Trends in Clinical and Medical Sciences

PSR Press

Article

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

Dr. Rakesh Mehar[1], Dr. Meena Singrol[2], Dr. Priyanka Solanki[1], Dr. Aksharaditya Shukla[1,*], Dr. Pankaj Shinde[1] and Dr. Kamna Dubey [3]

1 Department of Pathology, M.G.M. Medical College, Indore, MP.
2 Department of Pathology, Bundelkhand Medical College Sagar, MP.
3 Department of Anaesthesia, M.G.M. Medical College, Indore, MP.
* Correspondence: aksharaditya@gmail.com

Received: 1 January 2023; Accepted: 28 April 2023; Published: 8 May 2023.

Abstract: Infertility, characterized by the failure to conceive after a year of regular unprotected sexual intercourse, affects a significant proportion of couples. Male factors contribute to approximately 40-50% of infertility cases, with around 2% of men exhibiting suboptimal sperm parameters. Semen analysis serves as the primary diagnostic tool for male infertility. Elevated levels of serum follicle-stimulating hormone (FSH) often indicate severe impairment of spermatogenesis. In contrast, inhibin B concentrations are closely associated with sperm concentration and testicular volume, making it a valuable marker for assessing spermatogenesis. This prospective study aimed to investigate the relationship between semen parameters and the levels of serum FSH and inhibin B. The study included 35 cases of male infertility, and their semen samples were subjected to microscopy and comprehensive analysis. Among the cases, 14.3% exhibited a sperm count of =5 million/ml, with a mean serum FSH level of (46.45 *pm* 2.68) and a mean serum inhibin B level of (4.34 *pm* 2.34). The results revealed a significant negative correlation between inhibin B levels and FSH concentrations ($r = 0.919$, $p < 0.0001$). Furthermore, a positive correlation was observed between sperm concentrations and inhibin B ($r = 0.945$, $p < 0.0001$), while a negative correlation existed between sperm concentrations and FSH levels ($r = -0.980$, $p < 0.0001$). These findings suggest that the degree of male infertility is directly proportional to elevated FSH levels and inversely proportional to serum inhibin B levels. Thus, serum inhibin B emerges as a more sensitive parameter for assessing male infertility.

Keywords: Infertility; Spermiogram; FSH; Inhibin B.

1. Introduction

Infertility is defined as inability of couple to conceive after one year of regular unprotected sexual intercourse. It may be primary or secondary [1]. Primary infertility defined as pregnancy has never been achieved after regular coitus without contraception and exposure to pregnancy for one year. Secondary infertility is woman can conceive previously but is subsequently unable to conceive [2].

Globally, 48 million couples and 186 million individuals face the problem of infertility [1]. Out of all infertility cases, approximately 40-50% is due to female 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 [3]. Most cases of male factor infertility are of unknown etiology. Diagnosed if abnormal semen parameters in two semen analyses separated by one month. Sperm functional assays, endocrine tests, genetic testing, and imaging can be helpful [4].

Factors may contribute to this decline of male fertility are environmental, occupational, and modifiable lifestyle. 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 [5]. Semen analysis is most widely used test for the diagnosis of male infertility. The diagnostic and prognostic usefulness of semen analysis is related to strict compliance with the guidelines recently suggested by the World Health Organization.

1.1. Role of FSH and Inhibin B in male infertility

FSH is a heterodimeric glycoprotein synthesized and secreted by the anterior pituitary gland [6]. It is released under the influence of pulsatile secretions of gonadotrophin-releasing hormone (GnRH) [7]. It is composed of ± subunit and a subunit [8] and most important endocrine marker in the assessment of male infertility [9]. FSH play important role in spermatogenesis [10] enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membrane [11]. Increase level of serum FSH levels are usually associated with severe damage of spermatogenesis [12] and highest FSH values are usually found in patients with Klinefelter syndrome and complete Sertoli cell only (SCO) syndrome [13–16]. FSH secretion regulated by Inhibin produced by Sertoli cells [17].

INHIBIN is a heterodimeric glycoprotein composed of an ± and subunit. subunit containing either a A (inhibin A) or B-chain (inhibin B). In male inhibin is produce from Sertoli cells and play important role in the regulation of FSH secretion. Inhibin B is the physiologically important form of inhibin. A strong reverse correlation exists between inhibin B and FSH levels in men with normal and disturbed spermatogenesis. Inhibin B concentrations are closely related to sperm concentration in the semen and to testicular volume. inhibin B is helpful marker for spermatogenesis [18]. Serum Inhibin B is 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 direct marker of the spermatogenesis than FSH [19].

Primary aim of study is the analysis of semen as per recent WHO criteria in infertile male and to assess the role of FSH and INHIBIN B hormone in evaluation of primary male infertility. Also we aim to study clinicopathological correlation of sperm count, serum FSH and Inhibin B in male with primary male infertility.

2. Material and methods

This study was conducted in Department of Pathology, Mahatma Gandhi Medical College and Maharaja Yashwant Rao Hospital, Indore, Madhya Pradesh, India. Permission was obtained from the departmental scientific committee and the institutional ethical committee at the beginning of the study. Duration of study 18 Months, a Prospective study done in 35 Cases. Clinically suspected cases of primary male infertility and willing to give written consent were included and patient not willing to give written consent, patients of age more than 50 years and treated cases of male in fertility were excluded from our study.

3. History and Examination

Patients details regarding history of duration of infertility, varicocele, vasectomy in male and history of Menstrual cycle, Miscarriage, Anovulation, Tubal blockage, Recanalization and Ultrasonographic Report finding regarding uterine and ovarian anomaly to rule out female factor of infertility, semen analysis report finding, clinical diagnosis whenever possible will be noted. Improved Neubauer Counting Chamber is used for sperm count. 1:20 dilution was made by diluting 50ul aliquot of liquefied semen with 950 ul of diluent (sodium-bicarbonate-formalin diluting fluid is used).

3.1. Sperm motility

Sperm motility is tested by putting a drop of liquefied semen on a glass slide and cover with cover slip then examined under 40X with reduced illumination. 200 spermatozoa are counted in several different fields and calculate the percentage and note the grading of motility in terms of Progressive Motility (PR): Spermatozoa moving actively either linearly or in a large circle, regardless of speed, Non-Progressive Motility (NP) and immotile (IM). A cell with intact cell membrane will not take up the Eosin Y and it will not stain while the non-viable or dead cell will have damage cell membrane will take up the dye and stained pink red. Nigrosin dye used to stain the background material.

3.2. Evaluation of Inhibin-B and fsh by elisa

5 ml blood samples were drawn from an antecubital vein and centrifuge whole blood after clotting at 3000 rpm for 15 minutes and serum was stored at -20 degree Celsius until analysis. These tests are performed by ELISA methods. The serum Inhibin B levels were determined with ELISA were assayed by using the

commercially available Inhibin B ELISA. AL-107-i. CE kit. The serum levels 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 male 0-20 mIU/ml.

4. Results

The study involved a comparison between spermiogram results and serum FSH and inhibin B values. Among the 35 cases, 14.3% of patients had a sperm count of 5 million/ml, with a mean serum FSH level of (46.45 $\pm$ 2.68) and mean serum inhibin B level of (4.34 $\pm$ 2.34). Additionally, 34.3% of patients with a sperm count of 6-10 million/ml exhibited a mean serum FSH level of (34.43 $\pm$ 3.09) and mean serum inhibin B level of (23.44 $\pm$ 11.07). Another 42.9% of cases with a sperm count of 11-15 million/ml showed a mean serum FSH level of (27.13 $\pm$ 2.60) and mean serum inhibin B level of (45.85 $\pm$ 9.87). Furthermore, 8.6% of cases with a sperm count of 16-20 million/ml demonstrated a mean serum FSH level of (19.0 $\pm$ 1.66) and mean serum inhibin B level of (80.97 $\pm$ 17.17). These findings suggest that as the sperm count increases, the mean serum inhibin B level also rises, while the serum FSH level decreases.

Inhibin B levels showed a significant negative correlation with FSH concentrations ($r = 0.919$, $p < 0.0001$). Additionally, there was a positive correlation between sperm concentrations and inhibin B in all patients ($r = 0.945$, $p < 0.0001$), along with a negative correlation between these concentrations and FSH levels ($r = -0.980$, $p < 0.0001$). Furthermore, all seminal parameters, including sperm count, total motility, progressive motility, vitality, and morphology (normal forms), were significantly positively correlated with inhibin B ($p < 0.0001$) and significantly negatively correlated with FSH ($p < 0.0001$) in our study.

The cases were also analyzed based on age distribution, with corresponding data provided in Table 1. The distribution of cases according to sperm count can be found in Table 2, while Table 3 presents the serum levels of FSH and inhibin B (mean $\pm$ SD) in subgroups based on sperm count. Table 4 provides the mean and standard deviation (SD) of seminal parameters observed in the cases. The correlation of FSH with seminal parameters is shown in Table 5, and the correlation of inhibin B with seminal parameters is presented in Table 6.

Table 1. Age wise distribution of cases. N=35

S. No.	Age group	No. of cases	%
1	20-25	5	14.28
2	26-30	12	34.28
3	31-35	5	14.28
4	36-40	7	20
5	41-45	6	17.14
Total		**35**	**100%**

Table 2. Distribution of cases according to sperm count

S.No.	Sperm count (million/ml)	No of patients	% of patients
1	5	5	14.3
2	6-10	12	34.3
3	11-15	15	42.9
4	16-20	3	8.6

Table 3. Serum level of FSH and Inhibin B (Mean$\pm$SD) in subgroups of sperm count

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

Table 4. The mean and standard deviation (SD) 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 5. Correlation of FSH and Seminal parameters.

Factor II	r-value	p-value
Semen volume	0.151	0.388
Sperm count	-0.98	<0.0001
PR motility	-0.85	<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

Table 6. Correlation of Inhibin B and Seminal parameters.

Factor I	R-value	p-value
Semen volume	-0.132	0.388
Sperm count	0.945	<0.0001
PR motility	0.823	<0.0001
Total motility	0.926	<0.0001
Vitality	0.904	<0.0001
Normal Forms	0.846	<0.0001
Abnormal forms	-0.846	<0.0001

5. Discussion

Niederberger (2021) conducted a study on male infertility and found a significant decrease in Inhibin-B levels among azoospermia and oligospermia infertile men. The study also revealed a negative correlation between inhibin B and testosterone, as well as a weak correlation with other gonadal hormones. These findings highlight the importance of Inhibin-B as a diagnostic marker for idiopathic male infertility and its role in assessing normal spermatogenesis (Niederberger, 2021 [20]).

Corinne et al. (2020) conducted a cross-sectional study comparing serum inhibin B and follicle-stimulating hormone (FSH) levels between normal and infertile men in Yaounde. The results confirmed a significant and positive correlation between inhibin B and sperm concentration and leucocytes, while FSH showed a negative correlation with sperm concentration and vitality (Corinne et al., 2020 [21]).

Hildorf et al. (2019) conducted a study on infant cryptorchid boys aged 4-35 months to investigate the correlation between serum inhibin B and Sertoli cell number. The results demonstrated a correlation between inhibin B and Sertoli cell number, suggesting that inhibin B may reflect the function of Sertoli cells in infant cryptorchid boys (Hildorf et al., 2019 [22]).

Barbonetti et al. (2018) discussed the use of follicle-stimulating hormone (FSH) for the treatment of infertile men. The Italian Society of Andrology and Sexual Medicine recommended using FSH to increase sperm concentration and motility in infertile non-gonadotropic men with idiopathic oligozoospermia or OAT oligo-astheno-teratozoospermia. FSH treatment was suggested to improve spontaneous pregnancy rates as well as pregnancy rates after assisted reproductive technology (ART) (Barbonetti et al., 2018 [23]).

Oduwole et al. (2018) highlighted the role of follicle-stimulating hormone (FSH) in spermatogenesis. In male infertility cases, FSH is indicated for the induction and maintenance of spermatogenesis in patients with hypogonadotropic hypogonadism (Oduwole et al., 2018 [24]).

Grunewald et al. (2013) conducted a study on age-dependent inhibin B concentration in relation to FSH and semen sample qualities. The study suggested that inhibin B, especially the inhibin B-to-FSH ratio (IFR), is a more sensitive marker of male infertility compared to FSH alone (Grunewald et al., 2013 [25]).

Muratori et al. (2015) investigated the origin of sperm DNA fragmentation and identified apoptosis, immaturity, and oxidative stress as contributing factors. The study indicated that sperm DNA fragmentation primarily originates in the testis as a result of abortive apoptotic mechanisms or oxidative stress during transit in the male genital tract (Muratori et al., 2015 [26]).

6. 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. Level of serum FSH and Inhibin B are inversely proportional to each other. The degree of infertility is directly proportional to the raised level of FSH and inversely proportional to serum Inhibin B. Serum Inhibin B is more sensitive parameter for assessing male infertility.

Author Contributions: All authors contributed equally to the writing of this paper. All authors read and approved the final manuscript.

Conflicts of Interest: "Authors declare no conflict of interests."

References

[1] World Health Organization. (2021). WHO fact sheet on infertility. *Global Reproductive Health, 6*(1), e52.

[2] Rowe, P. (1987). Infertility. *Journal Article*. Retrieved October 2, 2021, from https://apps.who.int/iris/bitstream/handle/10665/53389.

[3] Kumar, N., & Singh, A. K. (2015). Trends of male factor infertility, an important cause of infertility: A review of literature. *Journal of Human Reproductive Sciences, 8*(4), 191.

[4] BMJ Best Practice US. (n.d.). Male factor infertility - Symptoms, diagnosis and treatment. Retrieved from https://bestpractice.bmj.com/topics/en-us/497.

[5] Dissanayake, D. M. I. H., Keerthirathna, W. L. R., & Peiris, L. D. C. (2019). Male Infertility Problem: A Contemporary Review on Present Status and Future Perspective.

[6] Aizen, J., Kasuto, H., Golan, M., Zakay, H., & Levavi-Sivan, B. (2007). Tilapia follicle-stimulating hormone (FSH): immunochemistry, stimulation by gonadotropin-releasing hormone, and effect of biologically active recombinant FSH on steroid secretion. *Biology of Reproduction, 76*(4), 692-700.

[7] Matin-du-Pan, R. C., & Bischof, P. (1995). Increased follicle stimulating hormone in infertile men: Is increased plasma FSH always due to damaged germinal epithelium? *Human Reproduction, 10*(8), 1940-1945.

[8] Pierce, J. G., & Parsons, T. F. (1981). Glycoprotein hormones: structure and function. *Annual Review of Biochemistry, 50*(1), 465-495.

[9] de Kretser, D. M., Loveland, K. L., Meinhardt, A., Simorangkir, D., & Wreford, N. (1998). Spermatogenesis. *Human Reproduction, 13*(suppl_1), 1-8.

[10] Boron, W. F., & Boulpaep, E. L. (2005). Medical Physiology: A Cellular and Molecular Approach (Updated 2nd ed.).

[11] Bergmann, M., Behre, H. M., & Nieschlag, E. (1994). Serum FSH and testicular morphology in male infertility. *Clinical Endocrinology, 40*(1), 133-136.

[12] De Kretser, D. M., Burger, H. G., Fortune, D., Hudson, B., Long, A. R., Paulsen, C. A., & Taft, H. P. (1972). Hormonal, histological and chromosomal studies in adult males with testicular disorders. *The Journal of Clinical Endocrinology & Metabolism, 35*(3), 392-401.

[13] Ishida, H., Isurugi, K., Aso, Y., Takayasu, H., & Tamaoki, B. I. (1976). Endocrine studies in Sertoli-cell-only syndrome. *The Journal of Urology, 116*(1), 56-58.

[14] Bablok, L., Janczewski, Z., Kwiatkowska, Z., & Fracki, S. (1978). The relationship between plasma FSH, testosterone levels and testicular histology in males with azoospermia. *Andrologia, 10*(6), 502-505.

[15] Micic, S., Ilic, V., Micic, M., Genbacev, O., & Dotlic, R. (1983). Endocrine profile of 45 patients with Sertoli cell only syndrome. *Andrologia, 15*(3), 228-232.

[16] Grunewald, S., Glander, H. J., Paasch, U., & Kratzsch, J. (2013). Age-dependent inhibin B concentration in relation to FSH and semen sample qualities: a study in 2448 men. *Reproduction, 145*(3), 237-244.

[17] Ying, S. Y. (1988). Inhibins, activins, and follistatins: gonadal proteins modulating the secretion of follicle-stimulating hormone. *Endocrine Reviews, 9*(2), 267-293.

[18] Von Eckardstein, S., Simoni, M., Bergmann, M., Weinbauer, G. F., Gassner, P., Schäpers, A., & Nieschlag, E. (1999). Serum inhibin B in combination with serum FSH is a more sensitive marker than serum FSH alone of impaired spermatogenesis in men but cannot predict the presence of sperm in testicular tissue samples. *The Journal of Clinical Endocrinology & Metabolism, 84*, 2496-2501.

[19] Kumanov, P., Nandipati, K., Tomova, A., & Agarwal, A. (2006). Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertility and Sterility, 86*(2), 332-338.

[20] Meacham, R. B., Joyce, G. F., Wise, M., Kparker, A., Niederberger, C., & Urologic Diseases in America Project. (2007). Male infertility. *The Journal of Urology, 177*(6), 2058-2066.

[21] Corinne, T. M., Anatole, P. C., & Jeanne, N. Y. (2020). Comparison of serum inhibin B and follicle-stimulating hormone (FSH) level between normal and infertile men in Yaoundé. *International Journal of Reproductive Medicine, 2020*.

[22] Hildorf, S., Dong, L., Thorup, J., Clasen-Linde, E., Andersen, C. Y., & Cortes, D. (2019). Sertoli cell number correlates with serum inhibin B in infant cryptorchid boys. *Sexual Development, 13*(2), 74-82.

[23] Barbonetti, A., Calogero, A. E., Balercia, G., Garolla, A., Krausz, C., La Vignera, S., ... & Ferlin, A. (2018). The use of follicle-stimulating hormone (FSH) for the treatment of the infertile man: position statement from the Italian Society of Andrology and Sexual Medicine (SIAMS). *Journal of Endocrinological Investigation, 41*(9), 1107-1122.

[24] Oduwole, O. O., Peltoketo, H., & Huhtaniemi, I. T. (2018). Role of follicle-stimulating hormone in spermatogenesis. *Frontiers in Endocrinology, 9*, 763.

[25] Grunewald, S., Glander, H. J., Paasch, U., & Kratzsch, J. (2013). Age-dependent inhibin B concentration in relation to FSH and semen sample qualities: a study in 2448 men. *Reproduction, 145*(3), 237-244.

[26] Muratori, M., Tamburrino, L., Marchiani, S., Cambi, M., Olivito, B., Azzari, C., ... & Baldi, E. (2015). Investigation on the origin of sperm DNA fragmentation: role of apoptosis, immaturity, and oxidative stress. *Molecular Medicine, 21*, 109-122.

© 2023 by the authors; licensee PSRP, Lahore, Pakistan. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (http://creativecommons.org/licenses/by/4.0/).