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RESEARCH ARTICLE

EVALUATION OF INHIBIN B AND FSH IN SERUM AND SEMINAL PLASMA IN DIFFERENT GROUPS OF INFERTILE MEN.

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Abstract

Background: Treatment of infertility-related hormonal dysfunction in men requires an understanding of the hormonal basis of spermatogenesis. The best method for accurately determining male androgenization status remains elusive.

Objective: This study is designed to evaluate the levels of serum and seminal plasma Inhibin B and FSH in relevance to sperm function parameters in different groups of infertile patients.

Subject, Material and Methods: This study was carried out in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies \ AL- Nahrain University, Baghdad-Iraq ; during the period of from September 2016 to July 2017. Forty five men were enrolled in this study, thirty of them were within infertility groups, and Fifteen were Normozoospermia as control. The infertile group subdivided into 11 male with oligozoospermia and 4 cryptozoospermia and 15 were patient with Azoospermia (9 obstructive and 6 non obstructive azoospermia). Comparison in the levels of serum and seminal plasma Inhibin B and FSH in relevance to sperm function parameters in different groups were done.

Results: There was highly significant difference between control group and study group in all seminal fluid parameters. The highest value of serum FSH was in NOA group and lowest value was in OA and normozoospermia and have highly significant value ($P=0.0001$). Serum Inhibin B shown highly significant value ($P=0.00947$) in comparison between groups and highest value seen in NOA and other groups have nearly convergent values. Both FSH and Inhibin B in seminal plasma have non-significant value when compared between control group and study sub groups.

Highly significant difference of FSH in serum and seminal plasma between fertile(control) group and infertile subgroups observed in each of oligozoospermia, cryptozoospermia and NOA and significant difference observed in normozoospermia and OA. Comparison of Inhibin B in serum and seminal plasma between fertile (control) group and infertile sub groups showed highly significant difference in each of cryptozoospermia, OA and NOA and significant difference in

normozoospermia and non-significant difference seen in oligozoospermia. Comparison of hormones in serum between fertile group and infertile subgroup showed significant value in Inhibin B but not in FSH. Highly significant positive correlation seen between age and level of FSH in serum, and significant positive correlation between age and FSH level in seminal plasma. Highly significant negative correlation between serum FSH and both of progressive motile sperm and normal morphology, and significant with negative correlation with sperm concentration, while significant positive correlation between FSH and non-progressive and immotile sperm. FSH in seminal plasma shown significant negative correlation with progressive motility, significant positive correlation with immotile sperm, and highly significant negative correlation with normal morphology. The most sensitive test was Inhibin B in serum while the most specific test was FSH in seminal plasma in relation to abnormal sperms parameters.

Conclusion: 1-High level of serum FSH and seminal plasma Inhibin B enhance to precise laboratory diagnosis for cryptozoospermia patient, particularly for those nominate for IVF or ICSI. 2-The best test used for detection of NOA is evaluation of Inhibin B in serum. 3-FSH increased with aging. 4-FSH have inverse relationship with progressive motile sperm as well as normal morphology of sperms. 5-Serum Inhibin B -FSH index is more sensitive than serum FSH alone, but sensitivity of seminal Inhibin B - FSH index is about double of seminal plasma FSH alone. 6- FSH test alone more specific test than Inhibin B-FSH index in both serum and seminal plasma.

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Introduction:-

Male fertility requires normal sperm production and sperm transport, and adequate sexual performance, functions that require normal levels of testosterone (for normal sperm production). Male infertility can be due to a number of factors, including abnormal spermatogenesis; reproductive tract anomalies or obstruction; inadequate sexual and ejaculatory functions; and impaired sperm motility⁽¹⁾.

Spermatogenesis and steroidogenesis are controlled by a master switch (gonadotropin releasing hormone (GnRH) pulse generator) that controls two separate and independent feedback systems: androgen production (LH-testosterone) and sperm production (FSH-inhibin)⁽²⁾.

Semen consists of spermatozoa suspended in a fluid medium called seminal plasma⁽³⁾. Seminal plasma is a complex fluid portion and mediates the chemical function of the ejaculate⁽³⁾. It contains an array of organic and inorganic chemicals, encompassing a number of biologically and immunologically active compounds, including hormones. The main groups of hormones detected in seminal plasma are classified as steroid hormones (sex steroids and immunomodulatory steroids) and Peptide hormones⁽⁴⁾. Sex steroid hormones are also produced from sex steroid precursors which origin from adrenal cortex⁽⁵⁾. Some of Peptide hormones serve as biomarkers of male fertility disorders, such as inhibin, activin, and antimüllerian hormone (AMH)⁽⁴⁾.

The initiation and maintenance of normal spermatogenesis is dependent on the synergistic effect of FSH and testosterone⁽⁶⁾. There are positive proportion between FSH and production of spermatozoa by preventing the apoptosis of type A spermatogonia⁽⁷⁾. In the male FSH is required for the determination of Sertoli cell number, and for induction and maintenance of normal sperm production⁽⁸⁾.

Inhibin B is gonadal dimeric polypeptide hormone of an α and β_B subunit^(7,9). It plays a key role in the regulation of the hypothalamic-pituitary-gonadal hormonal axis during male childhood and pubertal development, Inhibin B is a direct marker of the presence and function of Sertoli cells and appears to reflect testicular function in boys⁽⁹⁾. In addition to a negative correlation of Inhibin B with FSH, circulating inhibin B shows a positive correlation with sperm concentration in the ejaculate⁽¹⁰⁾.

Subject, Materials and Methods:-

Subject:-

The present study was carried out in the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies \ AL- Nahrain University, Baghdad-Iraq ; during the period of from September 2016 to August 2017. Forty five men were enrolled in this study, thirty of them were within infertility groups, and Fifteen were Normozoospermia as control. The infertile group subdivided into 11 male with oligozoospermia and 4 cryptozoospermia and 15 were patient with Azoospermia (9 obstructive and 6 non obstructive azoospermia, Forty five Men divided to five groups).

Evaluation of Male Infertility:-

Assessment of male infertility was done by specialist urologist depending on patient's history, physical examination, semen analysis and other specialized investigations, such as hormonal analysis.

Seminal Fluid Analysis:-

The semen samples were collected by masturbation into a clean, dry disposable Petri dish in a special room. Samples were transferred to the laboratory of seminal fluid analysis in the institute immediately and placed in an incubator at 37°C till complete liquefaction. All the samples were analyzed depending on the WHO2010 Laboratory Manual for the Examination and Processing of Human Semen.

Principle of Hormonal Assay:-

The assay was based on sandwich enzyme-linked immune-sorbent assay technology.

Statistical Analysis:-

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means. Estimate of correlation coefficient between variables in this study. and Microsoft Excel Work Sheet 2010). The results were expressed as mean \pm Standard error (SE). Paired sample t- test was applied by use SPSS statistical program to compare between two values. To study the correlation between the parameters within the same group, bivariate Pearson's correlation coefficient (two tailed) was used. The differences between the values were considered statistically significant if the P value is lower than 0.05 ($P < 0.05$).

Results:-

The mean age for patient group involved in this study was 28.93 ± 0.6 years with range from 21 to 38 years and the mean duration of infertility was 4.12 ± 0.181 years, however the mean age for control group was 32.06 ± 1.4 years with range from 27 to 45 years.

Semen samples were obtained from each male, Table (1) showed sperms function parameters for normozoospermic control group, mean sperms concentration was (concentration \pm SE) (62.5 ± 5.4 million /ml) million/ml, the mean of progressive sperm motility (%) (36.1 ± 1.6), the mean of non-progressive sperms motility (%) (40.4 ± 1.3), and the mean of immotile sperms (%) (23.5 ± 1.1) and the mean of normal sperms morphology was % (42.4 ± 4.1).

The study group (infertile group) observed the mean sperms concentration was (concentration \pm SE) (2.016 ± 3.639 million/ml), , the mean of progressive sperm motility (%) (6.73 ± 3.32), the mean of non-progressive sperms motility (%) (8.77 ± 2.38), and the mean of immotile sperms (%) (34 ± 7.44) and the mean of normal sperms morphology was % (7.2 ± 2.18). significant value ($p < 0.01$) of all parameters between control group and study group. higher values of FSH in serum were observed in the non-obstructive azoospermia (37.74 ± 3.32 IU/l) followed by cryptozoospermia (14.07 ± 8.55 IU/l) then oligozoospermia (9.64 ± 2.84 IU/l). Serum FSH range was relatively similar for obstructive azoospermia and normoazoospermia groups As it was (5.24 ± 1.54 IU/l), (4.76 ± 0.74 IU/l) respectively. While the lowest values for FSH were noticed in normozoospermia group.

The higher level of Inhibin B in serum observed in non-obstructive azoospermia (22.42 ± 20.59 pg/ml), Another groups have nearly convergent values (2.61 ± 0.58 pg/ml, 2.12 ± 0.38 pg/ml, 1.33 ± 0.78 pg/ml, 0.975 ± 0.14 pg/ml) for oligozoospermia, normozoospermia, obstructive azoospermia and cryptozoospermia respectively.

The higher level of FSH in seminal plasma was noticed in obstructive azoospermic group with Mean \pm SE of (1.33 ± 0.78 IU/ml), lower value was observed in non-obstructive azoospermic group (0.640 ± 0.06 IU/ml), Other values graded between these two groups as it was for cryptozoospermia group (0.975 ± 0.14 IU/ml), Oligozoospermia group (0.781 ± 0.09 IU/ml) and for normozoospermia, it was (0.780 ± 0.12 IU/ml). Statistical analysis shows a non-significant FSH variation among all groups of subjects (fertile and infertile).

Regarding seminal plasma Inhibin B the higher level was found in non-obstructive azoospermia with a mean \pm SE of (8.12 ± 4.09 pg/ml) followed by obstructive azoospermia (7.22 ± 2.21 pg/ml) then in cryptozoospermia was (7.03 ± 4.16 pg/ml), while it was (4.05 ± 0.82 pg/ml) in oligozoospermia and the lowest value was in normozoospermia (4.45 ± 0.65 pg/ml) these findings were statistically insignificant.

A high significant differences was observed between serum and seminal plasma in non-obstructive azoospermic group, Cryptozoospermia group and Oligozoospermia group with a P-value of <0.01 . These Findings run hand by hand with those observed in obstructive azoospermia group and normozoospermia group but with a P-value of <0.05 as shown in the table 1.

Table 1:-Comparison of FSH in serum and seminal plasma between fertile(control) group and infertile subgroups:

The Group	Mean \pm SE of FSH		T-Test	P-value
	Serum	Plasma		
Normozoospermia	4.76 ± 0.74	0.780 ± 0.12	2.174	0.0284*
Oligozoospermia	9.64 ± 2.84	0.781 ± 0.09	3.763	0.0031**
Cryptozoospermia	14.07 ± 8.55	0.975 ± 0.14	3.956	0.0001**
Obstructive azoospermia	5.24 ± 1.54	1.33 ± 0.78	2.308	0.0296*
Non-obstructive azoospermia	37.74 ± 3.32	0.640 ± 0.06	6.590	0.0001**

Table (2) illuminate highly significant value ($P < 0.01$) seen in each of non-obstructive azoospermia the difference between serum and seminal plasma was (5.387), then cryptozoospermia and obstructive azoospermia have the same difference between serum and seminal plasma (3.492) and the same significant value ($p < 0.01$) and considered a highly significant statistically, and the significant value ($p < 0.05$) seen in control group (normozoospermia) and its difference between serum and seminal plasma was (2.007).

A non-significant value ($p > 0.05$) seen only when compare between serum and seminal plasma in oligozoospermic group and the difference between them was (2.178) as it shown in the table below.

Table 2:-Comparison between serum and Seminal plasma in level of Inhibin B between fertile(control) group and infertile subgroups:

The Group	Mean \pm SE of Inhibin B		T-Test	P-value
	Serum	Plasma		
Normozoospermia	2.12 ± 0.38	4.45 ± 0.65	2.007	0.0415*
Oligozoospermia	2.61 ± 0.58	4.05 ± 0.82	2.178	0.084NS
Cryptozoospermia	0.975 ± 0.14	7.03 ± 4.16	3.492	0.0001**
Obstructive azoospermia	1.33 ± 0.78	7.22 ± 2.21	3.492	0.0001**
Non obstructive azoospermia	22.42 ± 20.59	8.12 ± 4.09	5.387	0.0001**

The comparison between fertile and infertile groups (main groups) in serum which include the following in FSH and inhibin B hormones. The mean and standard error of FSH in fertile men (4.76 ± 0.74 IU/ml) was significant ($p < 0.05$) in comparison with FSH in infertile groups men (13.62 ± 2.55 IU/ml).

The mean and standard error of Inhibin B in serum of fertile groups men (2.12 ± 0.38 pg/ml) was non-significant ($p > 0.05$) in comparison with Inhibin B in infertile groups men (5.04 ± 3.12 pg/ml).

Non-significant value in both hormones (Inhibin B and FSH) when compared between fertile and infertile subjects in seminal plasma. The Mean \pm SE of FSH result was (0.780 ± 0.12 IU/ml for fertile subject) and (0.957 ± 0.21 IU/ml for infertile patients), And the Mean \pm SE of inhibin B was (4.44 ± 0.65 IU/ml for fertile subjects) and it was (6.25 ± 1.09 IU/ml in infertile patients).

The correlation coefficient was positive with highly significant value ($p < 0.01$) between the age and FSH in serum. Also the correlation coefficient was positive correlation with significant value ($p < 0.05$) seen between age and FSH in seminal plasma.

The correlation coefficient was positive with non-significant values ($P > 0.05$) between age and inhibin in serum, The Negative correlation coefficient with non-significant value ($p > 0.05$) seen between age and Inhibin B in seminal plasma.

Negative correlation coefficient with highly significant value ($p < 0.01$) seen between normal morphology and FSH in serum and between motility of sperms and FSH too as shown in the table (4.9).

Positive correlation coefficient with significant value ($p < 0.05$) was seen between immotile sperm and FSH and the same correlation and significant value seen between non-progressive sperm and mentioned hormone (FSH).

Negative correlation coefficient with significant value ($P < 0.05$) seen between concentration of sperm with FSH in serum.

Positive correlation coefficient with non-significant value ($P > 0.05$) seen between Inhibin B and non-progressive sperm. Negative correlation with non-significant value ($P > 0.05$) seen between Inhibin B and each of: normal morphology, immotile sperm and progressive motile sperm respectively. Negative correlation coefficient with significant value ($P < 0.05$) seen between concentration of sperm with Inhibin B as shown in the table (4.10).

Negative correlation coefficient with highly significant value ($P < 0.01$) between normal morphology of sperm and FSH, positive correlation coefficient with significant value ($P < 0.05$) were seen between immotile sperm and FSH, negative correlation with significant value ($P < 0.05$) seen between progressive motile sperm and FSH.

FSH has non-significant value ($p > 0.05$) with negative correlation when compared with concentration of sperm, and has positive correlation with non-progressive motile sperm.

Positive correlation coefficient with non-significant value was seen between immotile sperm and Inhibin B. Negative correlation coefficient with non-significant value ($P > 0.05$) were seen between Inhibin B and each of: Normal morphology, concentration, progressive motile and non-progressive motile sperm.

Sensitivity and specificity of FSH and Inhibin B analysis:-

The table (3) was shown that the degree of sensitivity of FSH test with Inhibin B test together in serum (67.21%) were higher than FSH in serum alone (42.07%), while not effective when comparison between the sensitivity of these two test (68.22%) with sensitivity of Inhibin B in serum alone (68.22%), whilst FSH test in seminal plasma (31.86%) were less sensitive than when to be together with Inhibin B in seminal plasma (64.72%), the Inhibin B test in seminal plasma (54.09%) was less sensitive than Inhibin B test and FSH test together in seminal plasma (64.72%).

While the specificity of FSH test in serum (63.58%) were more specific than FSH and Inhibin B test together in serum (37.55%), The same think in FSH test in seminal plasma (68.41%) was more specific than both FSH test and Inhibin B test in seminal plasma (35.91%), nevertheless Inhibin B test in serum (37.44%) has nearly similar specificity when compare with test of FSH and Inhibin B in serum (37.55%), while Inhibin B test in seminal plasma (49.17%) has high specificity degree than FSH and Inhibin B together in seminal plasma (35.91%) as it shown in the table 3.

Table 3:-Sensitivity and Specificity of FSH and Inhibin B test in serum and seminal plasma:

Type of test	Sensitivity of test	Specificity of test
FSH test in serum	42.07	63.58
FSH test in seminal plasma	31.86	68.41
Inhibin B test in serum	68.22	37.44
Inhibin B test in seminal plasma	54.09	49.17
FSH test and Inhibin B test in serum	67.21	37.55
FSH test and Inhibin B test in seminal plasma	64.72	35.91

Discussion:-

In the present study the subjects were selected randomly and the median age of the fertile and infertile groups was quite similar and that identical to the observation of Nadia A.S. Aleisa,2013⁽¹¹⁾.

Nadia A.S. Aleisa,2013 agree with the present study when observed significant difference ($p < 0.01$) in the median values of sperm concentration, sperm motility and sperm abnormal morphology between the fertile and subfertile men⁽¹¹⁾, Kiran P. Nallella *et al*, 2006 also found significantly higher values for many of the sperm characteristics (concentration, motility, morphology) than the men with male factor infertility⁽¹²⁾.

Angela P. Cadavid J. *et al*, 2014. observed there was statistically significant decreased in sperm progressive and sperm count in the infertile group when compared with fertile male⁽¹³⁾ that mean they agree with our result but the last one also said there was non-significantly different between fertile and infertile groups in other semen parameters (Volume of ejaculate, Sperm vitality, Sperm motility, progressive motility, sperm motility, non-progressive, Sperm count, and Normal morphology) and that disagree with our observation because it was highly significant difference between fertile men and infertile men the difference may be due small number of the study group of the last authors.

Abasalt H. Colagaret *et al*, 2013 agree with the present study when observed highly significant deferens in sperm morphology and sperm count and sperm motility between fertile and infertile men⁽¹⁴⁾.

The expected values of FSH in normal men, is restricted between 1.0 - 14.0 IU/l, in our hormonal analysis kit this fact observed in all of normozoospermia, OA and oligozoospermia but cryptozoospermia have marginal serum FSH value between upper normal limiting and abnormal value, OA have slightly lower FSH serum level than normozoospermia that agree with Ahmet G. *et al*, 2013 when said normal levels of FSH and LH are expected in OA; however, FSH and LH can be low or elevated in NOA⁽¹⁵⁾, this finding is matching the result of Yong-Tong Zhu *et al*, 2016 when observed that FSH was remarkably elevated in Group NOA, moderate in Groups extremely severe oligozoospermia and cryptozoospermia, and lowest in Group OA⁽¹⁶⁾. Other finding of this study is higher values of FSH were observed in the NOA that motivated with Bromage SJ. *et al*, 2007 when say the serum concentration of FSH is inversely correlated with the impairment of spermatogenesis⁽¹⁷⁾.

Deffieux X *et al*, 2017. concluded that Inhibin B seems to be a useful marker of spermatogenesis⁽¹⁸⁾. Therefore, Yong-Tong Zhu *et al*, 2016. Noticed FSH and inhibin B concentrations conjointly reflected the capacity of spermatogenesis in cryptozoospermia and extremely severe oligozoospermia were similar, better than NOA but worse than OA⁽¹⁹⁾. This result is counter to our study because we observed the higher level of Inhibin B was in NOA and another groups have nearly convergent values this differ attributed to result of Santiago Brugo-Olmedo *et al*, 2001 when observed the patients with NOA has significantly higher levels of serum FSH and significantly lower levels of inhibin B. Mean inhibin B serum levels were significantly higher in patients with NOA who had spermatozoa on TESE (Testicular sperm extraction) than in those in whom no spermatozoa, but mean FSH serum levels did not have similar predictive power⁽²⁰⁾. This finding contradicts the results of Von Eckardstein *et al*, 1999 when observed that inhibin B measurement in serum, in which the level of inhibin B is higher in obstructive samples than in those without obstruction⁽²¹⁾.

Richard Hamplet *et al*, 2013 noticed both LH and FSH have been detected and measured in the human seminal plasma as early as in 1970s⁽²²⁾. P. Fossat *et al*, 2017 observation fit with result of present study when they observed non-significant difference was noted for seminal FSH values expressed either in nanograms per milliliter or nanograms per whole ejaculate between the normozoospermic group and the oligozoospermic group⁽²³⁾, our observation recorded non-significant value between fertile and all infertile groups (oligozoospermia, cryptozoospermia, obstructive azoospermia and non-obstructive azoospermia).

In the results of our study observed the higher level was found in NOA and lower level of Inhibin B recorded in oligozoospermia and normozoospermia that parallel to Le Lannou D. *et al*, 1979⁽²⁴⁾ when said that secretion of inhibin into the seminal fluid cannot be simply a result of passive transport from the circulation, This implies a concentrating effect by the prostate and/or the seminal vesicles. Most of the inhibin in the rete testis fluid is probably reabsorbed in the caput of the epididymis⁽²⁴⁾ this similar to of Yehia F. Al Garemet *et al*, 2002 who observed When subjects with obstructive azoospermia were compared to those without obstruction, there was no difference in seminal inhibin B concentrations, but same authors contrast with our result when they said seminal inhibin B to be

significantly higher in normozoospermic than in azoospermic samples, sustaining the relation between inhibin B secretion and sperm concentration⁽²⁵⁾. This difference of high level of Inhibin in NOA seems to be related to the developmental stage of the surrounding germ cells.

Jensen TK *et al*, 1997. FSH has been used as a marker of spermatogenesis, but the optimal criterion for serum level has still not been precisely determined⁽²⁶⁾. In the present study, we found highly significant ($p < 0.01$) difference in the levels of FSH within seminal plasma compared to serum in each of NOA, Oligozoospermia, cryptozoospermia and significant ($p < 0.05$) difference in both of normozoospermia and OA that conform with finding of Muhammad B. F., 2007 when observed there was significant ($p < 0.05$) reduction in the levels of gonadotropins within seminal plasma compared to serum⁽²⁷⁾.

The present study founded highly significant value between serum and seminal plasma Inhibin B in each of OA, NOA and cryptozoospermia that disagree with Deffieux *et al*, 2003 when observed seminal plasma it is not a better marker than the serum hormone, note that they compare between Inhibin B, Neutral alpha glucosidase (NAG), AMH and Fructose⁽¹⁸⁾, this difference may be due to high grading concentration of Inhibin B. Significant value seen in normozoospermia that agree with Deffieux *et al*, 2003 when observed that serum and seminal inhibin B levels are not predictive parameters for the selection of azoospermic men⁽¹⁸⁾. Oligozoospermia have non-significant value between serum and seminal plasma inhibin B that agree with Soudabeh S. *et al*, 2010, when suggest must identification of more specific molecular markers in seminal plasma to definitely evaluate the status of spermatogenesis is recommended⁽²⁸⁾, may be due to non-specific changes in inhibin B concentrations.

For initiation of spermatogenesis and maturation of spermatozoa, FSH is necessary. In the infertile men, higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage⁽²⁹⁾. That agree with the present study FSH level were significantly elevated in infertile males when compared with the levels in proven fertile controls.

The present study appear non-significant value of Inhibin B in serum between fertile and infertile group that disagree with Philip Kumanovet *et al*, 2006 revealed that inhibin B levels are significantly reduced in men with infertility problems, irrespective of etiology, compared with fertile men. So that the present study also disagree with them when said inhibin B levels are a more sensitive marker of male factor infertility than other available hormones, irrespective of the etiology⁽³⁰⁾, the difference in the result may be due to the contrast in the number of subjects or may be due to difference in the etiology of infertility.

In present study observed non-significant value of both hormones in seminal plasma between fertile and infertile group that agree with the result of Muhammad B. MR Fakhridin, 2007 that is about FSH in seminal plasma⁽²⁷⁾ but inhibin B concentrations showed significant difference in the seminal plasma of azoospermic (infertile) men in comparison with those of normospermic fertile men⁽²⁸⁾, the difference could be due to other hormonal disturbances such as testosterone.

In the present study we founded the highly significant value with positive correlation between FSH in serum and age that agree with Steven M. Pincus *et al*, 1997⁽³¹⁾.

In the present study we observed non-significant with positive correlation between age and Inhibin B in serum that disagree with S Grunewald *et al*, 2013 when finding that inhibin B showed a highly significantly negative correlation with age in subjects older than 30 years⁽²⁹⁾. And also disagree with Mahmoud *et al*. 2000 that study included only a smaller group of men detected a weak but significant correlation of serum inhibin B with age⁽³²⁾. This difference could be due to the age we are taken in the present study are younger than these other study.

In our result observed negative correlation with non-significant value between age and Inhibin B in serum that disagree with Masahiro Kondo *et al*, 2000 when observed that plasma concentrations of inhibin B increased gradually and were significantly correlated with age⁽³³⁾. This difference might be due to they were taking the male chimpanzees as model of study.

In the our study observed significant value with negative correlation between FSH and sperm concentration that agree with Anne-Laure Barbotin *et al*, 2015 when observed that the serum levels of FSH and LH are inversely (negative) associated with sperm concentration⁽³⁴⁾. The present study also match the result of the same

author when observed negative correlation with highly significant value between FSH with motility and morphology.

As well as the percentile and inverse relation between progressive motile sperm with non-progressive motile and immotile sperm that explains our result about significant positive correlation between FSH with non-progressive and immotile sperm.

In the present study observed significant value with negative correlation between Inhibin B and sperm concentration that inverses result of John D. Meeker *et al*, 2007 when observed the Inhibin B and free T4 are positively associated with sperm concentration⁽³⁵⁾. Also disagree with Anne-Laure Barbotin *et al*, 2015. they observed Inhibin B was positively correlated with the total sperm count⁽³⁴⁾. As well as disagree with them because they are revealed significant associations between hormone levels on one hand and progressive motility and normal forms on the other hand inverses of our result we are observed non-significant value between Inhibin B and progressive sperm and normal morphology.

Vasquez JM *et al*, 1986 observed that seminal FSH concentrations were positively correlated with sperm output but not sperm motility⁽³⁶⁾, that disagree with our result because we are found non-significant correlation between seminal FSH and concentration of semen but founded significant value with negative correlation with motility of sperm that confirm negative correlation between FSH level and concentration of sperm. Also we found highly significant negative correlation between seminal FSH and normal morphology of sperm that give important evidence about truth of theory said the FSH level is a useful initial marker for the evaluation of spermatogenesis. Elevated FSH levels are commonly seen in men with testicular diseases, and low levels are observed in those with central disorders⁽³⁷⁾.

Duvilla E. *et al*, 2008 observed a significant positive correlation was observed between Inhibin B concentration in the seminal plasma and sperm count⁽³⁸⁾. But Hu YA *et al*, 2003 notice that statistically positive correlation between sperm concentration with seminal plasma Inhibin B and serum Inhibin B⁽³⁹⁾. they disagree with present study because we find non-significant value between seminal plasma Inhibin B in and all seminal fluid parameters. This difference in result may be attributed to variability of infertile group in our study.

In the present study observed detection of Inhibin B in serum is more sensitive than other test that mean it useful test for detection type of infertility than other test that agree with Philip Kumanov *et al*, 2006 when observed that Inhibin B levels may be a better marker for evaluating male factor fertility than FSH and LH. In patients with infertility, measuring Inhibin B levels may provide useful information on spermatogenesis and possibly serve as a more direct marker of the spermatogenesis than FSH⁽⁴⁰⁾.

FSH-Inhibin B index in both serum and seminal plasma is also high sensitively than FSH alone in serum or seminal plasma in one hand or than Inhibin B in seminal plasma alone in the other hand that agree with Santiago Brugo-Olmedo *et al*, 2001 when said serum Inhibin B level seems to be more accurate than serum FSH level in prediction of the presence of testicular spermatozoa in patients with non-obstructive azoospermia⁽²⁰⁾. And also agree with Philip Kumanov *et al*, 2006 when recorded that Inhibin B and FSH together are a more sensitive marker for spermatogenesis than either one alone⁽⁴⁰⁾, but disagree with them about specificity because they observed Inhibin B-FSH index more specific test than each alone but in our study observed opposite result Inhibin B in seminal plasma was higher specific than other test even than FSH-Inhibin B index then FSH in serum also high specific than other test, this difference may be due to affecting of Inhibin B test by duration of taking sample.

Conclusion:-

High level of serum FSH and seminal plasma Inhibin B may be enhance to precise laboratory diagnosis for cryptozoospermia patient, particularly for those nominate for IVF or ICSI. The best test used for detection of NOA is evaluation of Inhibin B in serum. FSH increased with aging. FSH have inverse relationship with progressive motile sperm as well as normal morphology of sperms. Serum Inhibin B-FSH index is more sensitive than serum FSH alone, in the other hand sensitivity of seminal Inhibin B-FSH index is about double of seminal plasma FSH alone. FSH test alone more specific test than Inhibin B-FSH index in both serum and seminal plasma.

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