MAR Gynecology & Urology (2024) 6:3

Research Article

An Assessment of Serum FSH Levels in Males with Azoospermia and their Effect on Spermatogenesis

Rania Dahab *

*Correspondence to: Rania Dahab, Quttainah Medical Center (Department of Reproductive Medicine and Infertility) Kuwait.

Received: 25 March 2024

Published: 29 March 2024

Abstract

Background: *Azoospermia is seen in clinics and labs in patients that walk in for a semen analysis. Azoospermia is seen in many reports.*

Objective: *To evaluate if there is any association between hormonal disturbance in regards to FSH level and an Azoospermic semen analysis. The aims and objectives of the study are to investigate the presence of spermatogenesis among azzospermic patients and smokers and to investigate the high level of FSH among azzospermic patients.*

Material and Method: *The study design was observational. The study was conducted at Lahore Institute of Fertility and Endocrinology (LIFE). This study was held at Hameed Latif Hospital andrology Laboratory, New Garden Town Lahore. The sample size was 49 subjects coming for routine semen analysis.*

Result: *The results indicated relationship between FSH and Azoospermia .They were 28.57% patients have altered FSH level. And 72.43% patients have normal FSH level. There were 8.10% of patients who had high FSH level but showed evidence spermatogenesis. Another group of had normal FSH 24.48% but did not have any evidence of spermatogenesis.*

Conclusion: *It is concluded that FSH is strongly associated with spermatogenesis. Many other factors are involved like smoking habit. But these are not associated with spermatogenesis.*

Key Words: *Azoospermia, Follicle-stimulating Hormone, Obstructive, Non Obstructive*

Introduction

Azoospermia is a medical condition where a man does not have any measurable level of sperm in his semen, leading to very low fertility or even sterility. It is a major cause of male sub fertility, which prevents a man from getting a woman pregnant after 1 year of unprotected regular sex. This means that no birth control methods, such as birth control pills, diaphragms, condoms, or the rhythm method, have been used. It occurs in 5% of infertile men and may be caused by one or both of two conditions.

Obstructive azoospermia can occur due to genetic conditions such as congenital bilateral absence of the vas deferens, infections of the male reproductive system, trauma, smoking, drinking alcohol, and using illegal

drugs. Genetic conditions such as Klinefelter syndrome and Kallmann syndrome can also affect sperm production or development of reproductive organs. Abnormal hormone levels may be caused by disorders of the testicles, which may affect the production of sperm. Radiation used to treat cancer may affect sperm production.

Non-obstructive azoospermia can be caused by medications like steroids, antibiotics, and medicines used to treat inflammation or cancer. Smoking, drinking alcohol, and using illegal drugs may also cause problems with sperm production. Genetic conditions may affect sperm production or development of reproductive organs. Abnormal hormone levels may be caused by disorders of the testicles. Radiation used to treat cancer may affect sperm production. Retrograde ejaculation is a condition that causes semen to travel into the bladder instead of outside the body, usually caused by a problem with the neck of the bladder and may be due to spinal cord injuries, medicines, or diabetes. Pesticides, heavy metals, heat, and undescended testes can also affect sperm production.

Signs and symptoms of azoospermia include inability to get your partner pregnant, increased body fat, body hair, and breast tissue, clear, watery, or whitish discharge from the penis, presence of a mass or swelling on the scrotum that feels like a bag of worms (varicocele), stress or emotional pressure from not being able to conceive a child, small, soft, or cannot be felt testicles, and enlarged, twisted veins in the scrotum.

Diagnosis of azoospermia involves a physical exam, biopsy, blood tests, genetic testing, an MRI, spermatic venography, scrotal or transrectal ultrasound, and semen analysis. The follicle-stimulating hormone (FSH) blood test measures the level of FSH in the blood, which is released by the pituitary gland and regulates the development, growth, pubertal maturation, and reproductive processes of the body.

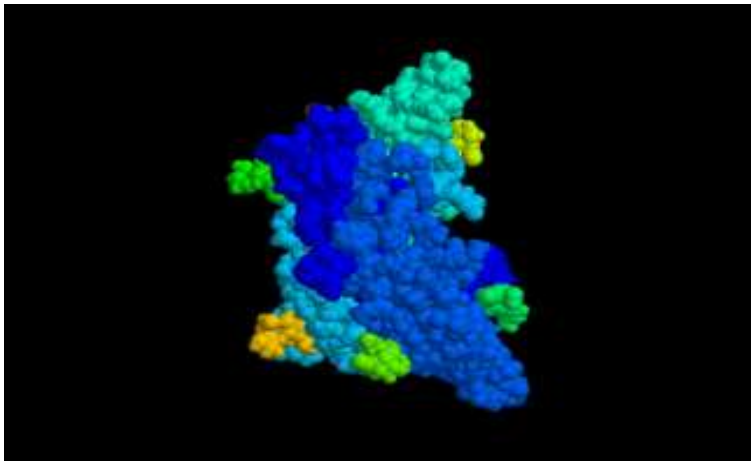

Figure 1: Follicle-stimulating hormone (Jiang et al., 2012)

FSH is a hormone that stimulates the growth and recruitment of immature ovarian follicles in the ovary. It is crucial in early antral follicles, which are 2-5 mm in diameter, to prevent apoptosis. In the luteal-follicle phase transition period, serum levels of progesterone and estrogen decrease, leading to FSH peaking around day three. The cohort of small antral follicles is large enough to produce enough Inhibin B to lower FSH serum levels. A sharp increase in estradiol production by the dominant follicle can cause a positive effect on the hypothalamus and pituitary, leading to rapid GnRH pulses and an LH surge. However, this decrease in serum FSH levels can cause smaller follicles to undergo atresia, as they lack sufficient sensitivity to FSH to survive. Occasionally, two follicles reach the 10 mm stage at the same time, and as both are equally sensitive to FSH, they survive and grow in the low FSH environment, potentially leading to non-identical (dizygotic) twins.

Stages	Reference Values (FSH)	Unit
Follicular	3.9-8.8	IU/L
Midcycle	4.5-22.5	IU/L
Luteal	1.8-5.1	IU/L
Postmenopausal	16.7-113.6	IU/L

Table No 1: *FSH Level in Female* (Dickerson *et al.*, 2008)

FSH, a hormone, stimulates the first division of meiosis in males, forming secondary spermatocytes. It also enhances androgen-binding protein production by Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes, crucial for spermatogenesis.

Reference Values

Age	Follicle-stimulating hormone	Unit
1-7 days:	< or =3.0 IU/L	IU/L
8-14 days	< or =1.4 IU/L	IU/L
15 days-3 years	< or =2.5 IU/L	IU/L
4-6 years	< or =6.7 IU/L	IU/L

7-8 years	< or =4.1 IU/L	IU/L
9-10 years	< or =4.5 IU/L	IU/L
11 years:	0.4-8.9 IU/L	IU/L
12 years	0.5-10.5 IU/L	IU/L
13 years:	0.7-10.8 IU/L	IU/L
14 years	0.5-10.5 IU/L	IU/L
15 years	0.4-18.5 IU/L	IU/L
16 years	< or =9.7 IU/L	IU/L
17 years	2.2-12.3 IU/L	IU/L
> or =18 years	1.0-12.0 IU/L	IU/L

Table No 2: *FSH Level in Male* (Boulpaep *et al.*, 2005)

High FSH levels are due to primary testicular failure. This can be the result of developmental defects in testicular growth or to testicular injury, as indicated below.

Developmental defects:

- Failure to develop gonads (gonadal agenesis)
- Chromosomal abnormality, such as Klinefelter syndrome

Testicular failure:

- Viral infection (mumps)
- Trauma
- Radiation exposure
- Chemotherapy
- Autoimmune disease
- Germ cell tumor

Spermatogenesis is the process of producing spermatozoa from male primordial germ cells through mitosis and meiosis. The process begins with spermatogonia, which yields primary spermatocytes. These spermatocytes divide meiotically into two secondary spermatocytes, which divide into two spermatids. These develop into mature spermatozoa, also known as sperm cells. Spermatozoa are the mature male gametes in sexually reproducing organisms and are the male version of gametogenesis. In mammals, spermatogenesis occurs in the seminiferous tubules of the male testes stepwise. It is highly dependent on optimal conditions and is essential for sexual reproduction. DNA methylation and histone modification regulate this process. Spermatogenesis starts at puberty and usually continues uninterrupted until death, with a slight decrease in produced sperm with age. This process produces mature male gametes, known as spermatozoa, which fertilize the female gamete, the oocyte, during conception to produce a single-celled individual called a zygote. In humans, chromosomal abnormalities resulting from incorrect spermatogenesis can lead to congenital defects, abnormal birth defects, and spontaneous abortion of the developing fetus.

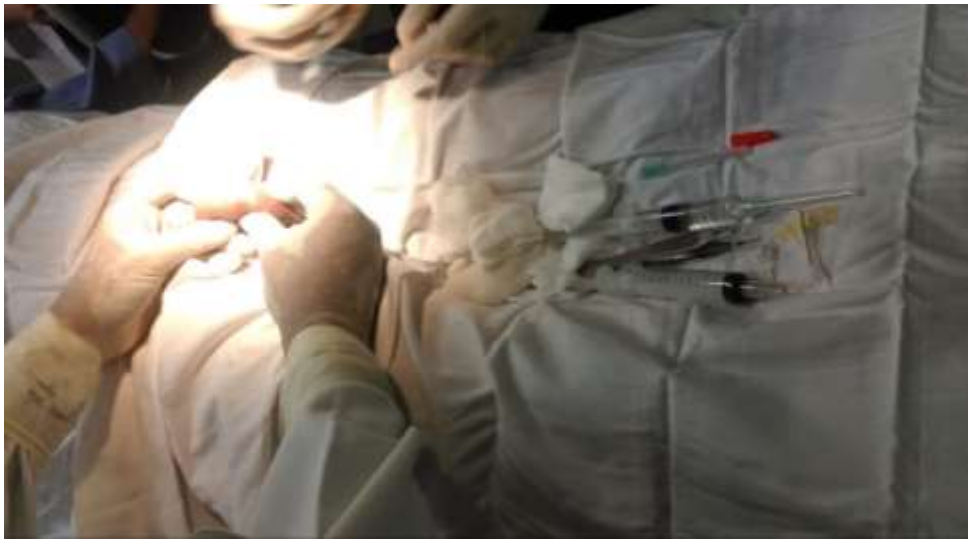

Fig No 2: *FNA* (Toulis, *et al.*, 2010)

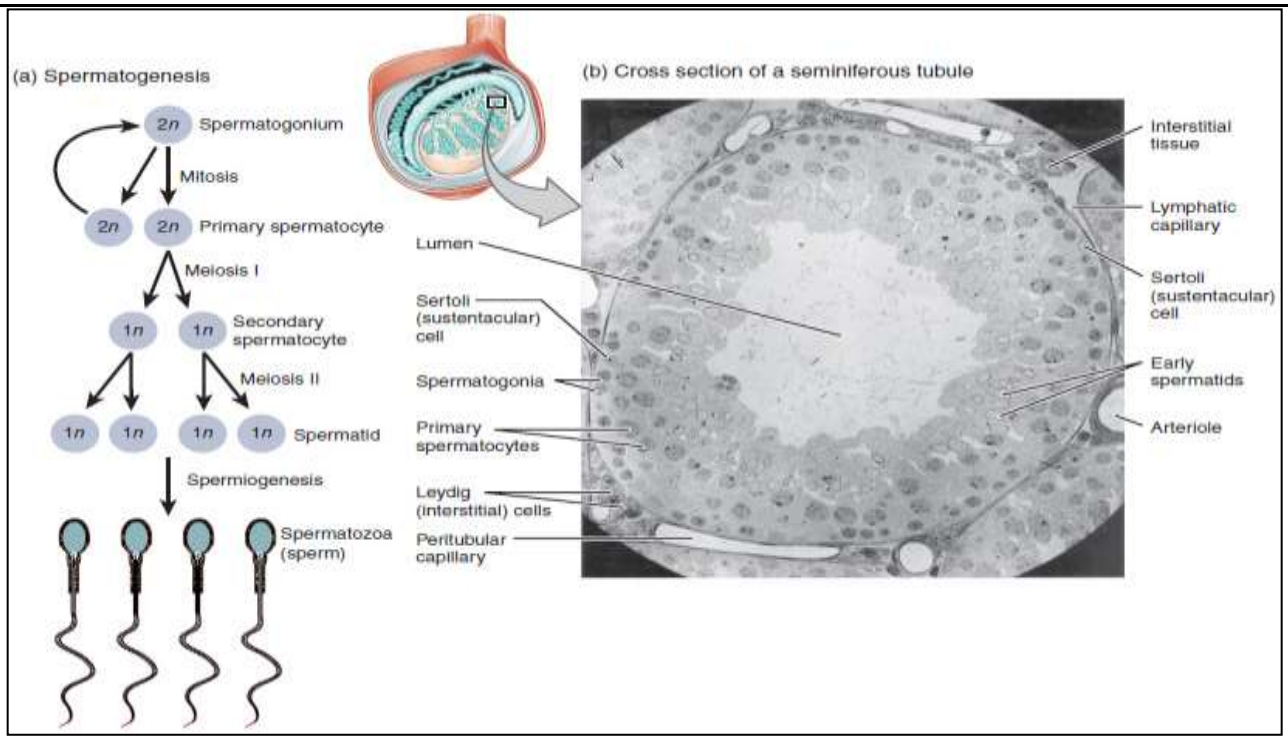

Fig No 3: *Spermatogenesis* (Toulis, *et al.*, 2010)

Mitosis is the process of cell duplication, where two daughter cells are formed with the same DNA and chromosomal content as the original diploid (2N) mother cell. In mammals, cells contain 46 chromosomes, 22 pairs of homologous autosomes, and one pair of sex chromosomes. Meiosis is a special process of reductional cell division, resulting in the formation of four gametes containing half (1N) the number of chromosomes found in somatic cells. Haploid gametes unite at fertilization to create a diploid zygote.

During spermatocytogenesis, primitive cells called spermatogonia proliferate by mitosis. The diploid number of primary spermatocytes is halved during meiosis, and a primary spermatocyte is transformed into two secondary spermatocytes during meiosis I, which are then converted into (1N) spermatids during meiosis II. Spermatocytes and spermatids tend to be larger than their ancestral spermatogonia.

Males have an almost unlimited capacity to produce germ cells, achieved by replenishment of spermatogonia early in mitosis. Spermiogenesis involves nuclear condensation, formation of the acrosomal cap, and development of a tail. Spermatocytes retain a rounded configuration throughout spermatocytogenesis, while

spermatids undergo a dramatic change in form during spermatogenesis, becoming streamline spermatozoa adapted for fertilization.

Spermiation is the process by which spermatozoa are released from the seminiferous epithelium into the lumen of the tubule. Most of the "excess baggage" (cytoplasm and organelles) of the spermatid is discarded within the seminiferous epithelium in the form of a residual body. A small amount of cytoplasmic material, the cytoplasmic droplet, remains attached within the neck region or around the middle piece as the spermatozoon makes its way into the epididymis. Seminiferous spermatozoa lack motility and fertilizing capacity.

The spermatogenic cycle and wave are distinct associations of sperm cells, with each stage following in an orderly sequence along the length of the tubule. The number of stages within a spermatogenic cycle and the number of cycles required for the completion of spermatogenesis varies between species. There are 12 different stages of the cycle in the bull of about 14 days each, with approximately four cycles within a given region of the tubule occurring before A1 spermatogonia is transformed into a spermatozoa.

Hormonal regulation plays a role in spermatogonia, with hypophysectomy arresting spermatocytogenesis at the primary spermatocyte stage. Hormonal effects on sperm cells are not direct but are mediated through Sertoli cells. The rate of production of spermatozoa is not influenced by endocrine therapy.

As sperm cells mature, they move between Sertoli cells from the basal toward the adluminal compartment of the seminiferous tubule. Occluding junctions that interconnect adjacent Sertoli cells shield secondary spermatocytes, spermatids, and spermatozoa from autoimmune recognition. The blood-testis barrier also acts to conserve certain products of Sertoli cells within the seminiferous tubule, such as ABP.

Temperature affects sperm cells, as they will not mature at core body temperature in most mammals. To adapt, the testes assume an external position, with uterine descent typically occurring during fetal or neonatal life. High ambient temperatures can sometimes be associated with infertility, but some mammals do not have scrotal sacs and the testes remain within the abdomen.

Materials and Methods

Study Design:

It was two months study in which diagnosed azzospermic patients + smokers were selected.

Setting:

The study was undertaken at Life department of Hameed Latif Hospital, Lahore, Pakistan.

Study Duration:

This study was started after the approval of synopsis and the time duration of the study was 3 month i.e. from 15 jun 2015 to 15 September 2015.

Sample Size:

A total of 49 azoospermic Patients samples were taken.

Data Collection Instruments:

The Performa was designed as a data collection tool in order to collect the information about the azoospermicin semen analysis and FSH in blood. The Performa was comprised demographic data.

Sample Collection:

Total 49 samples of azzospermic patients were selected. The diagnosis was based on testicular aspiration. **PESA** (percutaneous epididymal sperm aspiration): **TESA** (testicular sperm aspiration). Patients consent form and patient's Performa was dully filled to investigate the history of the patients. The samples were collected on the basis of inclusion and exclusion criteria.

Patient's inclusion criteria

1. Azzospermic diagnosed patients were selected.
2. Smokers and non Smokers
3. Gender (male).
4. Age > 50 years.
5. High fever patients.

Patient's exclusion criteria

1.Patients have hypertension.

2.Patients have Asthma

3. Patients have allergy.

Fig No 4*: Maceration the testicular tissue*

Enzymatic method:

1.Incubate the testicular tissue with collagenase (e.g. 0.8 mg of Clostridium histolyticum, type 1A per ml of medium) for 1.5–2 hours at 37 °C, vortexing every 30 minutes.

2. Centrifuge at 100g for 10 minutes and examine the pellet.

Mechanical method:

1. Macerate the testicular tissue in culture medium with glass cover slips until a fine slurry of dissociated tissue is produced.

2. Alternatively, strip the cells from the seminiferous tubules using fine needles (attached to disposable tuberculin syringes) bent parallel to the base of the culture dish.

3. Wash the specimens obtained by adding 1.5 ml of liquid with HAS culture medium.

4. Centrifuge at 300g for 8–10 minutes.

5. Remove the supernatant and resuspend the pellet in 0.5 ml of fresh culture medium.

6. Make a wet smear for observation

7. Count Total number of spermatozoa in per HPF's seen at x40

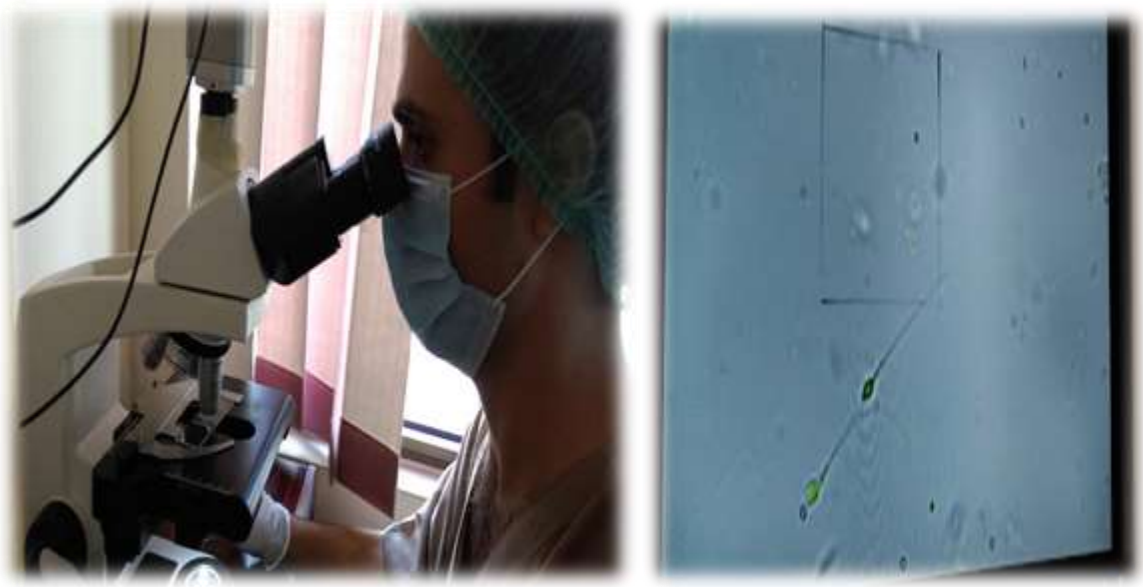

Fig No 5: *Observation of Spermatogenesis*

Morphology

Fixing the air-dried semen smear Immerse slides in triarylmethane fixative (as provided in the Diff-Quik kit method) for 15 seconds or 95% methanol alone for 1 hour. Drain the excess solution by placing slides vertically on absorbent paper. Staining the fixed semen smear sequentially immerses the slides in:

1. Rapid stain solution 1 for 10 seconds.

2. Rapid stain solution 2 for 5 seconds.

3. Running tap water 10 to 15 times to remove excess stain Drain the excess solution at each step by placing slides vertically on absorbent paper.

4. Mounting the stained smear.

5. Observe x100 oil-immersion bright field objectives and at least x10 eyepieces should be used.

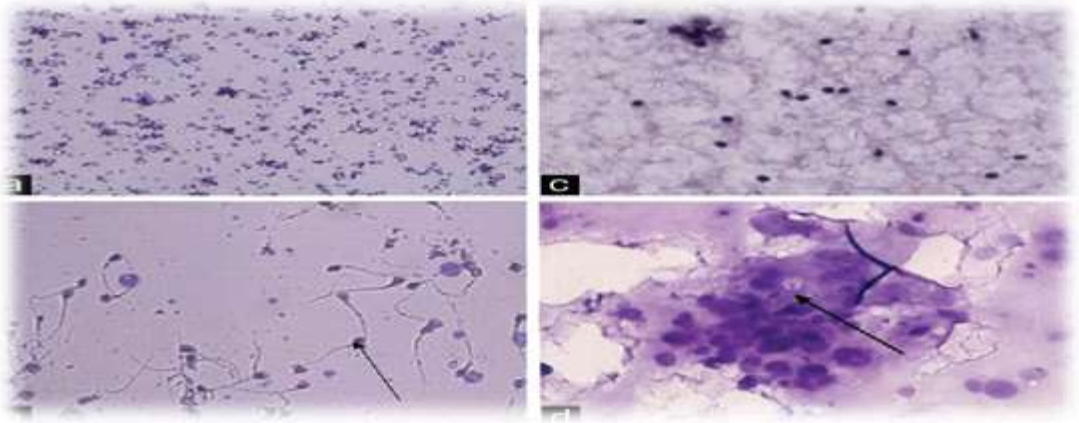

Fig No 6: *Diff-Quik rapid stain*

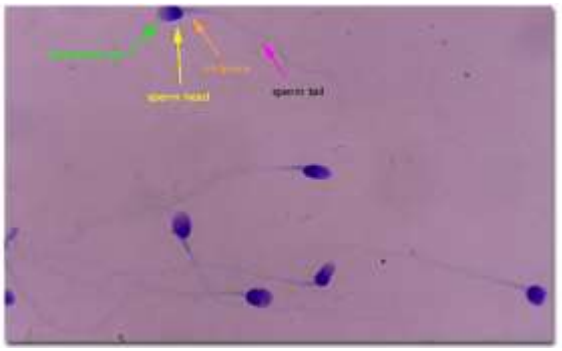

Fig No 7: *Giemsa Stain*

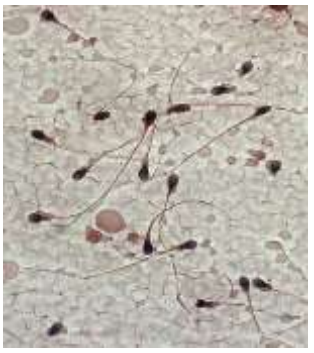

Fig No 8: *H &D Stain*

Results

The present study was conducted to find out effect of serum FSH on spermatogenesis in males with azoospermia. About 49 samples were collected.

The age description showed that minimum age found was 22 years and maximum age was 44 years with mean age 32.63±5.5 years.

	N	Minimum	Maximum	Mean	Std. Deviation
Age of Patient	49	22	44	32.63	5.510

Table No 3: *Descriptive Statistics of Age factor*

The history Performa showed that among 49 subjects, 31 (63.3%) were smokers and 18 (36.7%) were non-smokers.

	Frequency	Percent
Non-Smoker	18	36.7
Smoker	31	63.3
Total	49	100.0

Table No 4: *Smoker vs. Non Smokers*

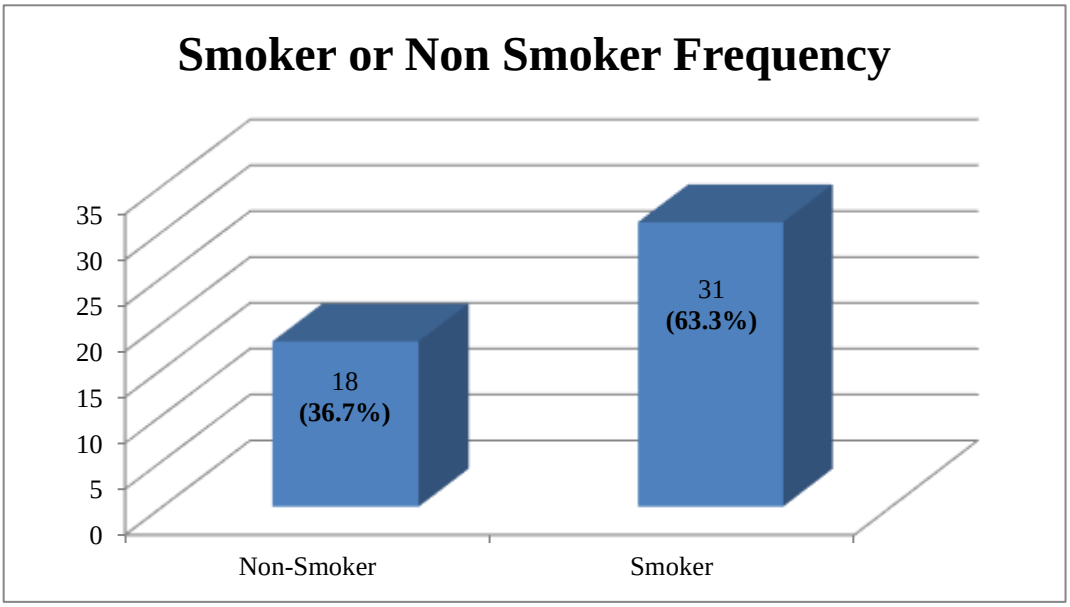

Fig No 9: *Smoker vs. Non Smokers*

The Logistic regression was applied to find out association between spermatogenesis and FSH level. The

statistical analysis shows that FSH level is strongly associated with spermatogenesis. Whereas when other historical factors are associated with spermatogenesis, it was seen that there is no association between history (like smokers & non- smokers) and spermatogenesis.

	Parameter (B)	S.E.	Sig.	Exp(B)	p-Value
FSH	-.134	.059	.024	.875	
History	-1.328	.711	.062	.265	$p < 0.05$
Constant	2.288	.768	.003	9.851	

Table No 5: *Statistical analysis for FSH studies*

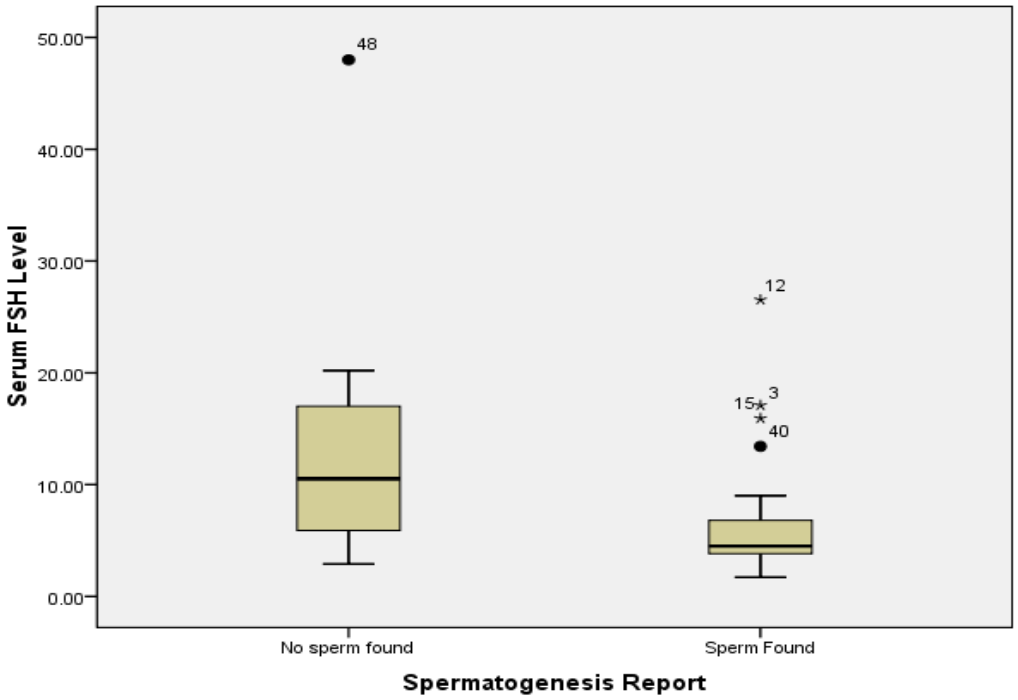

Fig No: 10

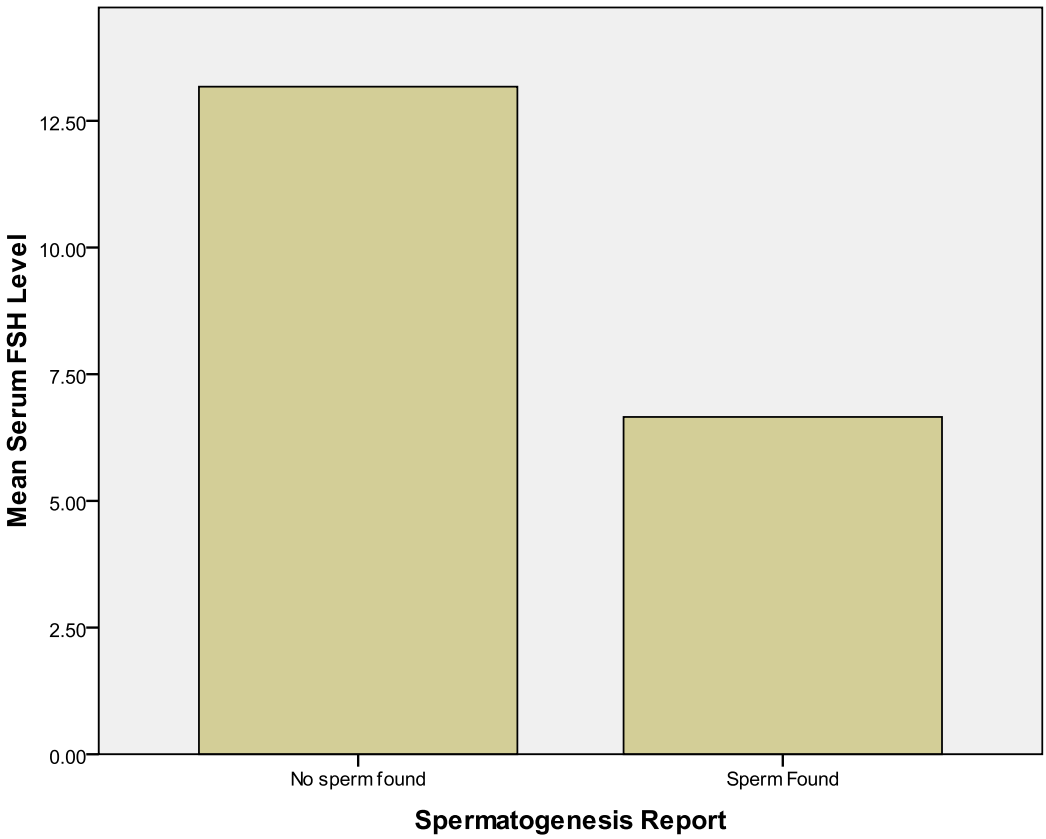

Fig No: 11

Discussion

Kretser, *et al.,* in 2013 explained the relationship between specific types of germinal cells in testicular biopsy specimens and serum FSH levels has been investigated in 49 men with infertility. Testicular biopsies were analyzed quantitatively by counting the germinal cells present in cross-sections of 25 different seminiferous tubules. These quantitative histological studies demonstrated that a significant correlation exists between serum FSH levels and the mean number of spermatogonia, primary spermatocytes, early and late spermatids per tubule when each group is taken singly. No correlation was evident between the serum FSH level and the mean number of Sertoli cells. The results indicate that the most significant correlation obtained was between the mean number of spermatogonia and serum FSH levels and suggest that correlations obtained between the more mature germinal cells and FSH levels may result from the former relationship (Kretser, *et al.*, 2013).

And our study also shows effect of serum FSH level on spermatogenesis, if spermatogenesis is high then

FSH level was low. It is concluded that FSH should be investigate to rule out a cause. If FSH is high, the patient can be referred a specialist for correction of FSH. If FSH is in normal rang an andrologist can performed fine needle aspiration to determine if spermatogenesis exist.

The results indicated relationship between FSH and Azoospermia .They were 28.57% patients have altered FSH level. And 72.43% patients have normal FSH level. There were 8.10% of patients who had high FSH level bt showed evidence spermatogenesis. Another group of had normal FSH 24.48% but did not have any evidence of spermatogenesis.

Kretser, et al., in 1989 stated that Serum concentrations of inhibin, The mean (±S.D) serum concentrations of inhibin in the normal men was 554 ± 156 U/l and did not differ significantly from those groups with oligospermia, azoospermia or Klinefelter's syndrome. Combined analyses of all groups did not reveal any significant correlation between serum concentrations of inhibin and FSH or with any other parameter measured. Serum concentrations of FSH and LH were positively correlated, and Leydig cell dysfunction, as evidenced by increased serum LH levels, low testosterone levels or a declining testosterone/LH ratio were found with severe spermatogenic damage. The failure of serum concentrations of inhibin to correlate with those of FSH levels or the degree of testicular damage raise questions as to the clinical value of this parameter alone (Kretser, et al.,1989).

Tony et al., in 2013 explained the aim of this review is to provide an integrative analysis of the role of FSH in the control of testicular function in higher primates, including man.. Inhibin B is the major component of the testicular negative feedback signal governing FSHβ gene expression and FSH secretion, and the evidence for this view is presented. The review concludes with the presentation of a model for the operation of the FSH-inhibin B feedback control system regulating sperm production postpubertally in and man, and with speculation on issues of clinical interest (Tony et al., in 2013).

Conclusion

Our study correlates with previous studies conducted by Kretser, et al., in 2013, 1989 and Tony et al., in 2013. We studied 49 patients of azoospermia, their result also show thas a higher FSH level effects spermatogenesis. This is evidence by no sperm when testicular biopsy was performed.

References

Borawski D, Bluth MH. Reproductive function and pregnancy. In: McPherson RA, Pincus MR, eds.Henry's Clinical Diagnosis and Management by Laboratory Methods

Boulpaep EL, Boron WF (2005). Medical physiology: a cellular and molecular approach. St. Louis, Mo: Elsevier Saunders. p. 1125.

Boulpaep EL, Boron WF (2005). Medical physiology: a cellular and molecular approach. St. Louis, Mo: Elsevier Saunders. p. 1125.

Carter, J.Stein (2014-01-14). “Mitosis”.biology.clc.uc.edu.

de Kretser DM, McLachlan RI, Robertson DM, Burger HG.J Endocrinol. 1989 Mar;120(3):517-23.

Dickerson LM, Shrader SP, Diaz VA (2008). "Chapter 8: Contraception". In Wells BG, DiPiro JT, Talbert RL, Yee GC, Matzke GR. Pharmacotherapy: a pathophysiologic approach. McGraw-Hill Medical. pp. 1313–28.

Dickerson LM, Shrader SP, Diaz VA (2008). "Chapter 8: Contraception". In Wells BG, DiPiro JT, Talbert RL, Yee GC, Matzke GR. Pharmacotherapy: a pathophysiologic approach. McGraw-Hill Medical. pp. 1313–28.

Dohle, Gert R (2010). "Male infertility in cancer patients: Review of the literature".International Journal of Urology 17 (4): 327–331. doi:10.1111/j.1442-2042.2010.02484.x.

Drabovich, A. P.; Dimitromanolakis, A.; Saraon, P.; Soosaipillai, A.; Batruch, I.; Mullen, B.; Jarvi, K.; Diamandis, E.P. (2013). "Differential Diagnosis of Azoospermia with Proteomic Biomarkers ECM1 and TEX101 Quantified in Seminal Plasma". Science TranslationalMedicine 5 (212):212ra160. doi:10.1126/scitranslmed.3006260.PMID 24259048.

Fowler PA, Sorsa-Leslie T, Harris W, Mason HD (December 2003). "Ovarian gonadotrophin surge-attenuating factor (GnSAF): where are we after 20 years of research?". Reproduction 126(6): 689–99. doi:10.1530/rep.0.1260689.

Fowler PA, Sorsa-Leslie T, Harris W, Mason HD (December 2003). "Ovarian gonadotrophin surge-attenuating factor (GnSAF): where are we after 20 years of research?". Reproduction 126 (6): 689–99. doi:10.1530/rep.0.1260689.

Fullerton, G.; Hamilton, M.; Maheshwari, A. (2010). "Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009?". Human Reproduction 25 (3): 588–597.doi:10.1093/humrep/dep431

Gruber HA, Farag AF. Evaluation of endocrine function. In: McPherson RA, Pincus MR, eds.Henry's Clinical Diagnosis and Management by Laboratory Methods

"Gonadotropin preparations: Past, present, and future perspectives". Fertility and Sterility 90 (5 Suppl): S13–20. 2008.

Haimov-Kochman, R.; Prus, D.; Farchat, M.; Bdolah, Y.; Hurwitz, A. (2010). "Reproductive outcome of men with azoospermia due to cryptorchidism using assisted techniques". International Journal of Andrology 33 (1): e139–e143.

Jarvi, K; Lo, K; Fischer, A; Grantmyre, J; Zini, A; Chow, V; Mak, V (2010)."CUA Guideline: The workup of azoospermic males". Canadian Urological Association journal 4 (3): 163–7

Jiang X, Liu H, Chen X, Chen PH, Fischer D, Sriraman V, Yu HN, Arkinstall S, He X (July 2012). "Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor". Proc Natl Acad Sci USA 109 (31): 12491–6.

Jiang X, Liu H, Chen X, Chen PH, Fischer D, Sriraman V, Yu HN, Arkinstall S, He X (July 2012). "Structure of follicle-stimulating hormone in complex with the entire ectodomain of its receptor". Proc Natl Acad Sci U S A 109 (31): 12491–6.

Lotti, F.; Maggi, M. (2014). "Ultrasound of the male genital tract in relation to male reproductive health". Human Reproduction Update 21 (1): 56–83.

Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine (2001).Infertility: Report on Evaluation of the Azoospermic Male (PDF). American Urological Association; American Society for Reproductive Medicine.

Melmed S, Kleinberg D, Ho K. Pituitary physiology and diagnostic evaluation. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, eds.Williams Textbook of Endocrinology

Menzies, F. M.; Shepherd, M. C.; Nibbs, R. J.; Nelson, S. M. (2010). "The role of mast cells and their mediators in reproduction, pregnancy and labour". Human Reproduction Update 17 (3): 383–396.

Padubidri; Daftary (2011). Shaw's Textbook of Gynaecology, 15e. p. 205.

Pierce, J G; Parsons, T F (June 1981). "Glycoprotein Hormones: Structure and Function". Annual Review of

Biochemistry 50 (1): 465–495.

Poongothai, J; Gopenath, TS; Manonayaki, S (2009). "Genetics of human male infertility". Singapore medical journal 50 (4): 336–47.

Practice Committee of ASRM, August 2008. "The management of infertility due to obstructive azzospermia" (PDF). Retrieved June 14, 2010.

Radu A, Pichon C, Camparo P, Antoine M, Allory Y, Couvelard A, Fromont G, Hai MT, Ghinea N (October 2010). "Expression of follicle-stimulating hormone receptor in tumor blood vessels".N. Engl. J. Med. 363 (17): 1621–30.