effects that may occur during puberty. The linear growth effects of testosterone are finite, with epiphysial closure when genetically determined height is achieved. Hypogonadism during puberty leads to imprecise closure of growth plates, leading to excessive height, long limbs, and disproportionate upper and lower body segments. Male secondary sexual characteristics can be staged by a system of development devised by Marshall and Tanner.  

2.1.6 Seminal fluid:
Semen, also known as seminal fluid, is an organic fluid that may contain spermatozoa. It is secreted by the gonads (sexual glands) and other sexual organs of males or hermaphroditic animals and can fertilize female ova. In humans, seminal fluid contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose are elements of seminal fluid which promote the survival of spermatozoa, and provide a medium through which they can move or "swim". Semen is produced and originates from the seminal vesicles, which is located in the pelvis. The process that results in the discharge of semen is called ejaculations.  

Semen is also a form of genetic material. In animals, semen has been collected for cryoconservation. Cryoconservation of animal genetic resources is a practice that calls for the collection of genetic material in efforts for conservation of a particular breed.
2.1.7 Semen Composition:

During the process of ejaculation, sperm passes through the ejaculatory ducts and mixes with fluids from the seminal vesicles, the prostate, and the bulbourethral glands to form the semen. The seminal vesicles produce a yellowish viscous fluid rich in fructose and other substances that makes up about 70% of human semen. The prostatic secretion, influenced by dihydrotestosterone, is a whitish (sometimes clear), thin fluid containing proteolytic enzymes, citric acid, acid phosphatase and lipids. The bulbourethral glands secrete a clear secretion into the lumen of the urethra to lubricate it (Turner, 2012).

Sertoli cells, which nurture and support developing spermatocytes, secrete a fluid into seminiferous tubules that helps transport sperm to the genital ducts. The ductuli efferentes possess cuboidal cells with microvilli and lysosomal granules that modify the ductal fluid by reabsorbing some fluid. Once the semen enters the ductus epididymis the principal cells which contain pinocytotic vessels indicating fluid reabsorption, secrete glycerophosphocholine which most likely inhibits premature capacitation. The accessory genital ducts, the seminal vesicles, prostate gland and the bulbourethral glands, produce most of the seminal fluid (Padam, 2013).

Seminal plasma of humans contains a complex range of organic and organic constituents: The seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract. The normal environment of the vagina is a hostile one for sperm cells, as it is very acidic (from the native microflora producing lactic acid), viscous, and patrolled by immune cells. The components in the seminal plasma attempt to compensate for this hostile environment. Basic amines such as putrescine, spermine spermidine and cadaverine are responsible for the smell and flavor of semen. These alkaline bases counteract and buffer the acidic environment.
of the vaginal canal, and protect DNA inside the sperm from acidic denaturation (Turner, 2010).

2.1.8 Semen analysis:

Semen analysis remains the single most useful and fundamental investigation in the search for the cause of male infertility. It is a simple test that assesses the formation and maturity of sperm as well as how the sperm interacts with the seminal fluid so it provides insight not only on sperm production (count), but sperm quality (motility, morphology) as well. The standard semen analysis has a sensitivity of 89.6%, that it is able to detect 9 out of 10 men with a genuine problem. The pathological causes for decreased sperm count arise from abnormality in the control mechanism of sperm production at pre-testicular, testicular or post-testicular level. In more than 90% of cases male infertility is due to either low sperm count or poor semen quality or combination of the two (Fauzia and Nishat, 2013).

Recent data confirm the decline in semen quality and quantity all over the world probably due to increased prevalence of sexually transmitted diseases (STDs) and urogenital infections. (Fauzia and Nishat, 2013). The semen analysis for fertility evaluation consists of both macroscopic and microscopic examination. Parameters reported include appearance, volume, viscosity, pH, sperm concentration and count, motility, and morphology (Suzan and Marjorie, 2008).

2.1.8.1 Appearance of semen:

Normal semen has a gray-white color, appears translucent, and has a characteristic musty odor. Increased white turbidity indicates the presence of white blood cells (WBCs) and infection within the reproductive tract. If required, specimen culturing is performed prior to continuing with the semen analysis. During the microscopic examination, WBCs must be differentiated from immature sperm (spermatids). The leukocyte
esterase reagent strip test may be useful to screen for the presence of WBCs. Varying amounts of red coloration are associated 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 the evaluation of motility (Suzan and Marjorie ., 2008).

2.1.8.2 Liquefaction of semen:
A fresh semen specimen is clotted and should liquefy within 30 to 60 minutes after collection; therefore, recording the time of collection is essential for evaluation of semen liquefaction. Analysis of the specimen cannot begin until after liquefaction has occurred. If after 2 hours the specimen has not liquefied, 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 a deficiency in prostatic enzymes and should be reported (Suzan and Marjorie ., 2008).

2.1.8.3 Volume of semen:
Normal semen volume ranges between 2 and 5 mL. It can be measured by pouring the specimen into a clean graduated cylinder calibrated in 0.1mL increments. Increased volume may be seen following periods of extended abstinence (Suzan and Marjorie ., 2008).

2.1.8.4 Viscosity of semen:
Specimen viscosity refers to the consistency of the fluid and may be related to specimen liquefaction. Incompletely liquefied specimens are clumped and highly viscous. The normal semen specimen should be easily drawn into a pipette and form droplets that do not appear clumped or stringy when discharged from the pipette. Normal droplets form a thin thread when released from the pipette. Droplets with threads longer that 2 centimeters are considered highly viscous. Ratings of 0 (watery) to 4 (gel-like) can be assigned to the viscosity report. Viscosity can also be
reported as low, normal, and high. Increased viscosity and incomplete liquefaction impede sperm motility (Suzan and Marjorie., 2008).

2.1.8.5 PH of semen:
The normal pH of semen is alkaline with a range of 7.2 to 8.0. Increased pH is indicative of infection within the reproductive tract. A decreased pH is associated with increased prostatic fluid. Semen for pH testing can be applied to the pH pad of a urinalysis reagent strip and the color compared with the manufacturer’s chart. Dedicated pH testing paper also can be used (Suzan and Marjorie., 2008).

2.1.8.6 Sperm Count:
Even though fertilization is accomplished by one spermatozoon, the actual number of sperm present in a semen specimen is a valid measurement of fertility. Normal values for sperm concentration are commonly listed as greater than 20 million sperm per milliliter, with concentrations between 10 and 20 million per milliliter considered borderline. The total sperm count for the ejaculate can be calculated by multiplying the sperm concentration by the specimen volume. Total sperm counts greater than 40 million per ejaculate are considered normal (20 million per milliliter 2 mL) (Suzan and Marjorie., 2008).

2.1.8.7 Sperm Motility:
The presence of sperm capable of forward, progressive movement is critical for fertility, because once presented to the cervix, the sperm must propel themselves through the cervical mucosa to the uterus, fallopian tubes, and ovum. Traditionally, clinical laboratory reporting of sperm motility has been a subjective evaluation performed by examining an undiluted specimen and determining the percentage of motile sperm and the quality of the motility. Assessment of sperm motility should be performed on well mixed, liquefied semen within 1 hour of specimen collection. The practice of examining sperm motility at timed intervals
over an extended period has been shown to serve no useful purpose. To provide continuity in reporting, laboratories should place a consistent amount of semen under the same size coverslip, such as 10 L under a 22×22 mm coverslip. The percentage of sperm showing actual forward movement can then be estimated after evaluating approximately 20 high-power fields. Motility is evaluated by both speed and direction. Grading can be done using a scale of 0 to 4, with 4 indicating rapid straight-line movement and 0 indicating no movement. A minimum motility of 50% with a rating of 2.0 after 1 hour is considered normal. The WHO uses a rating scale of a, b, c, d. Interpretation states that within 1 hour, 50% or more sperm should be motile in categories a, b, and c, or 25% or more should show progressive motility (a and b). The presence of a high percentage of immobile sperm and clumps of sperm requires further evaluation to determine sperm viability or the presence of sperm agglutinins. In recent years, instrumentation capable of performing computer-assisted semen analysis (CASA) has been developed. CASA provides objective determination of both sperm velocity and trajectory (direction of motion). Sperm concentration and morphology are also included in the analysis. Currently, CASA instrumentation is found primarily in laboratories that specialize in andrology and perform a high volume of semen analysis (Suzan and Marjorie., 2008).

2.1.8.8 Sperm Morphology:
Just as the presence of a normal number of sperm that are non-motile produces infertility, the presence of sperm that are morphologically incapable of fertilization also results in infertility. Sperm morphology is evaluated with respect to the structure of the head, neckpiece, mid piece and tail. Abnormalities in head morphology are associated with poor ovum penetration, whereas neckpiece, mid piece, and tail abnormalities affect motility. The normal sperm has an oval-shaped head approximately
5 m long and 3 m wide and a long, flagella tail approximately 45 m long. Critical to ovum penetration is the enzyme-containing *acrosomal cap* located at the tip of the head. The acrosomal cap should encompass approximately half of the head and covers approximately two thirds of the sperm nucleus. The neckpiece attaches the head to the tail and the midpiece. The midpiece is the thickest part of the tail because it is surrounded by a mitochondrial sheath that produces the energy required by the tail for motility. Sperm morphology is evaluated from a thinly smeared, stained slide under oil immersion. Staining can be performed using Wright’s, Giemsa, or Papanicolaou stain and is a matter of laboratory preference. Air-dried slides are stable for 24 hours. At least 200 sperm should be evaluated and the percentage of abnormal sperm reported. Routinely identified abnormalities in head structure include double heads, giant and amorphous heads, pinheads, tapered heads, and constricted heads. Abnormal sperm tails are frequently doubled, coiled, or bent. An abnormally long neckpiece may cause the sperm head to bend backward and interfere with motility. Additional parameters in the evaluation of sperm morphology include measurement of head, neck, and tail size, size of the acrosome, and the presence of vacuoles. Inclusion of these parameters is referred to as Kruger’s strict criteria. Performance of strict criteria evaluation requires the use of a stage micrometer or morphometry. At present, evaluation of sperm morphology using strict criteria is not routinely performed in the clinical laboratory but is recommended by the WHO. Strict criteria evaluation is a integral part of assisted reproduction evaluations. Normal values for sperm morphology depend on the method of evaluation used and vary from greater than 30% normal forms when using routine criteria to greater than 14% normal forms when using strict criteria (Suzan and Marjorie, 2008).
2.1.8.9 Sperm Viability:
Decreased sperm viability may be suspected when a specimen has a normal sperm concentration with markedly decreased motility. Viability is evaluated by mixing the specimen with an eosin-nigrosin stain preparing a smear, and counting the number of dead cells in 100 sperm. Living cells are not infiltrated by the dye and remain a bluish white color whereas dead cells stain red against the purple background. Normal viability requires 75% living cells and should correspond to the previously evaluated motility (Suzan and Marjorie., 2008).

2.2 Male infertility

2.2.1 Definition:
Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year, World Health Organization (WHO) (Jung wirth et al., 2015).
Infertility is a global problem. Although the estimates vary approximately 15% of couples attempting their first pregnancy meet with failure. Male factors are estimated to be the cause in up to 50% of the cases. About 25% of all infertility is caused by a sperm defect and 40-50% of infertility cases have a sperm defect as the main cause, or a contributing cause. If the sperm count or the motility is extremely low we usually assume this is the cause of the fertility issue. If the count or the motility is slightly low, it could be contributing factor, but the sperm might not be the only fertility issue in the couple. There would often be female fertility problems also (Padam., 2013).

2.2.2 Main causes of male factor infertility:

2.2.2.1 Pretesticular:
Hypothalamic disease:-
Gonadotrophin deficiency (Kallman syndrome) Pituitary disease.
Pituitary insufficiency (tumours, radiation, surgery).
Hyperprolactinaemia - Exogenous hormones (anabolic steroids, glucocorticoid excess, hyper-or hypothyroidism) (Karavolos et al., 2013).

2.2.2.2 Testicular:

Disorders of Sexual Development and Testicular Hypofunction:

Pubertal development could be premature (precocious) or delayed, even if development is normal at birth. 9,10 Detailed descriptions of the sequence of hormonal pubertal abnormalities of hair, genitals, and breasts are beyond the scope of this text. The differential diagnosis of hypogonadism includes a diverse group of disorders affecting the testicles.

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism incorporates a group of disorders characterized by low testosterone, elevated FSH or LH, and impaired sperm production.

Klinefelter’s Syndrome

Klinefelter’s syndrome occurs in about 1 of 400 men and is caused by the presence of an extra chromosome. The most common karyotype is 47,XY.11 Men with this disorder have small (∼2.5 cm), firm testicles.

Gynecomastia

(Enlargement of the male breast) can also be present at the time of diagnosis. Due to reduced production of testosterone, FSH and LH levels are elevated. These men also have azoospermia and resultant sterility. Men with mosaicism may produce some sperm and pregnancies have been reported with such men. Elevated levels of FSH and LH induced increased aromatase activity, resulting in elevated estrogen levels. Men with Klinefelter’s syndrome may have reduced bone density and breast cancer.

Testicular Feminization Syndrome:

Testicular feminization syndrome is the most severe form of androgen resistance syndrome, resulting in lack of testosterone action in the target
tissue. As a result of the lack of testosterone effect, the physical development pursues the female phenotype, with fully developed breast and female distribution of fat and hair. Most men present for evaluation of primary amenorrhea, at which time the lack of female internal genitalia becomes apparent. The testicles are often undescended, and failure to promptly remove these organs results in malignant transformation. Biochemical evaluation reveal normal levels of testosterone with elevated FSH and LH levels. There is no utility or response to administration of exogenous testosterone.

5-Reductase Deficiency
The genotype in 5- reductase deficiency is XY. A reduction in levels of the enzyme 5- reductase results in decreased testosterone levels. Physical development is similar to the female phenotype until puberty when residual enzyme activity sufficiently converts testosterone to dihydrotestosterone, resulting in development of a male phenotype.

Myotonic Dystrophy
Myotonic dystrophy is inherited in an autosomal dominant fashion and presents with hypogonadism, muscle weakness, frontal balding, diabetes and muscle dystonia. Testicular failure typically presents in the fourth decade of life.

Hypogonadotropic Hypogonadism
The hallmark of disorders of hypogonadotropic hypogonadism is the occurrence of low testosterone levels together with low or inappropriately normal FSH or LH level.

2.2.2.3 Post-testicular (obstruction):

Congenital
- Cystic fibrosis, congenital absence of the vas deferens (CAVD) and Young’s syndrome.
Acquired
- Vasectomy, Infection (Chlamydia, gonorrhea) and Iatrogenic vasal injury.

Disorders of sperm function or motility
- Maturation defect, Immunological infertility, Immotile cilia syndrome
  And Globozoospermia.

Sexual dysfunction:
Timing and frequency, Erectile/ ejaculatory dysfunction, Diabetes mellitus
multiple sclerosis and spinal cord/pelvic injuries (Karavolos et al., 2013).

2.2.2.4 Hormonal investigation:
Endocrine malfunctions are more prevalent in infertile men than in the
general population, but are still quite uncommon. Hormonal screening
can be limited to determining follicle stimulating hormone (FSH),
luteinizing hormone (LH), and testosterone levels in case of abnormal
semen parameters (Jungwirth et al., 2010).

2.3 Gonadotropin Hormones:
2.3.1 Follicle - stimulating hormone (FSH):
Follicle-stimulating hormone (FSH) is a hormone released by the
anterior pituitary gland via stimulation from gonadotrophin releasing
hormone and potentially other factors. It is released in a pulsatile fashion
and is regulated in part by glycoproteins including activin and inhibin.
FSH reflects the status of spermatogenesis as a result of the feedback
between the testis and hypothalamus/pituitary glands. FSH acts on
Sertoli cells in the seminiferous tubules to initiate spermatogenesis
(John et al., 2007).
2.3.2 **Luteinizing hormone (LH):**

Luteinizing hormone (LH) is a hormone released by gonadotropic cells in the anterior pituitary gland. In males it stimulates Leydig cells production of testosterone. It acts synergistically with FSH, induces production of testosterone. Testosterone, acting with FSH, has paracrine effects on the seminiferous and Sertoli cells inducing spermatogenesis. Exogenous overuse or abuse of testosterone, such as occurs with some athletes, will reduce the high intratesticular concentration of testosterone, leading to reduction of sperm (Padam., 2013).

2.4 **Hormonal control of spermatogenesis:**

Spermatogenesis is primarily controlled by the gonadotropins—FSH and LH. LH indirectly affects spermatogenesis by stimulating endogenous testosterone production. The Sertoli cells possess specific high-affinity FSH receptors and produce androgen-binding protein, which carries androgens intracellularly, serves as an androgen reservoir within the seminiferous tubule, and transports testosterone from the testes into the epididymal tubule. The physical proximity of the Leydig cells to the seminiferous tubules, and the elaboration by the Sertoli cells of ABP, maintain an extremely high level of androgen concentration within the microenvironment of the developing spermatozoa (Dale and Frcs., 2005). Hormonal requirements for the initiation and maintenance of spermatogenesis appear to be different. In humans, FSH is necessary for the maintenance of quantitatively normal sperm production and is particularly important for initiating spermatogenesis in pubertal males and reinitiating spermatogenesis in men whose germinal epithelium has regressed after hypophysectomy. **Qualitative** sperm production can be achieved by replacement of either FSH or LH alone. However, both FSH
and LH are necessary to maintain *quantitative* normal spermatogenesis in humans (Dale and Frcs., 2005).

The first, and rate-limiting, step in the testicular steroidogenesis is the conversion of cholesterol to pregnenolone. This cholesterol is either trapped by endocytosis from the blood or synthesized within the Leydig cells. The LH binds to the glycoprotein receptor in the cell wall and induces intracellular cyclic AMP production that, in turn, activates protein kinase A, which catalyzes protein phosphorylation. This latter step induces testosterone synthesis. The testicular steroidogenesis pathway is similar to the pathway in the adrenal cortex and they share the same enzymatic systems (Bishop., 2010).

Testosterone is the principal androgen hormone in the blood. It is largely bound, with 2%–3% free. About 50% of testosterone is bound to albumin and about 45% is bound to sex hormone–binding globulin (SHBG). The concentration of binding protein determines the level of total testosterone but not the free testosterone levels during laboratory estimation. Testosterone and inhibin are the two hormones secreted by the testes that provide feedback control to the hypothalamus and pituitary. Testosterone concentration fluctuates in a circadian fashion, reflecting the parallel rhythms of LH and FSH levels. This fact should be considered when interpreting serum levels of testosterone: the highest level is found at about 8 AM and correlates with most laboratory normal ranges. (Padam ., 2013)

### 2.5 Regulation of spermatogenesis:

Sperm are formed in the seminiferous tubules, from germinal cells called spermatogonia. Spermatogonia divide by mitosis into primary spermatocytes, which in turn undergo two reduction divisions (meiosis I and II) to form spermatids. By the process of spermiogenesis .
spermatids transform into mature cytoplasm-free sperm with condensed DNA in the head, an apical acrosome and a tail. Normal spermatogenesis is under the influence of follicular stimulating hormone (FSH) and testosterone. FSH binds to Sertoli cells and increases spermatogonial number and maturation to spermatocytes, but it is unable to complete spermatogenesis alone. Luteinizing hormone (LH) is necessary for testosterone production by the Leydig cells, and plays an essential role in spermatid maturation (Karavolos et al., 2013).

The entire spermatogenic process, including transit in the ductal testicular system takes approximately 3 months. This is important to bear in mind when advising individuals on the potential effect of lifestyle changes on semen quality improvement (Karavolos et al., 2013).

2.6 Association of FSH and LH in male infertility:

The management of infertility problems has become an increasingly important part of health services during the past two decades in the most countries. Recent studies have focused on semen quality of men in the general population. However, most studies of semen quality and reproductive hormones in unselected populations have little previously reported. (Fakherildin, M.B., 2007).

Gonadotropins comprise follicle stimulating hormone (FSH) and luteinizing hormone (LH) produced by the pituitary gland. In men, they are essential for spermatogenesis and testosterone secretion. Circulating FSH has long been considered a valuable marker for Sertoli cell function and spermatogenesis, but FSH is influenced by hypothalamic function, as well as testicular factors and steroidal hormones. However, an increased concentration of LH in circulation accompanying idiopathic oligozoospermia suggests that LH secretion may be linked to the factors regulation spermatogenesis. It has generally been observed that testis size
and function is associated with gonadotropic activities that are necessary for initiation and maintenance of spermatogenesis. Previous studies had shown no specific pattern in the serum or seminal plasma hormone profiles of men with infertility and it is debatable whether there is a need to perform routine seminal hormone assays in the management of men with infertility. Furthermore, low concentration of sexual hormones may increase the apoptosis of germ cells, which can induce male infertility.

The significance of the correlation between the levels of LH and FSH in seminal plasma and sperm concentration and motility is unknown. Although, seminal sex hormone might be more sensitive indices to assess the direct effect on sperm functions and extent of feedback inhibition on hypothalamus-pituitary-testis axis. Thus, to investigate the possible causes of the deterioration of male fertility and make attempts to prevent further decay, they can be studied (Fakherildin, M.B., 2007).
3-Materials and methods

3.1 Study design:
A cross-sectional case - control study conducted between May to September 2017.

3.2 Study area and period:
This study was conducted in Khartoum state. Banoon fertility center.

3.3 Study population:
Study done on Sudanese men with infertility who attended to Banoon center in the period of the study.

One hundred and fifty males randomly were enrolled in this study and then classified to two groups. Seventy five men with infertility as cases group and seventy five men apparently healthy as control group.

3.4 Inclusion criteria:
Sample were collected from individuals attended the fertility center.

3.5 Exclusion criteria:
Subjects with diabetes mellitus, renal diseases, hypertension and thyroid disease or any other endocrine disease have been excluded from the study also patients who received hormonal therapy.

3.6 Ethical considerations:
Study was approved from scientific committee of the Sudan university of Science and Technology, verbal informed consent was obtained and all Participants were informed by the aim of study.

3.7 Collection of Samples:
One hundred and fifty blood samples (3ml) were collected in plain containers. Then serum was obtained by centrifugation at 4000 rpm and transferred to plain containers, and stored in -20°C until use.
3.9 Method of hormones estimation:

3.9.1 Principle of test:
The (FSH and LH) quantitative test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a polyclonal Anti (FSH, LH) antibody for solid phase (microtiter wells) immobilization and a mouse monoclonal anti(FSH –LH) antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in (FSH, LH) molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells were washed to remove unbound labeled antibodies. A solution of substrate was added and resulting in the development of blue color. Then stop solution was added to stop the reaction and color changed to yellow spectrophotometrically at 450 nm. The concentration of (FSH, LH) is directly proportional to the color intensity of the test sample (Knobi et al.; 1980).

3.9.2 Procedure of the test:
Appendix π.

3.10 Quality control:
Control sera was run with the samples to ensure Q.C.

3.11 Statistics analysis:
Data analysis was performed using SPSS version 22 software. Descriptive statistics on subject demographics were calculated, Mean, SD T-test and ANOVA were employed to compare mean concentration. Pearson's correlation was applied to correlate between study variable
4- Results

4.1 Results

This study included 150 males (75 males with normal semen) and (75 males with abnormal semen). The study included infertile males with age range (21-62) year and normal fertile male with age group (23-59) year. The age of infertile group was classified to different groups to identify the relationship between gonadotropins and age. (33-42) year was the largest group (42%) followed by age group (43-62) year which represented (33%) and (21-32) year age group was represented (25%) as it shows in figure (4-1) One way - ANOVA test was used to analysis the data. Statistical analysis shows no significant association between gonadotropins and age, with p.value (0.77) for FSH, and p.value (0.66) for LH. As it shows in table (4-4).

The serum levels of FSH in both groups of azoospermia and oligospermia was increased and inversely correlated with sperm count. The mean of serum FSH in azoospermia was (18.1±9.1 mIU/mL) and in oligospermia group was (14.1 ± 9.4 mIU/mL) which is high when compared to control group (6.4 ± 7.03 mIU/mL) with p.value (0.00) (0.00) as it shows in table (4-1).

The levels of LH in both groups was elevated and inversely correlated with sperm count. The mean of LH in azoospermia group was (14.4 ± 7.08 mIU/mL), and the mean of LH in oligospermia group was (11.1 ± 7.1 mIU/mL) with p.value (0.02) (0.03) respectively, independent samples T.test was used to analysis data. As it shows it table (4-2).

The study also include patients from different areas in Sudan, urban area and rural area to identify relationship between gonadotropins and
residence. The majority of patients were from urban area represented (75%) while the rest of patients were from rural area represented (25%) as it shows in figure (4-2). Statistical analysis shows no significant differences between gonatropins and residence with p.value (0.60) for FSH, and p.value (0.065) for LH.

According to occupation the result in table (4-3) revealed that the majority of total group were the group of unidentified group works represented (62.7%) followed by labor intensive work group (28%) followed by drivers (9.3%). Statical analysis did not show any significant difference between occupation and gonadotropins. The p.value was (0.22) for LH and (0.46) for FSH.

Figure (4-3) and figure (4-4) shows strong negative correlation between (FSH, LH) and sperm count with r (-0.12) (-0.24) respectively and p.value (0.04) (0.03) respectively.
Figure (4-1) Distribution of age groups per years among patients.

- 33-42
- 43-62
- 21-32
Figure (4-2) Distribution of the patients according to residence

- Urban: 75%
- Rural: 25%
Table (4.1): Compression between means of FSH levels in Sudanese men with azoospermia, oligospermia and control group

<table>
<thead>
<tr>
<th>Sperm count report</th>
<th>Mean ± S.D</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia (52)</td>
<td>18.1 ± 9.1 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
<tr>
<td>Normospermia (75)</td>
<td>6.4 ± 7.03 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
<tr>
<td>Oligospermia (23)</td>
<td>14.1 ± 9.4 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
</tbody>
</table>

Independent sample T.test was used to compare between two means.
p.value significant at level ≤ 0.05

Table (4.2): Compression between means of LH levels in Sudanese men with Azoospermia, Oligospermia and control group.

<table>
<thead>
<tr>
<th>Sperm count report</th>
<th>Mean ± S.D</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoospermia (52)</td>
<td>14.4 ±7.08 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
<tr>
<td>Normospermia (75)</td>
<td>6.4 ± 7.03 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
<tr>
<td>Oligospermia (23)</td>
<td>11.1 ± 7.1 mIU/mL</td>
<td>6.4 ± 7.03 mIU/mL</td>
</tr>
</tbody>
</table>

Independent sample T.test was used to compare between two means
p.value significant at level ≤ 0.05
### Table (4.3) Compression between means of serum FSH among infertile male according to occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FSH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Driver (n =7)</td>
<td>3.0 ± 1.7</td>
<td>0.46</td>
</tr>
<tr>
<td>Labor intensive work (n =15)</td>
<td>4.2 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Other (n=53)</td>
<td>3.7 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

- One way - Anova was used to compare between the means
- P.value considered significant ≤ 0.05

### Table (4.4) Compression between means serum LH among infertile male according to occupation

<table>
<thead>
<tr>
<th>Occupation</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LH</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Driver (n =7)</td>
<td>4.7 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Labor intensive work (n =15)</td>
<td>3.7 ± 2.08</td>
<td>0.22</td>
</tr>
<tr>
<td>Other (n=53)</td>
<td>3.3 ± 2.03</td>
<td></td>
</tr>
</tbody>
</table>

- One way - Anova was used to compare between the means
- P.value considered significant ≤ 0.05
Table (4.5) : Compression between means of serum LH among infertile males according to age groups

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH</td>
<td>21 - 32</td>
<td>11.7 ± 4.6</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>33 - 42</td>
<td>13.1 ± 8.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43 - 62</td>
<td>13.4 ± 7.2</td>
<td></td>
</tr>
</tbody>
</table>

- One way - Anova was used to compare between the means
- P.value considered significant ≤ 0.05

Table (4.6) : Compression between means of serum FSH among infertile males according to age groups

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>21-32</td>
<td>15.2 ± 7.9</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>33-42</td>
<td>17.7 ± 9.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43-62</td>
<td>17.4 ± 10.4</td>
<td></td>
</tr>
</tbody>
</table>

- One way - Anova was used to compare between the means
- P.value considered significant ≤ 0.05
Table (4.7) : Compression between means of FSH among infertile Males according to residence

<table>
<thead>
<tr>
<th>Residence</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>3.3 ± 1.9</td>
<td>0.065</td>
</tr>
<tr>
<td>Rural</td>
<td>4.3 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

- Independent samples T.test was used to compare between two means.
- p.value significant at level ≤ 0.05

Table (4.8) : Compression between means of LH among infertile Males according to residence

<table>
<thead>
<tr>
<th>Residence</th>
<th>Mean ± S.D</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>3.6 ± 1.8</td>
<td>0.23</td>
</tr>
<tr>
<td>Rural</td>
<td>4.2 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

- Independent samples T.test was used to compare between two means.
- p.value significant at level ≤ 0.05
Figure (4-3): Scatter plot between LH and sperm count in oligospermia group with p.value 0.03 and r -0.24
Figure (4-4) : Scatter plot between FSH and sperm count in oligospermia group with p.value 0.04 and r -0.12
5-Discussion-Conclusion and Recommendations

5.1 Discussion:

It is extremely important for the evaluation of male infertility to consider the reproductive hormone levels. It was reported that these hormones have a major role in male spermatogenesis. LH, FSH, and testosterone evaluation are useful in management of male infertility (Suzan and Marjorie, 2008).

The present study revealed that levels of gonadotropins (FSH and LH) are increased in Sudanese infertile males compared to normal fertile males and there was an inverse association between (FSH, LH) levels and sperm count, which is in agreement with (Ramesh et al., 2004) who found that there was an inverse relationship between gonadotropins and sperm count.

In addition, it revealed that statistical relationship between gonadotropins and age was insignificant, which is in agreement with (S vaninetti et al., 2000), who stated that the relationship between serum gonadotropin levels and age was not linear and serum gonadotropins remained stable up to 70 years.

The relationship between residence and sperm count was insignificant which is in agreement with (Adeeb, 2010) who found no association between sperm count and residence.

Our study found that relationship between occupation and sperm count was insignificant, and this is in contrast with (Ola et al., 2016) who stated that male fertility represented by quality of semen might be affected by occupational and environmental risk factors, the contrast in study may be due to different occupation were enrolled in the two studies.
5.2 Conclusion:
From this study it can be concluded that the levels of gonadoropins are increased in infertile male and there were inverse association between serum level of FSH, LH and sperm count. The relation between gonadoropins level, age, residence and occupation is insignificant.

5.3 Recommendations:

1. Use both (FSH, LH) as tool of diagnosis of male infertility.
2. Estimate both FSH and LH as a indicator of testicular function.
3. Other pituitary and gonadal hormones should be studied to identify the relationship between pituitary, gonads and semen.
Sudan University for Science and Technology
Serum Gonadotropins Levels among Sudanese Men with Infertility

(1) Questionnaire:

NO:1

Demographic data:

- Age
  1. 25-36
  2. 37-49
  3. 50-61
- Occupation
  1. Driver
  2. Lobar intensive work
  3. Other
- Resident
  1. Urban
  2. rural
- Married duration:
  1. 0-2 year
  2. 3-5 year
  3. Above 5 year

Semen report:

- Count:
  1. <15
  2. 15-16
  3. >16
- Others:
  1. Diabetes mellitus and hypertension.
  2. Thyroid disorders
  3. Patients who received hormonal therapy.
References


