Journal of Cardiovascular Disease Research

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

Assessment of level of FSH and Inhibin B in the evaluation of primary male infertility with reference to spermiogram

Author's Name, Academic Titles & Afflictions:

1. Meena Singrol, Junior Resident, Pathology Department, Bundelkhand Medical College, Sagar, India
2. Jyoti Namdev, Senior Resident, Pathology Department, Bundelkhand Medical College, Sagar, India
3. Nancy Mourya, Assistant Professor, Pathology Department, Bundelkhand Medical College, Sagar, India
4. Smita Doharey, Senior Resident, Pathology Department, Shrimant Rajmata Vijayaraje Scindia Medical College & Hospital, Shivpuri, India
5. Puja Singh*, Associate Professor, Pathology Department, Bundelkhand Medical College, Sagar, India
6. Rakesh Mehar, Associate Professor, Pathology Department, Mahatma Gandhi Medical College, Indore, India
7. Pankaj Shinde, Associate Professor, Pathology Department, Mahatma Gandhi Medical College, Indore, India
8. Sumitra Yadav, Professor, Obstetrics and Gynaecology Department, Mahatma Gandhi Medical College, Indore, India

* Corresponding Author:

Dr. Puja Singh
Address: Pathology Department, Bundelkhand Medical College, Shivaji ward, Tilli Road, Sagar, M.P. 470003, India Email: dr.pujasingh@gmail.com Phone: +91. 95160 94970

ABSTRACT

Introduction

Globally, 48 million couples and 186 million individuals face the problem of infertility. Out of all infertility cases, approximately 40–50% are due to the "male factor" and 2% of all men will exhibit suboptimal sperm parameters. It may be one or a combination of low sperm concentration, poor sperm motility, or abnormal morphology.

In recent years, the development of computerized systems provides an objective and rapid method for semen analysis, suitable for the study of more sophisticated parameters of sperm motility. Electron microscopy should be performed for the evaluation of ultrastructural abnormalities of spermatozoa in men with infertility of uncertain origin.

Method

Semen was examined macroscopically as well as under the microscope as per WHO guidelines for the count, vitality, and morphology by using routine microscopy with PAP stain and Eosin nigrosin dye. The FSH and Inhibin B test was performed using sandwich Elisa by commercially available kit (Qualisa™ FSH) kit and AL-107-i. CE kit respectively.

Result

The majority of patients were seen in the age group of 26-30 years. Total motility of sperms, progressive motile sperms, and semen vitality between 11-20 % was seen in a maximum of 33, 30, and 24 patients respectively. 26 cases had a normal form of sperm between 0-5%. The FSH level and Inhibin B level were lowest and highest at 16-20% of sperm count.

Conclusion

Infertility is one of the biggest causes of stress among married couples. The parameters of male infertility can be easily diagnosed by a simple semen examination. The FSH and Inhibin B-level investigations provide a better mode of treatment for male infertility.

Keywords: Male infertility, Semen examination, FSH, and Inhibin B.

INTRODUCTION

Infertility is defined as the inability of a couple to conceive after one year of regular unprotected sexual intercourse. It may be primary or secondary. [1]

The clinical definition of male factor infertility is the presence of abnormal semen parameters in the male partner of a couple unable to achieve conception after 1 year of unprotected intercourse. The World Health Organization defines

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

male factor infertility as the presence of ≥1 abnormality in the semen analysis or the presence of inadequate sexual or ejaculatory function. [2]

Most cases of male factor infertility are of unknown etiology and are commonly diagnosed if abnormal semen parameters are in 2 semen analyses separated by 1 month. Sperm functional assays, endocrine tests, genetic testing, and imaging can be helpful. [3]

Lifestyle factors associated with male infertility include smoking cigarettes, alcohol intake, use of illicit drugs, obesity, psychological stress, advanced paternal age, diet composition, and coffee consumption. Among other factors are testicular heat stress, intense cycling training, lack of sleep, and exposure to electromagnetic radiation from mobile phones. [4]

Nutritional deficiencies such as lack of sufficient vitamin C, zinc, or folic acid, Genetics and certain genetic disorders such as cystic fibrosis, Klinefelter syndrome, exposure to toxins, harmful chemicals, Frequent exposure to heavy metals such as lead (Pb), arsenic (As) or cadmium (Cd), which stops the production of the enzyme that is essential in the formation of the membrane covering the sperm head. Frequent infections, Radiation treatment and chemotherapy, Tube structural abnormalities, and Varicocele, may not cause infertility, but it is suspected to affect sperm count. All these factors are associated with low sperm concentration. [5]

Semen is composed of two components: spermatozoa and seminal fluid. Spermatozoa are made by the seminiferous tubules of the testis, and the seminal fluid produced by the accessory glands that nourish the sperm. [6] In semen analysis, we use the terms sperm count, which reflects the number of spermatozoa in the semen sample, and, the volume of these men-reflects the amount of seminal fluid produced**.** [7]

FSH is a heterodimeric glycoprotein synthesized and secreted by the anterior pituitary gland. [8] It is released under the influence of pulsatile secretions of gonadotrophin-releasing hormone (GnRH). [9] It is composed of an α subunit and a β subunit and is the most important endocrine marker in the assessment of male infertility. [10,11]

FSH play important role in spermatogenesis. [12] It enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membrane. [13]

INHIBIN is a heterodimeric glycoprotein composed of an α and β subunit. β subunit containing either a βA (Inhibin A) or βB-chain (Inhibin B). Serum Inhibin B is a better marker for assessing male factor fertility than FSH and LH. In patients with infertility, measuring Inhibin B levels may provide useful information on spermatogenesis and it is a direct marker of spermatogenesis than FSH. [14]

AIMS & OBJECTIVES

1. Analysis of semen as per recent WHO criteria in infertile males.
2. To assess the role of FSH and Inhibin B hormone in the evaluation of primary male infertility.
3. To study the clinicopathological correlation of sperm count, serum FSH and Inhibin B in males with primary male infertility.

MATERIAL AND METHODS

The study was conducted over 18 months of duration between June 2020 to December 2021. A prospective study was carried out. A total of 95 cases were studied.

Inclusion Criteria
All clinically suspected cases of primary male infertility patients who were willing to give written consent.

Exclusion Criteria

1. Patients who are not willing to give written consent.
2. Patients of old age group (50 years and above)

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

3. Treated cases of male's infertility.

Semen analysis

The semen sample is collected into a clean, wide-mouthed container made of glass or plastic after 2 to 7 days of sexual abstinence. After the collection, the container is labeled with the patient's name and identification number and the date and time of collection. The specimen container is placed at 37 °C in an incubator while the semen liquefies. Smears of the raw semen were stained by using the Pap stain to assess sperm morphology using World Health Organization criteria. A sperm vitality test was done by using eosin-nigrosin dye.

Evaluation of Inhibin -B and FSH by Elisa 5 ml blood samples were drawn from an antecubital vein and centrifuged after clotting at 3000 rpm for 15 minutes and serum was stored at -20 degrees Celsius until analysis. These tests are performed by ELISA methods. The serum Inhibin B levels were determined with ELISA and were assayed by using the commercially available Inhibin B ELISA - AL-107-i. CE kit. Serum Inhibin B was measured in a quantitative three-step sandwich-type immunoassay. The Inhibin B assay has intraassay and interassay coefficients of variation 3.89 % variation and 7.42% respectively. The Inhibin B assay has a detection limit of 1.6 pg/mL.

The serum levels of FSH were determined with ELISA (Qualisa™ FSH) kit. This kit is a sandwich-based enzyme-linked immunosorbent assay. The minimum detection of limit by this assay is 2.5 mIU/ml. Expected value and sensitivity of FSH in males 0-20 mIU/ml. By using the absorbance of the value of each specimen determine the corresponding concentration of FSH in mIU/ml.

RESULTS

The study shows age group involvement from 20-45 years. The maximum number of cases 24, were seen in the age group of 26-30 and the least number of cases 17 were seen in two groups 31-35 and 41-45.

As per the sperm count the highest number of cases 32 was seen for a sperm count of 11-15 million/ml followed by 28.43 % of cases with a sperm count of 6-10 million/ml. Less than 5 million / ml of sperm count has been seen in 25 cases and 11.57 % of patients had 16-20 million/ml of sperm count. Table 1 shows the correlation between FSH and Inhibin B along with sperm count. The table clearly shows the fall in the FSH level along with the rise in the Inhibin level as the semen count increases.

Table 1: Serum level of FSH and Inhibin B (Mean ± SD) in subgroups of sperm count

S.No.	Sperm count (million/ml)	FSH (mean ± SD) (mIU/ml)	Inhibin B (Mean ± SD) (pg/ml)
1.	≤5	46.45 ± 2.68	4.34 ± 2.34
2.	6-10	34.43 ± 3.09	23.44 ± 11.07
3.	11-15	27.13 ± 2.60	45.85 ± 9.87
4.	16-20	19.00 ± 1.66	80.97 ± 17.17

The total motility of sperm between 11-20 % has the highest number of patients 33. 15.78 % of cases were seen with more than 40% of sperm total motility. The total number of cases was 18 and 17 with total motility of 21- 30 % and 31 – 40 % respectively. A total of 30 and 29 patients were associated with 0-10 % and 11-20 % of progressively motile sperm. 16.85 and 20 .05% of cases were seen in association with 31-40% and 21-30 % of progressive motile sperms. 24 cases showed 11-20 % of sperm vitality. 21.04% of cases were seen with 31-40% of sperm vitality.18 cases were seen with both 0-10% and 21-30% of the vitality of sperms. Only 15 cases were seen with more than 40 % of vitality. 15 cases were seen with normal sperm morphology between 16-25%. A total of 26 cases were

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

associated with 26% of normal sperm morphology. Table 2 shows the various parameters of sperm examination along with their mean and standard deviation.

Table 2: Distribution Characteristics (Mean and Standard Deviation) of seminal parameters of cases

S. No.	Factors	Mean ± SD
1.	S.C. (Sperm count)	10.51±3.98
2.	Sperm motility	19.17 ±11.15
3.	PR Motility	13.17±8.24
4.	Sperm Vitality	22.42±12.41
5.	NF (normal forms)	8.23±6.84
6.	AF (abnormal forms)	91.77±6.84

Table 3 shows the correlation between all the seminal parameters along with FSH showing the r-value and p-value. The p-value is significant in most of the parameters as it is less than 0.0001. Table 4 shows the correlation between Inhibin B and the various seminal parameters calculating the r-value and the p-value. Once more, the p-value is significant in most of the parameters as it is less than 0.0001.

Table 3: Correlation of FSH and Seminal parameters

Factor I	Factor II	r-value	p-value
FSH	Semen volume	0.151	0.388
	Sperm count	-0.980	<0.0001
	PR motility	-0.850	<0.0001
	Total motility	-0.942	<0.0001
	Vitality	-0.939	<0.0001
	Normal Forms	0.837	<0.0001
	Abnormal forms	-0.837	<0.0001

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

Table 4: Correlation of Inhibin B and Seminal parameters

Factor I	Factor II	r-value	p-value
Inhibin B	Semen volume	-0.132	0.388
	Sperm count	0.945	<0.0001
	PR motility	0.823	<0.0001
	Total motility	0.926	<0.0001

Figures 1-A and 1-B show the morphology of sperm stained with eosin–nigrosin stain. The image clearly shows the white active and motile sperm whereas the dead sperm has taken the red stain. Figure 2 shows the pap-stained semen showing the diving spermatid and the mature spermatocyte as indicated by the blue and red arrows respectively.

Figure 1-A: Showing Live sperms stained White. (x100)

Figure 1-B: Showing Dead sperms stained Pink Red (Eosin and nigrosin stain). (x100)

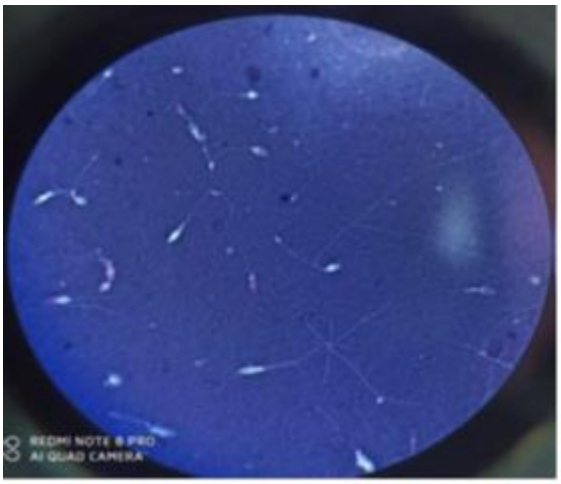


FIGURE 1-A

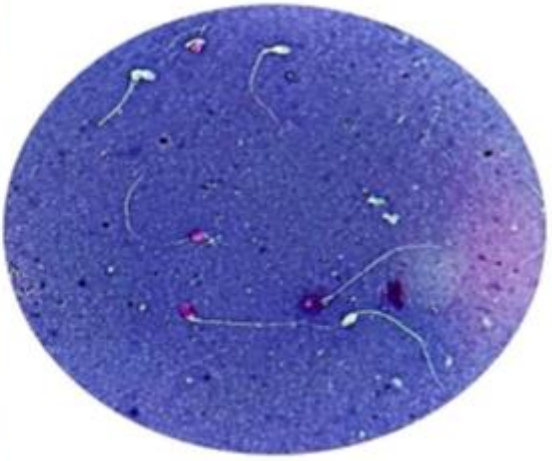
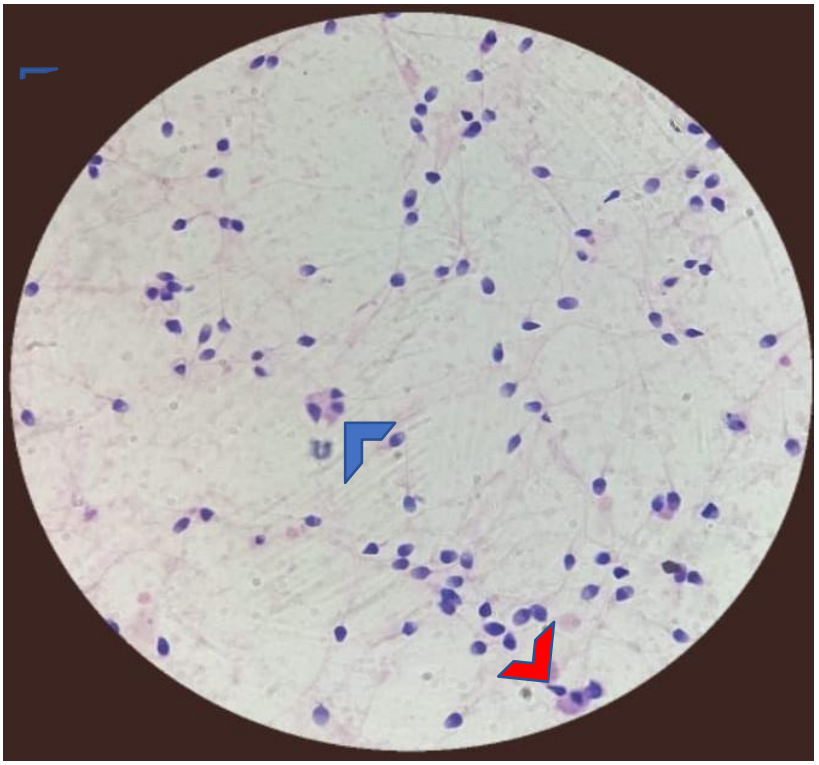


FIGURE 1-B

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

Figure 2: Showing dividing spermatid (blue) and spermatocyte (red). (x100)



DISCUSSION

Infertility is a great social stigma and a major reproductive health problem worldwide reports suggest that infertility affects 10–15% of couples. Approximately 50% of these are accountable to the male partner. [15]

The present study shows that the serum levels of FSH are inversely associated with sperm count, motility, vitality and morphology, while Inhibin B is positively associated with sperm concentration. Our findings for Inhibin B, FSH, and sperm concentrations are consistent with previous studies. [8,9,14]

Subhan et al, 1995 studies serum of 161 oligospermic men and analysed for hormones LH and FSH and the androgen testosterone. The hormonal analysis indicated FSH leva els showed negative correlation to the sperm concentration. The present studies support these findings. In all patients there was a negative correlation between sperm concentrations and FSH ($r = -0.980$, $p < 0.0001$) shown in Table 3. [16]

Mahmoud et al. 1998, studied forty-seven infertile men were evaluated by semen analysis along with hormone determinations, Inhibin B in all 47 infertile men were measured. Higher Inhibin B (median, range: 160.3, 81.8–328.5 pg/mL vs. 94.9, 15.6– 389.7 pg/mL, $P < 0.024$) and lower FSH ($P = 0.001$) were detected in men with sperm concentrations >20 million/mL ($n = 9$), compared to oligozoospermia (n=38). Inhibin B significantly negative correlated with FSH and positively correlated with sperm concentration in yet another study by Pierik et al., 1998 Serum Inhibin B levels were closely correlated with the serum FSH levels ($r = -0.78$, $P < 0.001$), confirming the role of Inhibin B as feedback signal for FSH production. Inhibin B levels were significantly correlated with the total sperm count ($r = 0.54$ and $P < 0.001$), comparable to our study. In our study serum FSH and Inhibin B level measured in 95 primary infertile males in which mean Inhibin B and FSH levels are (35.24 ±22.83) and (31.69±8.05) respectively. In our study Inhibin B is also significantly negatively correlated with FSH. [17,18]

Similar to Mahmoud et al and Pierik et al our present study also support that Inhibin B significantly negatively correlated with FSH and positively correlated with sperm concentration. Correlation between FSH and Inhibin B ($r = -0.919$ $p < 0.0001$) and the correlation between sperm concentration and Inhibin B ($r = 0.945$ $p = < 0.0001$) is also seen. In the present study, we also mention the correlation of FSH and Inhibin B with other seminal parameters. [17,18]

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

Our findings for Inhibin B, FSH, sperm motility and sperm morphology are consistent with two previous studies that is Jensen et al (2004) have reported significant correlations of FSH and Inhibin B with sperm concentration, motility, and morphology among 1558 young Danish men who reported for military service. These findings support the present study. In our study we found, a significant positive correlation between Inhibin B and sperm count, sperm total motility, sperm motility and morphology (normal forms). In present study we also found significant positive correlation between Inhibin B and live sperms that is vitality which is not observed in the previous study. [19]

In another study in 2003, Uhler et al. reported significant correlations between FSH and Inhibin B with concentration, motility and morphology which is similar to our study in which Inhibin B positively correlated with sperm count, motility and normal forms of sperms while FSH is negatively correlated with all these factors. In our study we found correlation between FSH and vitality which is negatively correlated ($r = -0.939$, $P < 0.001$) which is not mentioned in that study. [20]

In a study by Kumanov et al. in 2006, FSH level were found to be significantly negatively correlated with semen parameters. The same study stated that Inhibin B levels were a stronger indicator of infertility than FSH and LH levels. Findings are similar to our study in which semen parameters (sperm count, progressive motility, total motility and vitality) are negatively correlated with FSH. [21]

Meeker et al. in 2007 reported in their study significant negative correlations of FSH with concentration, motility and also about morphology these findings are similar to present study. In our study we found morphology (normal forms) of sperms is negatively correlated with FSH (r=-0.837 P=<0.0001). [22]

CONCLUSION

The results of present study are comparable to other series of studies regarding correlation of serum level of FSH and Inhibin B with sperm count, total motility, progressive motility, normal and abnormal morphological forms.

Out of 95 cases 14.3 % of patients have sperm count ≤5 million/ml and have mean serum FSH level is (46.45±2.68) and mean serum Inhibin B level is (4.34±2.34), 34.3% patients in which sperm count is 6-10 million/ml having mean serum FSH level is (34.43±3.09) and mean serum Inhibin B level is (23.44±11.07) another 42.9% cases have sperm count 11-15 million/ml having mean serum FSH level is (27.13±2.60) and mean serum Inhibin B level is (45.85±9.87) and 8.6% cases have sperm count 16-20 million/ml having mean serum FSH level is (19.0±1.66) and mean serum Inhibin B level is (80.97±17.17) concluded that as the sperm count is raised, mean serum Inhibin B level is also increasing and serum FSH level is decreasing.

Inhibin B levels were significantly negatively correlated to FSH concentrations ($r = 0.919$, $p < 0.0001$) (Table 4). In all patients there was a positive correlation between sperm concentrations and Inhibin B ($r = 0.945$, $p < 0.0001$) and a negative correlation between these concentrations and FSH levels ($r = -0.980$, $p < 0.0001$). In our study all the seminal parameters that is sperm count, total motility, progressive motility, vitality and morphology (normal forms) are significantly (p=<0.0001) positively correlated with Inhibin B and significantly (p=<0.0001) negatively correlated with FSH.

REFERENCES:

1. World Health Organization. WHO fact sheet on infertility. Global Reproductive Health. 2021; 6(1): p e52. DOI: 10.1097/GRH.0000000000000052.

2. Selice R, Ferlin A, Garolla A, Caretta N, Foresta C. Effects of endogenous FSH on normal human spermatogenesis in adults. Int J Androl. 2011; 34(6 Pt 2): e511-7. doi: 10.1111/j.1365-2605.2010.01134.x.

3. BMJ Best Practice US. Male factor infertility - Symptoms, diagnosis and treatment. Rev ed. [Internet]. 2022. Available from: https://bestpractice.bmj.com/topics/en-us/497.

4. Dissanayake DMIH, Keerthirathna WLR, Peiris LDC. Male Infertility Problem: A Contemporary Review on Present Status and Future Perspective. Gender and the Genome. 2019; 3(5): 247028971986824. DOI:10.1177/2470289719868240.

5. Muratori M, Tamburrino L, Marchiani S, Cambi M, Olivito B, Azzari C, Forti G, Baldi E. Investigation

ISSN:0975-3583,0976-2833 VOL14,ISSUE06,2023

on the Origin of Sperm DNA Fragmentation: Role of Apoptosis, Immaturity and Oxidative Stress. Mol Med. 2015; 21(1):109-22. doi: 10.2119/molmed.2014.00158.

6. Schjenken JE, Robertson SA. Seminal Fluid Signalling in the Female Reproductive Tract: Implications for Reproductive Success and Offspring Health. Adv Exp Med Biol. 2015; 868:127-58. doi: 10.1007/978-3-319-18881-2_6.

7. Kliesch S. Diagnosis of male infertility: diagnostic work-up of the infertile man. European Urology Supplements. 2014; 13(4): 73-82. DOI: 10.1016/j.eursup.2014.08.002.

8. Aizen J, Kasuto H, Golan M, Zakay H, Levavi-Sivan B. Tilapia follicle-stimulating hormone (FSH): immunochemistry, stimulation by gonadotropin-releasing hormone, and effect of biologically active recombinant FSH on steroid secretion. Biol Reprod. 2007; 76(4): 692-700. doi: 10.1095/biolreprod.106.055822.

9. Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of Follicle-Stimulating Hormone in Spermatogenesis. Front Endocrinol (Lausanne). 2018; 9:763. doi: 10.3389/fendo.2018.00763.

10. Pierce JG, Parsons TF. Glycoprotein hormones: structure and function. Annu Rev Biochem. 1981; 50: 465-95. doi: 10.1146/annurev.bi.50.070181.002341.

11. Nieschlag, E. Care for the infertile male. Clinical Endocrinology. 1993; 38: 123-133. DOI: 10.1111/j.1365-2265.1993.tb00983.x

12. Hamilton JA, Cissen M, Brandes M, Smeenk JM, de Bruin JP, Kremer JA, Nelen WL, Hamilton CJ. Total motile sperm count: a better indicator for the severity of male factor infertility than the WHO sperm classification system. Hum Reprod. 2015; 30(5): 1110-21. doi: 10.1093/humrep/dev058.

13. Corinne TM, Anatole PC, Jeanne NY. Comparison of serum Inhibin B and follicle-stimulating hormone (FSH) level between normal and infertile men in yaounde. International journal of reproductive medicine. 2020. DOI: 10.1155/2020/4765809

14. Niederberger C. Male infertility. The Journal of urology. 2021 Sep 8:10-97.

15. Abid S, Maitra A, Meherji P, Patel Z, Kadam S, Shah J, Shah R, Kulkarni V, Baburao V, Gokral J. Clinical and laboratory evaluation of idiopathic male infertility in a secondary referral center in India. J Clin Lab Anal. 2008;22(1):29-38. doi: 10.1002/jcla.20216.

16. Subhan F, Tahir F, Ahmad R, Khan ZD. Oligospermia and its relation with hormonal profile. J Pak Med Assoc. 1995; 45(9): 246-7.

17. Mahmoud AM, Comhaire FH, Depuydt CE. The clinical and biologic significance of serum Inhibin s in subfertile men. Reprod Toxicol. 1998 Nov-Dec;12(6):591-9. doi: 10.1016/s0890-6238(98)00042-2.

18. Pierik FH, Vreeburg JT, Stijnen T, De Jong FH, Weber RF. Serum Inhibin B as a marker of spermatogenesis. J Clin Endocrinol Metab. 1998 Sep;83(9):3110-4. doi: 10.1210/jcem.83.9.5121.

19. Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. Fertil Steril. 2004; 82(4): 863-70. doi: 10.1016/j.fertnstert.2004.03.056.

20. Uhler ML, Zinaman MJ, Brown CC, Clegg ED. Relationship between sperm characteristics and hormonal parameters in normal couples. Fertil Steril. 2003 Jun;79 Suppl 3:1535-42. doi: 10.1016/s0015-0282(03)00336-4.

21. Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. Fertil Steril. 2006 Aug;86(2):332-8. doi: 10.1016/j.fertnstert.2006.01.022.

22. Meeker JD, Godfrey-Bailey L, Hauser R. Relationships between serum hormone levels and semen quality among men from an infertility clinic. J Androl. 2007; 28(3): 397-406. doi: 10.2164/jandrol.106.001545.