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Original Article

Association of Serum Inhibin B and Primary Male Reproductive Hormone with Semen Quality in Normal and Infertile Men

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ABSTRACT

Background: Inhibin B, which is produced in the testis from Sertoli cells, functions in the negative feedback process for the release of Follicle Stimulating Hormone (FSH). These hormones appear to be crucial indicators for the health of somniferous tubules, where spermatozoa were formed from germ cells. The present study designed to assess the role of inhibin B, FSH, testosterone and Luteinizing Hormone (LH) on semen quality (sperm morphology, concentration, and total motility of sperms) in normal and infertile men. **Materials and Methods:** This study was conducted in the department of reproductive medicine with a cross-sectional study design. In normal and infertile groups, the participants were enrolled as 1:2 ratios. Following 4-5 days of sexual abstinence, men's fresh semen samples were obtained, and the samples were then tested for semen quality using the World Health Organization (WHO) 2010 handbook. Using the Chemiluminescent Immune Assay (CLIA) and Enzyme-Linked Immunosorbent Assay (ELIZA) approach, blood samples were taken, serum was separated for inhibin B, FSH, testosterone and luteinizing hormone analysis, and the correlation was evaluated using the Spearman's correlation test. **Results:** It was found that inhibin B level was higher in normal group (p=0.0005) whereas FSH level was higher in infertile group (p=0.001). It was found that, a significant and hostile correlation among inhibin B and FSH in the normal and infertile group. In infertile group inhibin B shows the positive correlation with total sperm concentration in semen (r=0.175* p=0.023) and adversative correlation with non-progressive motility (r=0.184* p=0.050). FSH noted hostile correlation with tail defects (r=-0.235* p=0.012). In normal group, it was noted that optimistic correlation between inhibin B and progressive motility (r=0.280* p=0.035) and there are no negative correlations were noted. Testosterone had negative correlation with FSH and LH. Testosterone showed positive correlation with sperm concentration. The sperm concentration and total motility shown optimistic correlation with LH. **Conclusion:** Consistent with other study, this study finding infers the stout correlation between the inhibin B and FSH with semen quality in infertile male in South India.

Keywords: Spermatozoa, Total sperm concentration, Motility, Vitality, Hormones.

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INTRODUCTION

Inhibin is a heterodimeric glycoprotein formed by alpha and beta subunits. Alpha (α) subunit is glycosylated and beta (β) subunit is non-glycosylated and beta subunits are of two types: A and beta-B. Inhibin's are classified into two types, those are A and B. Alpha and beta-A and alpha and beta-B are dimers of inhibin-A and inhibin-b, respectively. These di-sulphide bonds hold those alpha and beta together. Inhibin B, which is only produced by the testicles and has a pronounced diurnal fluctuation in adults, is associated to testosterone. Gonadotropin-Releasing Hormone (GnRH) is released by the hypothalamus and works on the anterior pituitary gland to cause the production of LH and FSH. In order to make Androgen Binding Protein (ABP), which aids in sperm formation, FSH works on Sertoli cells and activates them. LH causes Leydig cells to become active and releases testosterone. Sertoli cells, also known as nurse cells, aid in the maintenance of sperm cells and the formation of the blood-testis barrier. Male Sertoli cells also generate Inhibin B, which controls blood

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FSH levels through a pituitary negative feedback mechanism.[1] A blood marker for spermatogenesis and Sertoli cell activity is inhibin B. Inhibin B levels in serum rise after delivery, albeit this is only partially correlated with rising FSH levels. The levels of inhibin B later reduced during puberty. Inhibin B secretion is induced by FSH treatment in healthy men, but it is considerably more pronounced in men with mild hypogonadism. When infertile males with FSH were treated, the level of Inhibin B did not rise. The degree of spermatogenetic damage often correlates with inhibin B levels, with the arrest having the lowest levels at the earliest stages.[2] When blood levels of Inhibin B and FSH were compared in healthy and infertile patients, a positive association between Inhibin B and sperm vitality and a negative correlation between FSH and sperm vitality were found.[3] Both Inhibin B and FSH were stronger and more reliable indicators of spermatogenesis than each one by itself.[2] Contradictory evidence suggests that inhibin-B is a useful diagnostic tool that can also provide information on the control of the pituitary-gonadal axis and testicular function.[4]

The most important of these regulatory pathways is the Hypothalamic-Pituitary-Gonadal (HPG) axis, which regulates vital processes through the action of heterodimeric glycoproteins, follicle-stimulating hormone, luteinizing hormone, and the preservation of testosterone concentration in intratesticular region. A common component and a hormone-specific subunit make up these two hormones. The interstitium of the testis having the endocrine cells namely Leydig cells, produce testosterone under the influence of the LH hormone. Testosterone is necessary for male virilization, and it also initiates and sustains spermatogenesis when combined with FSH. T and FSH work together to affect Sertoli cells, which define the wall of the seminiferous tubules that support germ cells developing into mature sperm throughout time. The serum Inhibin B levels have limited therapeutic value for certain individuals despite being a useful spermatogenesis indicator.[5,6] with this background this study was planned to assess the correlation between inhibin B, FSH, testosterone and Luteinizing Hormone (LH) on semen quality in infertile men.

MATERIALS AND METHODS

Study design

This study conducted in the department of reproductive medicine and surgery, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University) for the period of 12 months with cross-sectional study design.

Ethical approval

Before the commencement of this study, the protocol was approved by the Institutional Ethics Committee (IEC) of Sri Ramchandra Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India. IEC No: CSP/19/NOV/81/411. The written informed consent was obtained from all the study participants.

Sample size and subject recruitment

A convenient sample of 57 infertile men's and 57 normal men's were screened in 1:1 ratios. The age of 21 to 45 years married male with bilateral testis were included. Patients were divided into groups based on the World Health Organization's (WHO) 2010 standard reference values for the characteristics of spermatozoa, which include sperm volume of 2 mL or more, pH of 7–8, sperm concentration of 15 million or more per mL, total number of 39 million or more per ejaculate, motility of 40% or more progressive motility, morphology of 4% or more, and viability of 58% or more live spermatozoa. Patients who met with the above criteria were included in the fertile group and who is not met this criterion were included in the infertile group. participants with alcoholic and smoking history, diabetes, hypertension, mumps, orchitis TB, varicocele and taking treatment for infertility were excluded from the study.

Data collection procedure

After getting written informed consent from the patient, the demographic details such as, patient's age, education and occupation were collected in a specially designed data collection form.

Collection of semen samples

Semen was obtained by masturbating into a sterile plastic container in the department lab following 4-5 days of abstention from sexual activity. Based on the information provided by the patient during study enrolment, the statistics on sexual abstinence were recorded. For analysis, the obtained semen sample was liquefied at 37°C for a minimum of 30 min and a maximum of 1 hr.

Analysis of semen quality

The parameters have been analyzed by using World Health Organization (WHO) 2010 manual. Based on Kruger's morphology classification defects in the sperm head, mid piece and tail were assessed. If any abnormalities were detected it is categorized as abnormal report.

Blood sample collection

4 mL of venous blood were collected and stored for 30 min at room temperature. The blood sample was then centrifuged for 15 min at 3000 rpm. The serum was collected and stored in the microfuge tube and stored at -20°C.

Determination of Inhibin B

Human inhibin B CLIA kit was used to measure the serum inhibin B levels (Cat: MBS2531981 96T). This is a sandwich test with three steps that is antibody specific. The relevant

micro CLIA plate wells are filled with standards or samples, followed by a particular antibody. Next each microplate well is sequentially added to and treated with an Avidin-Horseradish Peroxidase (HRP) combination and a biotinylated detection antibody specific for INHB. The fluorescence of an Avidin-HRP combination and a biotinylated detection antibody will be seen. The Chemiluminescence Immunoassay Analyzer measures the Relative Light Unit (RLU) value spectrophotometrically. The RLU value and INHB concentration are positively correlated.

Determination of FSH

The human FSH CLIA kit (Ct: E-CL-H0760), which used the sandwich-CLIA concept, was used to measure the serum FSH. After that, each microplate well receives an addition of an HRP combination and a biotinylated detection antibody specific for Human FSH. There will be fluorescence only in the wells containing human FSH, biotinylated detection antibody, and Avidin-HRP conjugate. The chemiluminescence immunoassay analyzer measures the Relative Light Unit (RLU) value. The level of human FSH is positively correlated with the RLU score.

Determination of Testosterone

Human testosterone ELISA kit (MBS494249) was used to measure the serum testosterone utilizing the competitive binding concept. An exclusive antigenic location on the testosterone molecule is the target of a particular antibody that has been used to coat the microtiter wells. The testosterone in the additional sample competes with the additional enzyme conjugate, which is testosterone coupled to horseradish peroxidase, for binding to the coated antibody during the first incubation. following a washing process to get rid of any unbound substances. The substrate solution is incubated with the solid phase. By adding stop solution, the colorimetric process is stopped, and the optical density of the ensuing yellow product is measured. The relationship between colour intensity and analyte concentration in a sample is inverse.

Determination of Luteinizing hormone

Using a human LH ELISA kit (LS-F20640) that adhered to the sandwich-ELISA principle, the serum LH was determined. A target-specific capture antibody has been pre-coated in each well of the provided microtiter plate. With the addition of standards or samples, the capture antibody in the wells is bound by the target antigen. Through washing, a sample or unbound standard is eliminated. A biotin-conjugated detecting antibody later binds to the antigen that was collected. Any non-bound biotinylated detection antibody should be removed with water. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added, and the biotin is subsequently bound by it. Unbound Avidin-HRP conjugate is washed away. The subsequent interaction of the HRP enzyme with a TMB substrate results in the development of colour. The addition of a sulfuric acid stop solution halts the colour development reaction, and the Optical Density (OD) of the well is then measured at a wavelength of 450 nm + 2 nm.

Statistical Analysis

The collected data were entered in the Excel worksheet. Using IBM SPSS Statistics for Windows, Version 23.0, the data were analyzed (Armonk, NY: IBM Corp). Frequency analysis, percentage analysis, and mean and S.D. were employed for categorical variables and continuous variables, respectively, to characterize the data using descriptive statistics. The unpaired sample t-test was employed to determine the significant difference between the bivariate samples in independent groups. In order to evaluate the monotonic component of semen characteristics, Spearman's correlation test was performed.

RESULTS

Table 1 summarizes the macroscopic and microscopic finding of semen samples between normal and infertile group. Abstinence, volume and liquefaction time were found to be not significant among the study group ($p>0.05$). Whereas, the sperm concentration, total motility of both progressive and non-progressive, immotile concentration, and vitality was found to be highly significant ($p<0.005$) between normal and infertile group.

The distribution of the population according to the level of hormone FSH and Inhibin B which was shown in Table 2. It was found that inhibin B and testosterone levels was found to be higher in normal group ($p=0.0005$) whereas FSH and LH levels was observed higher in infertile group ($p=0.001$, $p=0.0005$).

The correlation of hormone level with seminal characteristics in overall population have been studies in Table 3. It was found the positive correlation of inhibin B with sperm concentration ($r=0.194^{*}$ $p=0.011$), total motility ($r=0.157^{*}$ $p=0.041$), progressive motility ($r=0.244^{**}$ $p=0.001$), normal sperm ($r=0.203^{**}$ $p=0.008$) and vitality ($r=0.187^{*}$ $p=0.014$). whereas a negative correlation was seen in FSH with sperm concentration ($r=-0.148$ $p=0.043$), normal sperm ($r=-0.186^{*}$ $p=0.015$) and abnormal sperm ($r=-0.155^{*}$ $p=0.043$). Testosterone have positive correlation with sperm concentration ($r=0.172^{*}$ $p=0.025$) and vitality ($r=0.173^{*}$ $p=0.024$) in overall population. LH had optimistic correlation with sperm concentration ($r=0.223^{**}$ $p=0.003$) and total motility ($r=0.225^{**}$ $p=0.003$), progressive motility ($r=0.290^{**}$ $p=0.005$), normal morphology ($r=0.331^{**}$ $p=0.0005$), and vitality ($r=0.236^{**}$ $p=0.002$) in overall population. Also had negative correlation with immotile sperm concentration ($r=-0.319^{**}$ $p=0.005$) and tail defects ($r=-0.160^{*}$ $p=0.037$).

DISCUSSION

The >50 % of participants were observed with the age of 31-35 years. FSH level increases with years and inhibin B level were decreased.[3] Men who are older experience diminished testicular

Table 1: Macroscopic and microscopic finding of semen samples between normal and infertile group.

Sl. No	Macroscopic Characteristics	Normal group (*n*=57)		Infertile group (*n*=57)		*p*-value
		Mean	SD	Mean	SD	
1.	Abstinence (in days)	5.16	4.59	4.70	4.37	0.663
2.	Volume (mL)	2.67	1.35	2.59	1.66	0.751
3.	Liquefaction time (Min)	14.79	2.04	15.00	0.00	0.440
4.	Sperm concentration (Mil/mL)	39.00	17.79	16.56	14.36	0.0005**
5.	Total motility (%)	71.33	8.96	49.29	19.30	0.0005**
6.	Progressive motility (%)	40.96	7.34	24.40	13.79	0.0005**
7.	Non-progressive motility (%)	30.74	7.33	25.60	9.10	0.0005**
8.	Immotile concentration (%)	28.93	9.67	48.27	17.90	0.0005**
9.	Vitality (%)	83.49	6.80	62.69	17.91	0.0005**
10.	Total Motile Sperm Concentration (TMSC), (Millions)	75.04	60.98	24.49	34.24	0.0005**

p value < 0.05 indicates significant, < 0.005 indicates highly significant.

Table 2: Distribution of the population according to the level of hormone FSH and Inhibin B.

Sl. No	Anti- oxidative Biomarkers	Normal group (*n*=57)		Infertile group (*n*=57)		*p*-value
		Mean	SD	Mean	SD	
1.	Inhibin B (pg/mL)	165.53	58.11	121.39	34.49	0.0005**
2.	Follicle stimulating hormone (IU/L)	6.52	2.73	7.91	2.32	0.001*
3.	Testosterone (ng/ml)	5.81	4.27	4.53	6.82	0.0005**
4.	Luteinizing Hormone (mIU/mL)	5.65	2.11	7.40	1.91	0.0005**

p value < 0.05 indicates significant, < 0.005 indicates highly significant.

function and metabolism as well as age-related morphological changes in the testes.[4] In contrast, this study revealed there is no differences in age between the normal and infertile group. Inhibin B controls the release of FSH by a negative feedback mechanism. It is unclear how germ cells affect the synthesis of inhibin B Yet, both FSH and spermatogenic status are factors that affect inhibin B production in adults. Throughout life, inhibin B production is controlled differently.[7] It was found a significant and adverse correlation among inhibin B and FSH in the normal and infertile group. FSH levels in infertile males were substantially higher than those in the normal group, whereas Inhibin B levels were much lower. This finding was extremely consistent with the literature, which showed a substantial negative association between inhibin B and FSH as well as a new diagnostic marker for evaluating spermatogenesis and fertility.[8]

In the overall population, Although FSH was adversely connected with sperm concentration, normal and aberrant forms of sperm, inhibin B was substantially and positively correlated with sperm concentration, total motility, progressive motility, and normal forms of sperm. These results corroborated the study's findings, which showed a positive link between inhibin B levels and sperm parameters and a negative correlation between FSH levels and sperm parameters.[9,10] Inhibin B has positive correlation with sperm concentration in infertile group and progressive motility with normal group. This finding was in contrast to the study stated that there is no correlation in normal group and sperm motility.[2,3] Compared to FSH, testosterone and LH, the amount of inhibin B may be a more reliable indicator for assessing male aspect fertility. Inhibin B measurements in infertile individuals may be useful in understanding spermatogenesis and may even be a more precise indication of spermatogenesis than FSH. The inhibin B-FSH index may also be an unfailing indicator of male factor infertility in addition to inhibin B.[9] Inhibin B appears to reflect testicular activity, particularly in spermatogenesis, sperm count, and concentration. It is a direct marker of Sertoli cell existence and function.[11]

Testosterone from the testes negatively modulates the synthesis of GnRH in the hypothalamus, which has a negative feedback impact on the production of FSH and LH. Steroid hormones significantly suppress LH synthesis more than they do FSH. It is thought that testosterone reduces the number of GnRH pulses. This finding was like the study detailed that the steroid, which primarily work

Table 3: Correlation between hormone level and semen parameters by using spearman correlation test.

Sl. No	Semen Parameters	Population	Inhibin B (r, p-value)	FSH (r, p-value)	Testosterone (r, p-value)	LH (r, p-value)
1.	Volume (mL)	Overall population	0.022 (0.777)	0.023 (0.767)	0.021 (0.784)	0.069 (0.368)
		Normal group	-0.084 (0.536)	0.202 (0.132)	-0.170 (0.207)	-0.014 (0.920)
		Infertile group	0.079 (0.401)	-0.051 (0.589)	0.063 (0.503)	0.094 (0.322)
2.	Sperm concentration (Mil/mL)	Overall population	0.194* (0.011)	-0.148 (0.053)	0.172* (0.025)	0.223** (0.003)
		Normal group	0.008 (0.953)	-0.142 (0.293)	0.101 (0.454)	0.050 (0.713)
		Infertile group	-0.145 (0.123)	0.095 (0.312)	-0.028 (0.768)	-0.005 (0.960)
3.	Total motility (%)	Overall population	0.157* (0.041)	-0.054 (0.479)	0.134 (0.082)	0.225** (0.003)
		Normal group	0.016 (0.908)	0.169 (0.209)	-0.196 (0.144)	0.241 (0.071)
		Infertile group	-0.162 (0.084)	0.092 (0.330)	-0.002 (0.984)	-0.009 (0.922)
4.	Progressive motility (%)	Overall population	0.244** (0.001)	-0.119 (0.120)	0.091 (0.235)	0.290** (0.005)
		Normal group	0.280* (0.035)	-0.068 (0.617)	0.027 (0.841)	0.057 (0.673)
		Infertile group	-0.106 (0.262)	0.057 (0.548)	-0.105 (0.267)	0.126 (0.182)
5.	Non-progressive motility (%)	Overall population	-0.051 (0.511)	-0.006 (0.938)	0.006 (0.949)	0.078 (0.312)
		Normal group	-0.242 (0.070)	0.138 (0.306)	0.024 (0.858)	0.264* (0.048)
		Infertile group	-0.184* (0.050)	0.039 (0.683)	0.189* (0.027)	-0.133 (0.158)
6.	Immotile (%)	Overall population	-0.133 (0.084)	0.061 (0.428)	-0.101 (0.188)	-0.319** (0.005)
		Normal group	0.051 (0.706)	-0.165 (0.220)	0.086 (0.526)	-0.267* (0.045)
		Infertile group	0.159 (.091)	-0.063 (0.508)	0.051 (0.588)	-0.141 (0.136)
7.	Normal (%)	Overall population	0.203** (0.008)	-0.186* (0.015)	0.145 (0.058)	0.331** (0.0005)
		Normal group	-0.186 (0.166	0.024 (0.862)	-0.213 (0.112)	0.292* (0.027)
		Infertile group	-0.107 (0.256)	-0.039 (0.683)	-0.035 (0.713)	0.017 (0.861)
8.	Abnormal (%)	Overall population	-0.083 (0.283)	0.155* (0.043)	-0.130 (0.089)	-0.149 (0.052)
		Normal group	0.120 (0.372)	0.037 (0.783)	-0.103 (0.447)	0-.080 (0.555)

Sl. No	Semen Parameters	Population	Inhibin B (r, p-value)	FSH (r, p-value)	Testosterone (r, p-value)	LH (r, p-value)
		Infertile group	-0.068 (0.470)	0.171 (0.069)	-0.009 (0.922)	0.010 (0.915)
9.	Head defects (%)	Overall population	-0.025 (0.741)	0.036 (0.637)	-0.170* (0.022)	0.011 (0.887)
		Normal group	-0.155 (0.249)	-0.194 (0.148)	0.044 (0.745)	0.055 (0.683)
		Infertile group	0.168 (0.074)	0.118 (0.210)	-0.121 (0.200)	0.058 (0.539)
10.	Midpiece defects (%)	Overall population	0.010 (0.892)	0.083 (0.283)	0.096 (0.212)	-0.045 (0.558)
		Normal group	0.193 (0.150)	0.204 (0.129)	0-.038 (0.779)	-0.126 (0.351)
		Infertile group	-0.072 (0.446)	0.010 (0.916)	0.178* (0.014)	0.007 (0.939)
11.	Tail defects (%)	Overall population	-0.027 (0.724)	-0.134 (0.081)	-0.168* (0.028)	-0.160* (0.037)
		Normal group	-	-	-	-
		Infertile group	0.068 (0.473)	-0.235* (0.012)	-0.144 (0.127)	-0.132 (0.161)
12.	Vitality (%)	Overall population	0.187* (0.014)	-0.074 (0.336)	0.173* (0.024)	0.236** (0.002)
		Normal group	0.010 (0.943)	0.028 (0.836)	-0.009 (0.948)	0.247 (0.064)
		Infertile group	-0.105 (0.266)	0.103 (0.273)	0.021 (0.825)	0.008 (0.935)
13.	TMSC (Millions)	Overall population	0.132 (0.086)	-0.099 (0.198)	0.090 (0.243)	0.210** (0.006)
		Normal group	-0.105 (0.439)	0.035 (0.796)	0.002 (0.990)	0.044 (0.744)
		Infertile group	-0.069 (0.467)	0.017 (0.854)	-0.101 (0.286)	0.042 (0.661)

p value < 0.05 indicates significant, < 0.005 indicates highly significant.

within the central nervous system to decrease GnRH release, have a negative feedback regulatory effect on LH secretion.[12] Testosterone directly suppresses the FSH secretion and also Inhibin B and testosterone harmonious to negatively regulate the FSH secretion. In line with previous studies,[13-15] it was found that decline in semen parameters like sperm concentration, motility, morphology, and vitality in infertile group. There is a significant difference observed between normal and infertile group.

The previous literature stated that the reproductive hormones have strong association with semen quality parameters. Specifically, Inhibin B, FSH, testosterone and luteinizing hormone were well thought out that is more significant markers for testicular function.[1] Similar to testosterone, serum inhibin B has a distinct diurnal fluctuation[2].The functional parameters of the semen is increased by Inhibin B and FSH. Measurement of these endocrine markers may be useful for assessing semen quality.[1]

CONCLUSION

The findings of this study showed that the infertile group had low quality semen characteristics, particularly in sperm concentration, motility, morphology, vitality, and TMSC. Inhibin B and FSH were shown to have a substantial negative relationship. Inhibin B level was less and FSH level were more in infertile group. This finding clearly indicates that impaired spermatogenic function in infertile group. Inhibin B and FSH are good indicators for assessing spermatogenesis when unable to perform the semen analysis but can't assure that these hormones may be the indicator of fertility potential in men. In Leydig cells, luteinizing hormone stimulates the release of testosterone in

male. The synthesis of GnRH in the hypothalamus is adversely modulated by testosterone from the testes, which has negative feedback effects on the production of FSH and LH. LH production is far more inhibited by steroid hormones than FSH production is. It is believed that testosterone works by reducing the frequency of GnRH pulses. These results may help to rationalize drug therapy and dependable counselling to infertile patients.

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ABBREVIATIONS

LH: Luteinizing hormone; **FSH:** Follicle stimulating hormone; **CLIA:** Chemiluminescent immunoassay; **ELIZA:** Enzyme-linked immunosorbent assay.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests with respect to research, authorship, and/or publication of this article.

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