Sudan University of Science andTechnology
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Assessment of FSH in Sudanese with abnormal semen analysis at Shendi city-Sudan

تقييم مستوى الهرمون المنبه للجريب فى السودانين الذين لديهم تحليل السائل المنوي غير طبيعي بمدينة شنديفيالسودان

A dissertation submitted for partial fulfillment for the requirement ofM.Sc Degree in Medical Laboratory science- Clinical chemistry

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بسم الله الرحمن الرحيم

قال تعالى :

" يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ "

سورة المجادلة الآية رقْم 11

Dedication

To the soul of my Mother

To my Father

Brother and Sister

Friends and Teacher

ACKNOWLEDGMENTS

First, I thank the Almighty Allah, for enabling me to reach this level of the academic ladder. It is all because of His power, mercy and grace. This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this theisi, therefore it is with immense gratitude that I acknowledge the support and help of those individuals. Firstly I would like to thank my supervisor Dr.Abdelkariem Abubaker for his unlimited support, invaluable guidance and comment during the study. And grateful thanks for my friends and my colleague.

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Abstract

Background: Infertility is the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.Reasons for a male infertility:Congenitalfactors,Acquired urogenital abnormalities,Urogenital tract infections, Increased scrotal temperature ,Endocrine disturbances**,**Systemicdiseases.

Objective: To assess serum FSH levels in Sudanese male with abnormal semen analysis at Shendi city-Sudan

Materials and Methods: In a cross-sectional study population115 males randomly were enrolled in this study. Then were classified based on semen sample result into two groups, group one normal semen analysis result (61 males)and group two abnormal semen analysis result (54 males.The parameters measured were semen analysis and FSH hormone.

Results: The levels of FSH in abnormal semen analysis were increased significantly with ($p< 0.005$) when compare with the normal range value. The results revealed that, there was inversely association between FSH levelssperm count and total sperm count with significant p–value (0.021 and 0.030) respectively.

Conclusion: The study concluded that, there is inverse association between serum level of FSH, low sperm count and motility this could contribute the pathogenesis of male infertility. Thehighlevel of FSH may be an indication of testicular problems as the cause of infertility in the studied subjects.

مستخلص البحث

خليفه الدراسه: العقم هو عدم القدره علي النشاط الجنسي للزوجين غير المستخدمين لموانع الحمل لتحقيق الحمل في سنه واحده واسباب العقم عند الذكور: عوامل خلقيه،التهابات الجهاز البولي التناسلي المكتسبه،زيادة درجه حراره الصفن، اضطرابات الغدد الصماء، والامراض المزمنه .

الهدف: تقييم مستويات الهرمون المنبه للجريب في الذكور السودانين مع تحليل السائل المنوي الغير طبيعي في مدينه شندي .

المواد والطرق: اجريت دراسه مستعرضه ل 115 ذكر عشوائيا تم تسجيلهم في هذه الدراسه ،وصنفو اعتمادا علي نتيجه تحليل السائل المنوي الي مجموعتين،مجموعه طبيعيه تحليل السائل المنوي61 ومجموعه غير طبيعيه 54 مع قياس الهرمون المنبه للجريب.

النتائج: مستويات الهرمون المنبه للجريب في زياده عند الذكور غير طبيعيين السائل المنوي مع مقارنه مع الذكور الطبعيين السائل المنوي ($p< 0.005$)قيمه احتماليه

اوضحت الدراسه ان هنالك علاقه عكسيه بين الهرمون المنبه للجريب مع مستويات عدد ومجموع الحيوانات المنويه مع قيم احتماليه (0.021 و0.030) علي التوالي

الخلاصة: خلصت الدراسه ان هناك علاقه عكسيه بين الهرمون المنبه للجريب وقله عدد وحركة الحيوانات المنويه .

Chapter One:

Introduction

&

Literature review

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 tumour, orchitis),Urogenital tract infections,Increased scrotal temperature (e.g. as a consequence of varicocele); Endocrine disturbances,Genetic abnormalities

Immunological factors**,**Systemicdiseases**,**Exogenous factors (medications, toxins, irradiation, lifestyle factors)Idiopathic (40-50% of cases) (Jungwirth andDiemer,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. Azospermia: Absence of spermatozoa in ejaculate, Criptozoospermia: Presence of spermatozoa after high speed centrifuge, which is absentinfreshsample, Necrozoospermia: Less vital, more immotile spermatozoon in ejaculate. Leukospermia: Presence of leukocyte in ejaculatein an amount greater than reference values and Aspermia: Absence of ejaculate (Omer et al.,*2012).*

Spermatogenesis is primarily controlled by the gonadotropins—FSH and LH.

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 MALE INFERTILITY

1-2-1Definition

Infertility is the inability of a sexually active, non-contracepting couple to achieve spontaneous pregnancy in one year, World Health Organization (WHO) **(**Jungwirth 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 befemale fertility problems also (Padam,2013).

1-2-2Main causes of male factor infertility:

1-2-2-1Pretesticular:

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).

1-2-2-2Testicular:

Congenital Genetic

- Chromosomal (Kleinfelter syndrome 47, XXY)

- Y chromosome microdeletions

- Noonan syndrome (male Turner syndrome 45, XO)

Other

- Cryptorchidism

Acquired

- Injury (orchitis, torsion, trauma)

- Varicocele

- Systemic disease (renal failure, liver failure)

- Chemotherapy, radiotherapy

- Testicular tumours

-Idiopathic(Karavolos et al,2013).

1-2-2-3Post-testicular (obstruction):

Congenital

- Cystic fibrosis, congenital absence of the vas deferens (CAVD)

- Young's syndrome

Acquired

- Vasectomy

- Infection (chlamydia, gonorrhoea)

- Iatrogenic vasal injury

Disorders of sperm function or motility

- Immotile cilia syndrome

- Maturation defects

- Immunological infertility

- Globozoospermia

Sexual dysfunction

- Timing and frequency

- Erectile/ ejaculatory dysfunction

- Diabetes mellitus, multiple sclerosis, spinal cord/pelvic injuries (Karavolos et al,2013).

1-3Abnormal semen analysis:

The misconception that infertility is typically associated with the female is commonly faced in the management of infertile men. It is uncommon for a patient to present for an infertility evaluation with an abnormal semen analysis report before an extensive female partner workup has been performed. Additionally, a man is usually considered fertile based only on seminal parameters without a physical exam. This behavior may lead to a delay in both the exact diagnosis and in possible specific infertility treatment.Moreover, male factor infertility can result from an underlying medical condition that is often treatable but could possibly be life-threatening(Cocuzza et al,2013).

The responsibility of male factor in couple's infertility has been exponentially rising in recent years due to a comprehensive evaluation of reproductive male function and improved diagnostic tools. Despite thisimprovement in diagnosis, azoospermia is always the most challenging topic associated with infertility treatment. Several conditions that interfere with spermatogenesis and reduce sperm production and qualitycan lead to azoospermia. Azoospermia may also occur because of a reproductive tract obstruction (Cocuzza et al, 2013).

1-3-1Abnormal semen status was classified as follows:

Oligozoospermic (sperm concentration $<20\times10^6$/mL), severe oligozoospermic (sperm concentration $<5\times10^6$/mL), Asthenozoospermic (percentage of a+b grade sperm<50%), Teratozoospermic (the percentage of morphologically normal sperm<15%), Cryptozoospermic (spermatozoa absent from fresh preparations but observed in a centrifuged pellet), Oligoastheonozoospermic (sperm concentration $<20\times10^6$/mL and percentage of (a+b) grade sperm <50%), Oligoteratozoospermic (sperm concentration $<20\times10^6$/mL, and the percentage of morphologically normal sperm <15%) (Fadlalla *et al,2014).*Azoospermia, defined as the absence of sperm in the ejaculate, is identified in approximately 1% of all men and in 10 to 15% of infertile males (Cocuzza et al ,2013). Oligoasthenoteratozoospermia (OAT) syndrome refers to a combination of abnormalities in the three semen parameters and is a common phenotype for infertile men.OAT syndrome includes oligozoospermia, asthenozoospermia, teratozoospermia, oligoasthenozoospermia, asthenoteratozoospermia, oligoteratozoospermia, and oligoasthenoteratozoospermia (Wei et al,2013).Necrospermia, all the spermatozoa present are dead (Bodalet al,2014).Leukospermia, Presence of leukocyte in ejaculate in an amount greater than reference values. Aspermia, Absence of ejaculate (Omer et al,*2012).*

1-4 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).

Thesemen analysis for fertility evaluation consists of both macroscopicand microscopic examination. Parameters reported include appearance, volume, viscosity**,** pH, sperm concentration and count, motility, and morphology(Suzan andMarjorie,2008).

1-4-1Appearance:

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).

1-4-2 Liquefaction:

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 liquified,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).

1-4-3 Volume:

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).

1-4-4 Viscosity:

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).

1-4-5 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 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).

1-4-6 Sperm Concentration/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).

1-4-7 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).

1-4-8 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, midpiece, and tail. Abnormalities in head morphology are associated with poor ovum penetration, whereas neckpiece, midpiece, and tail abnormalities affect motility.The normal sperm has an oval-shaped head approximately 5 m long and 3 m wide and a long, flagellar 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 andcovers approximatelytwothirds 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 sheaththat 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 theWHO. Strict criteria evaluation is an intregal 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 andMarjorie,2008).

1-4-9 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).

1-5 Lower reference limits (5th centiles andtheir 95% confidence intervals) for semencharacteristics*(WHO Manual for Semen Analysis, 5th edition, 2010)*

Parameter	**Lower reference limit(range)**
Semen volume (mL)	1.5 (1.4-1.7)
Total sperm number (10^6 per ejaculate)	39.0 (33.0 - 46.0)
Sperm concentration (10^6 per mL)	15.0 (12.0-16.0)
Total motility (Progressive and Non progressive, %)	40 (38-42)
Progressive motility (%)	32 (31-34)
Vitality (live spermatozoa, %)	58 (55-63)
Sperm morphology (normal forms, %)	4 (3.0-4.0)
Other consensus threshold values	
Ph	> 7.2
Peroxidase-positive leukocytes (10^6 per mL)	< 1.0
MAR test (motile spermatozoa with bound particles, %)	< 50
Immunobead test (motile spermatozoa with bound beads, %)	< 50
Seminal zinc (μmol/ejaculate)	≥ 2.4
Seminal fructose (μmol/ejaculate)	≥13
Seminal neutral glucosidase (mU/ ejaculate)	≥20

(Jungwirthet al, 2010).

1-6Frequency of semen analyses:

If values are normal according to WHO criteria, one test should suffice. If the results are abnormal, semen analysis should be repeated. It is important to distinguish between oligozoospermia (< 15 million spermatozoa/mL), asthenozoospermia (< 40% motile spermatozoa), and teratozoospermia (< 4% normal forms). Quite often, all three pathologies occur simultaneously asoligo-astheno-teratozoospermia (OAT) syndrome. In extreme cases of OAT syndrome (< 1 million spermatozoa/mL), just as with azoospermia, there is an increased incidence of genetic abnormalities and obstruction of the male genital tract(Jungwirthet al .,2010).

1-7 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).

1-7-1FSH:

Follicle-stimulating hormone (FSH) is ahormone 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 etal,2007).

1-8 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).

1-9 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. 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).

1-10 Male Gonad:

Regulated by SRY (sex-determining region on the Y chromosome), the primary sex cords enter the medulla and differentiate into the seminiferous cords. These are the precursors to the seminiferous tubules where sperm will be produced. The parts of the primary sex cords that extend deepest into the medulla form the rete testes, the first in a series of structures by which sperm leave the testes in adulthood. As the seminiferous tubules are forming, the primordial germ cell(PGC) enter the gonad and associate with the tubules. The PGC will give rise to sperm (after puberty), the cords give rise to the "sustentacular cells" of the tubules,the Sertoli cells. [(11)]In the presence of Sertoli cells, the germ cells remain in meiotic arrest and are inactive spermatogonia until puberty. This phenomenon is due to the secretion by the Sertoli cells of antimüllerianhormone. AMH also signals for mesenchymal cells (intermediate mesoderm) to differentiate into Leydig cells which secret testosterone (Ann-Judith,2002).

1-11 Male gonadal function:

Spermatogenesis and its control

Spermatogenesis takes place in the seminiferous tubules,and requires normal functioning of both the leydig and the sertoli cells.Laydig cells produce testosterone,the principal androgen,under the control of LH.Sertoli cells provide other testicular cells with nutrients,and also produce several regulatory proteins, of which inhibin and androgen binding protein(ABP) are the best characterized.Sertoli cell function is regulated by FSH.Testosterone has crucial paracrine actions in the testes which are required for normal spermatogenesis and fertility (Geoffreyet al,2005).

The entire hypothalamic-pituitary –testicular axis most normally for spermatogenesis .Gn-RH from the hypothalamus stimulates the release of LH and FSH. Its effect on LH release is more marked than on FSH release. The secretion of Gn-RH,and thus of LH.Occurs in pulses; the secretion of FSH is less markedly pulsatile. The amplitude and frequency of the pulses of LH release appear to be important in exerting effects on testosterone production(Geoffrey et al, 2005).The secretion of LH is under negative feedback control from plasma (free testosterone), and the release of FSH is inhibited by inhibin and stimulated by activin,both released by sertoli cells,high testicular,(testosterone) is ensured by the anatomical proximity of leydig,sertoli and spermatogenic cells,and by the local release of ABP. Inhibin is a dimeric glycopeptide comprising a 20 KDa alpha –subunit and a 15 KDa beta-subunit. Two formsof beta-subunit occur, thus two forms of inhibin(inhibin A and B)occur.Although in males, inhibin B is the most important form in plasma, both FSH and testosterone are necessary for inhibin production in normal men.Activinis a dimer of inhibin beta-subunits and has a stimulatory action of FSH release(Geoffrey et al, 2005).

1-12 Abnormal semen analysis in previous study:

The study done by john D for Relationships Between Serum Hormone Levels and Semen Quality Among Men From an Infertility Clinic shows that the serum levels of FSH are inversely associated with sperm concentration, motility, and morphology,their findings for FSH, and sperm concentrations are consistent with those in several previous studies (Sina et al, 1975; Subhan et al, 1995; Jensen et al, 1997; Mahmoud et al, 1998; Pierik et al, 1998; Mabeck et al, 2005), while only two previous studies have reported associations of FSH with sperm motility and morphology. Jensen et al (2004) have reported significant correlations of FSH, with sperm concentration, motility, and morphology, in another study, Uhler et al (2003) also have reported significant bivariate correlations of FSH with sperm concentration, motility, and morphology(JOHN et al,2007).

Study done by Tzu-Chun Wei a,c, William J. Huang a,b,c,*, Alex T.L. Lin a,c, Kuang-Kuo Chen a,c , for The role of hormones on semen parameters in patients with idiopathic or varicocele-related oligoasthenoteratozoospermia (OAT) syndromethey found there was a general trend of elevated FSH levels in patients with OAT syndromes, either with or without varicoceles . This phenomenon is supported by the context that FSH shows inverse correlation with all the semen parameters. For human spermatogenesis, FSH appears more effective than LH in supporting spermatocytes. They hypothesize that a higher FSH may imply a more sensitive hormonal axis feedback, which results in better sperm production in a patient with OAT syndrome(Weiet al,2013).

The study done by Jennifer Gordetsky Edwin van Wijngaarden* and Jeanne O ' Brien† for Redefining abnormal follicle-stimulating hormone in the male infertility population found a correlation between increasing FSH levels and abnormal semen parameters. The study is the first to attempt to redefine the 'normal' reference range of FSH. In the study, an FSH level >4.5 IU/L associations with abnormal sperm concentration and morphology. Although the 'normal' range for FSH is qualified as a value in the range 1.4 – 18.1 IU/L, the study has shown that an FSH level >4.5 IU/L is predictive of an abnormal semen analysis in terms of morphology and sperm concentration. Therefore, we suggest that an FSH level >4.5 IU/L in the male should be considered as an abnormal value when evaluating patients with possible male infertility (Jenniferet al , 2011).

Study done by V.Novero*et al.* for Relationship between serum follicle stimulating hormone in the male and standard sperm parameters, and the results of intracytoplasmic sperm injection, their study has confirmed the inverse relationship between serum FSH concentration and sperm concentration.Furthermore, it has shown that no relationship exists between FSH concentration and progressive sperm motility, sperm morphology and the results (fertilization,

cleavage,implantation, and pregnancy rates) of ICSI. The need to measure serum FSH concentration in the male has to be reviewed in patients being prepared for ICSI because FSH will be of no benefit in predicting the success or failure of treatment. It may, however, be useful in distinguishing obstructive from non-obstructive azoospermia (Novero*et al*,1997).

Study done by DhananjayVasantraoBhale*, Roshan Kumar Mahat** for Evaluation of LH, FSH and Testosterone in Infertile Males was shown higher concentration of FSH to be a reliable indicator of germinal epithelial damage,also was showed to be associated and clearly indicate significant increase in FSH with azoospermia and severe oligozoospermia. From this study high plasma levels of FSH, low sperm count are pathognomonic of male infertility. The high level of FSH is an indication of testicular problems as the cause of infertility(Dhananjay and Roshan, 2013)

1-13 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 FSH is important.

1-14 **Objectives**

1-14-1General objective:

The assess serum FSH levels in Sudanese male with abnormal semen analysis at Shendi city-Sudan

1-14-2 Specific objectives:

- To determine FSH in patient with azoospermia, oligozoospermia and asthenozoospermia
- To correlate between level FSH and sperm count
- To correlate between level FSH and sperm motility

Chapter Two:

Materials

&

Methods

2-1Study aria:

This study was conducted in Shendi city during 2016, Shendi is situated on the east bank of the River Nile 150 Km northeast of capital Khartoum,and it is covering area of 30 km^2

2-2Study design:

A descriptive cross-sectional study conducted between March to May2016

2-3Study population:

study done on males who were attended the fertility clinic for infertility study in the period of the study.

2-4 Sample size:

A 115 males randomly were enrolled in this study,and then classified based on semen sample resultto two groups, group one normal semen analysis result (61 males)and group two abnormal semenanalysis result (54 males)

2-5 Inclusion criteria:

Sample were collected from individuals attended the fertility center

2-6Exclusion criteria:

Subjects with diabetes mellitus, renal diseases, and hypertension and thyroid disease have been excluded from the study.

2-7 Collection of Samples:

A 115 Semen sample was collected by masturbation into sterile plastic containers Subjects were instructed to abstain from ejaculation for at least 48 hours prior to producing the semen sample. And 54 Blood samples (3ml) were collected in heparin containers. Then plasma obtain by centrifuged at 4000 RPM ,and transferred to plain containers, and stored in -20° until use.

2-8 Ethical Considerations:

Study was approved from ethical committee of the Sudan University of Science and Technology, verbal informed consent was obtained and all patients were informed by aims of the study

2-9 Method of sperm count:

10 µl from semen add to 2 ml of semen diluent, mix and take drop on semen chamber, wait 2 min and count under microscope by X^{10}

2-10 TOSOH Automated immunoassay systems:

2-10-1Principle of FSH estimation:

The ST AIA – PACK FSH is a tow side immunoenzymometric assay witchis performed entirely in the ST AIA – PACK FSH test cups. FSH present in the test sample is pound with monoclonal antibody immopelized on a magnetic sold face and enzyme – labeled monoclonal antibody in test cups. 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 FSH concentration in the test sample. A standard curve is constructed and unknown sample concentrations are calculated using this curve.

2-11 Statistical Analysis

Dataanalysis was performed using statistical package of social science (SPSS computer program), frequencies, Means, SD, and Pearson's correlation have been used to compareand correlate between parameters and study variables

Chapter Three:

Results

3- Results

This study included 115 males (61males normal semen) and (54males) abnormal semen and FSH).The serum levels of FSH were inversely correlate with sperm concentration and motility. The mean of serum FSH in azoospermia was (17.96±12.46 m IU/mL) which is significantly high compared to reference values (1.4-5.5m IU/mL), p.value was 0.000, and in Oligoasthenozoospermia was (9.26±6.10 m IU/mL) and p.value was 0.000, the mean of serum FSH was (5.0±2.29 m IU/mL) with p.value 0.005 in asthenozoospermia, and in Sevreoligozoospermia was (10.33±4.04 m IU/mL) with p.value of 0.000, and oligozoospermia group the mean of serum FSH was (8.13±4.00 m IU/mL) and p.value was 0.001, for these and referring to (Figure 3.1) and (Figure 3.2) there was statistically significant negatively correlation between serum FSH and abnormal semen analysis parameter (r= -0.315, *p*-value 0.021) and (r=-0.295, *p*-value 0.030) respectively. Serum FSH level is not correlated with age with R (0.564) and p (0.000) in (Figure 3.3).

Table (3.1) mean cncentrationof FSH among abnormal semen analysis

Parameter	Mean	Std. Deviation	Reference value	P-value
Azoospermia	17.96	12.46	1.4-5.5	0.000
Oligoasthenozoospermia	9.26	6.10	1.4-5.5	0.000
Ashenozoospermia	5.00	2.29	1.4-5.5	0.005
Sevreoligozoospermia	10.33	4.04	1.4-5.5	0.000
Oligozoospermia	8.13	4.00	1.4-5.5	0.001

Table (3-2) volume frequency and percentage

Type	Frequency	Percentage (%)
< 2	36	31.3
2-4	74	64.3
5-7	5	4.3
Total	115	100.0

Table (3-3) Liquefaction frequency and percentage

Type	Frequency	Percentage (%)
< 30	106	92.2
31-60	6	5.2
>61	3	2.6
Total	115	100.0

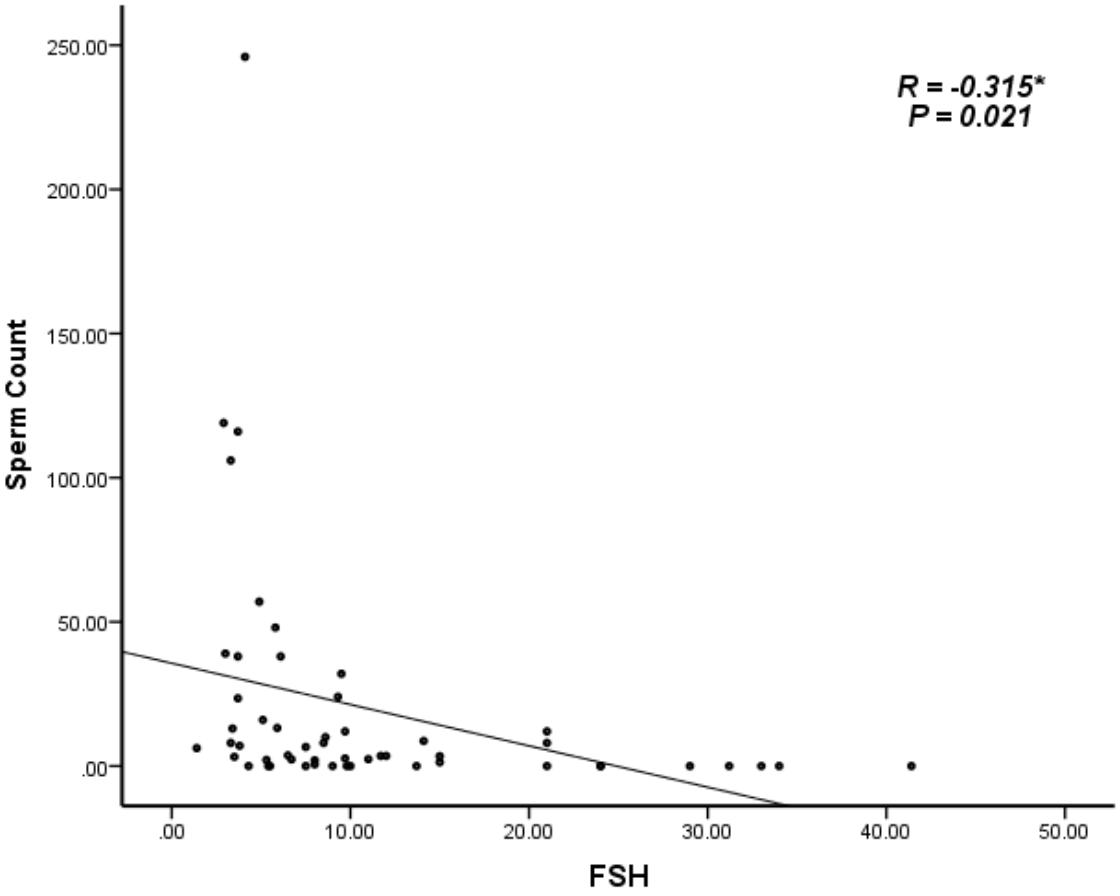

Figure (3.1) the result shows that there was negative correlation between FSH and Sperm count with R (-0.315) and p (0.021)

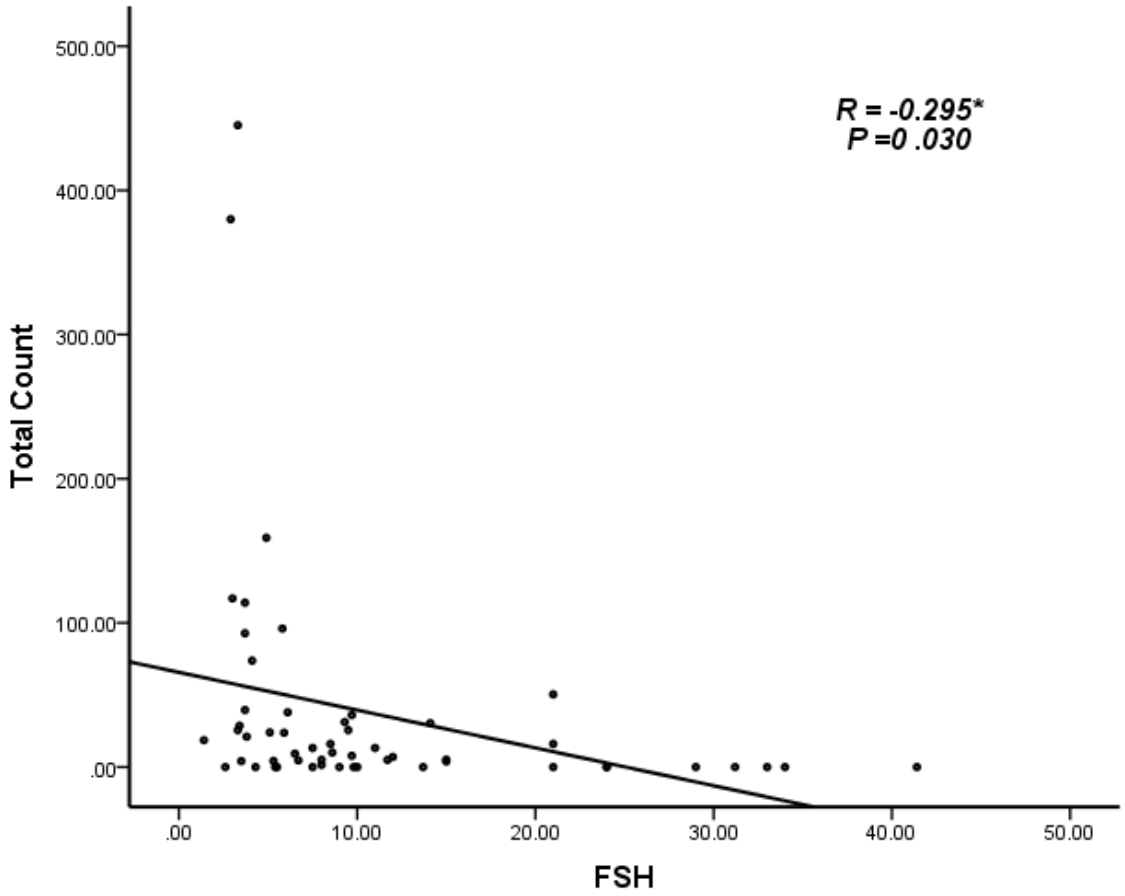

Figure (3.2) the result shows that there was negative correlation between FSH and Total Sperm count with R (-0.295) andP (0.030)

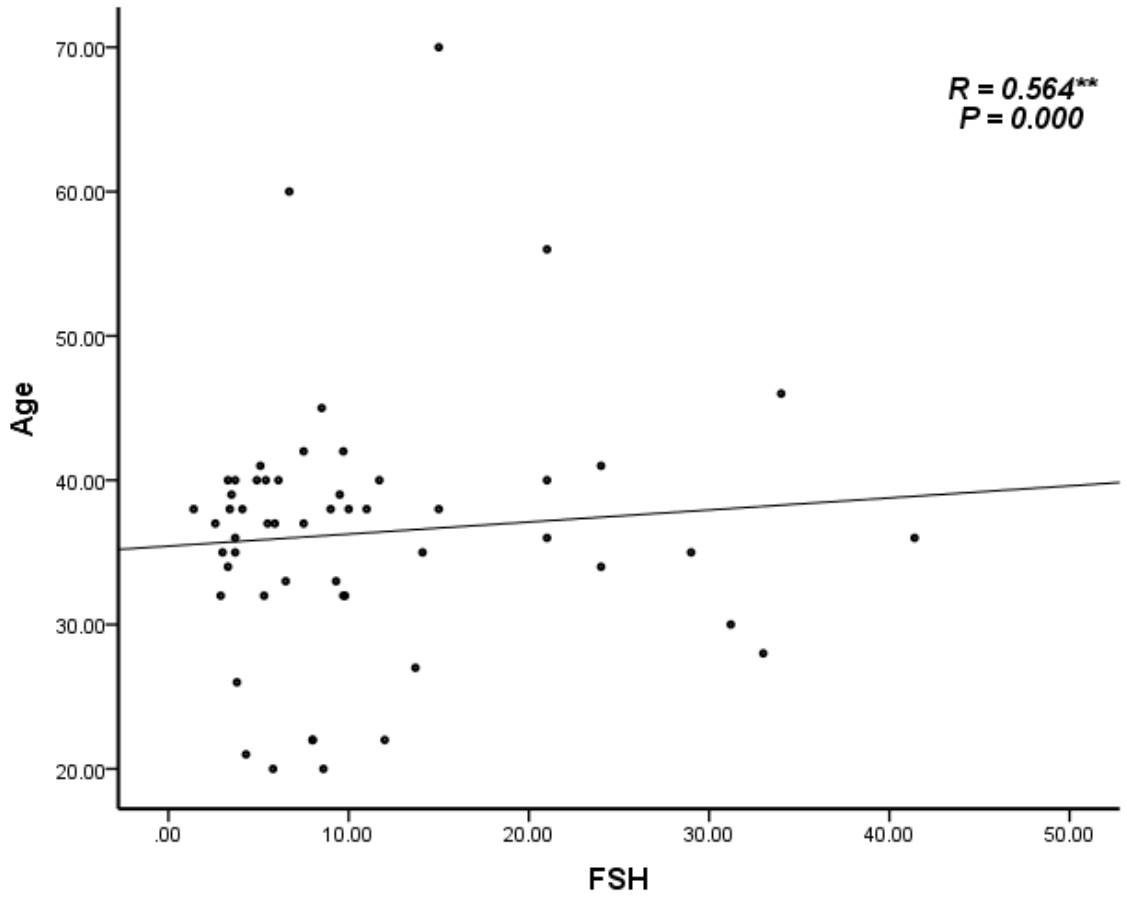

Figure (3.3) the result shows that there was positivecorrelation between FSH and Agewith R (0.564) and p (0.000)

Chapter four:

Discussions

&

Conclusion

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Recommendations

4-1Discussion.

It is extremely important evaluationmale infertility to consider the reproductive hormone levels.It reported that these hormone have a major role in male spermatogenesis.LH, FSH and Testosteroneevaluation isuseful in management of male infertility. For initiation of spermatogenesis and maturation of spermatozoa, FSH is necessary of infertile men.Higher concentration of FSH is considered to be reliable indicator for germinal epithelial damage, and shown to be associated with Azoospermia and severe oligozoospermia (Dhananjay andRoshan, 2013).

This present study revealed that, there was inversely association between FSH levels and abnormal semen parameterswith significant *p*–value.

Our finding was in agreement with previous reports who stated that, there was inversely association between serum FSH and semen concentration and motility(JOHN et *al*. 2007; Sina et al, 1975; Subhan et al, 1995; Jensen et al, 1997; Mahmoud et al, 1998; Pierik et al, 1998; Mabeck et al, 2005) shows that the serum levels of FSH are inversely associated with sperm concentration, motility, and morphology, while other previous studies have reported associations of FSH with sperm motility and morphology(Jensen et al ,2004) have reported significant correlations of FSH, with sperm concentration, motility, and morphology , In another study, (Uhler et al ,2003) also have reported significant bivariate correlations of FSH with sperm concentration, motility, and morphology. In contrast withother study reported found a correlation between increasing FSH levels and abnormal semen parameters (Jennifer *et al*,2011) we found a correlation between increasing FSH levels and abnormal semen parameters. the study is the first to attempt to redefine the ‘ normal ’ reference range of FSH. In the study, an FSH level >4.5 IU/L associations with abnormal sperm concentration and morphology. Although the ‘ normal ’ range for FSH is qualified as a value in the range 1.4 – 18.1 IU/L, the study has shown that an FSH level >4.5 IU/L is predictive of an abnormal semen analysis in terms of morphology and sperm concentration. Therefore, we suggest that an FSH level >4.5 IU/L in the male

should be considered as an abnormal value when evaluating patients with possible male infertility(Wei. et al, 2013) we found there was a general trend of elevated FSH levels in patients with OAT syndromes, either with or without varicoceles . this phenomenon is supported by the context that FSH shows inverse correlation with all the semen parameters. For human spermatogenesis, FSH appears more effective than LH in supporting spermatocytes.

The results of present study provide evidence that, serum FSH level was significantly inversely in abnormal semen. There was agreement correlation between sperm count and total sperm count with serum FSH levels because the R=0.315, p = 0.021 and R = -0.295, P = 0.030 respectively.

4-2Conclusion

From this study it can be concluded that there is inverse association between serum level of FSH,low sperm count and motility are pathognomonic of male infertility. Thehighlevel of FSH may be an indication of testicular problems as the cause of infertility in the studied subjects.

4-3 Recommendations

Use of FSH estimation as indicator of spermatogenesis.

Other pituitary and gonadal hormons should be studied to identify the relationship.

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Appendixes

Appendix (1)

Sudan University of Science and Technology

College of Graduate studies

M.SC of medical laboratory

Questionnaire

-Patient code

-Age:

-Time from married and sexual activity:

Less than 1 year more than 2 years

-Disease:

Diabetes mellitus and hypertension

Thyroid disease/any other endocrine problem

Chronic renal failure

Others

-family history of infertility:

Yes( ) No( )

-predisposing factors:

Trauma

Surgery

Others

-Duration of treatment

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