COMPARATIVE STUDY OF SOME BIOCHEMICAL MARKERS IN SEMINAL PLASMA AND SERUM FOR THREE GROUPS OF INFERTILITY MEN PATIENT.

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Abstract- High rates of infertility and pollution in Iraq in particular, and in the countries of the region in general, because of the wars, led to the emergence of many difficulties in the diagnosis of infertility, so the objective of this study was to find out which methods used to diagnose infertility is better, whether in serum, semen or both.This study determined the main fertility hormones and some biochemical markers in both blood and seminal plasma by ELISA method, and Total protein was measured by spectrophotometer.The current study showed the biochemical markers were an approximate match in the levels of its concentrations in both seminal plasma and blood serum. In addition, there was a significant increase ($p<0.05$) in the level of FSH, LH, Prolactin and Total protein in both Azoospermia and Oligospermia in comparison with the control group (Normospermia) in seminal plasma and serum.whereas there was a significant decrease ($p<0.05$) in the level of (Testosterone, Inhibin B, AMH, and Natural α- Gluc) in both Azoospermia and Oligospermia comparison with the control group in seminal plasma and serum. The correlations of both serum and seminal biochemical markers with seminal plasma parameters were somewhat similar. BMI has a significant increase ($p< 0.05$) in both Azoospermia (29.80± 3.68 Kg/m²), and Oligospermia (27.96± 2.42 Kg/m²) comparison with Normospermia (22.10± 3.09 Kg/m²). In conclusion, estimation of reproductive hormones in seminal plasma along with serum is a crucial tool in order to better identify and accurate treatment for male infertility disorders such as impaired spermatogenesis, especially in case of seminal (LH, Inhibin B, and AMH) were higher than those in serum. As well as high BMI level has a mischievous effect on semen quality and concentrations of reproductive hormones.

Keywords- BMI, AMH, Inhibin B, NAG.

I. INTRODUCTION

The male infertility factors are about 30% of infertile couples, and 20% of infertility factors is due to both partners. [45].Two types of infertility, Primary infertility the couple is an inability to carry a baby and does not predate uterogestation yet, or unable to the procurement a live birth. Secondary infertility the couple is an unaptness to bear a child in spite of having the earlier pregnancy, or unable to obtain another child. [7, 38].Male infertility factors are numerous such as varicocele that present in 2-22% of infertile males [30] that result from decrease level of Testosterone in serum. Other factors such as hormonal disorders, environmental, genetic, coital and idiopathic factors which constitute about 25 % of male infertility. [7] Another researcher classified the causes of infertility into four classes: the male factor, female factor, congregated factor and idiopathic factor [36]. Large numbers of protein biomarkers, thousands of essential proteins and tissue-specific proteins found in seminal plasma, which represent accurate indicators for pathologic status related to the reproductive system [12]. This study deals with a number of biochemical markers hormones (Follicle Stimulating Hormone, Luteinizing Hormone, Testosterone, Inhibin B, Prolactin, and Anti Mullerian hormone,) with other biochemical markers (neutral α –glucosidase enzyme and total protein). Each of these biochemical markers has the important role in spermatogenesis and determines part of the problem that related to male infertility, along with the seminal plasma parameters.Many of the previous studies did not address the seminal plasma as hormonal indicators enough to study, but most it tended to focus on the serum, so this study will rely on seminal plasma as the main hormonal indicator (destination search) along with serum. [56]. Therefore, the objective of the study is to identify hormonal changes, and its relationships in each of three infertility groups (Azoospermia, Oligospermia, and Normospermia) and to find the relationships between BMI and biochemical markers in both seminal plasma and serum.. Follicle-stimulating hormone (FSH).It is a pituitary glycoprotein hormone (heterodimer) consists of both α-subunit and a specific β-subunit-like LH hormone; it secreted under the influence of GnRH (releasing by the hypothalamus) on the anterior pituitary gland. FSH stimulates release of inhibin B (by Sertoli cells) that has negative feedback on anterior pituitary gland to decrease FSH production. Spermatogenesis rely on both FSH and high level of Testosterone [20] Serum FSH is a major tool for diagnosing the types of male infertility especially in Azoospermia which has a great role in determining the complement of spermatogenesis in the testis (seminiferous tubules) [45,7].FSH secretion is controlled by (inhibin B and Testosterone) which have negative feedback to regulate the release of FSH. While LH, Testosterone and FSH act as regulators for germ cells development and male fertility [8]. Luteinizing hormone (LH)Called (Lutrophin).It is a pituitary glycoprotein hormone (heterodimer). It consists of both α-subunit

and a specific β-subunit which is secreted by gonadotrophic cells in the anterior pituitary gland and exerts its effect on Leydig cells to synthesize Testosterone hormone, the receptor which sites on the cell surface of both hypothalamus and pituitary glands bind to circulating Testosterone and decrease amount of releasing LH. [51].Testosterone hormone (T).It is secreted by the Leydig cell under the influence of LH, and acts as a negative feedback factor on the hypothalamus to reduce the release of GnRH, also it acts directly on the anterior pituitary gland in order to inhibit release of the LH hormone [51].The effects of Testosterone are numerous and very wide, it acts on stimulating growth of bone connective tissue, muscle cell and on skin to increase the size and secretion of sebaceous gland, and to stimulate growth and development of testes, prostate gland, seminal vesicles and penis, Testosterone has importantl function in spermatogenesis and developing of secondary sex characteristics. [30] . Anti-Mullerian hormone (AMH). Anti-Mullerian hormone also called Mullerian-inhibiting hormone (MIH) is produced by Sertoli cells, it has an important role in determining the sex of the fetus. Testicular Sertoli cells play a major role in synthesized AMH. Serum FSH and AMH can be used to indicate the case of spermatogenesis[20].The evaluation of AMH should be made carefully in Azoospermia and Oligospermia Anti- Mullerian hormone can be used as an indicator of protein production and development of Sertoli cells. [7] AMH has a great role in inducing regression of the Mullerian canal. Other roles are represented by regulating Testosterone synthesis and developing Leydig cells. AMH has been shown to regulate the production of sex hormones. [3] Inhibin B hormone (inh B):Inhibin B is a dimer component has B subunit which identical to activin. It is an important hormone which secreted by Sertoli cells under the effect of FSH, and it acts directly as inhibiting factor (has negative feedback) on the anterior pituitary gland in order to decrease the level of FSH, Inhibin has a common α-subunit with a βa-subunit so called (Inhibin A) or with β b-subunit called (Inhibin B), synthesis of Inhibin B increases by influence of FSH, when the level of inhibin B (which has down regulate to FSH) is rising lead to decrease FSH releasing from the pituitary gland.[37].Natural α- glucosidase enzyme (NAG): It is secreted in the epididymis, and it reflects the employment status of the epididymis.[34] The functions of accessory sex glands can be determined by the investigation of fructose, NAG and acid phosphatase. Epididymis (especially corpus and cauda) is the major source of α- glucosidase. The value of α- glucosidase was lower in case distal ductal obstruction. Natural α – Glucosidase and fructose are the best pointer to accessory glands functions and obstruction in reproduction duct, epididymis represents the major source to secret α –Glucosidase, low α –Glucosidase in semen reflect distal ductal obstruction. [45].Causes of male infertility may include decreased sperm count that accounts for about 90% of causes, other causes are such as infections, ejaculatory malfunction, ductal obstruction and disorder of the accessory glands. [42] Prolactin hormone (PRL): Prolactin hormone was regulated by inhibiting factor Dopamine which regards Prolactin Inhibiting Factor (PIF), increase level of Estrogens in male accompanied by increasing of Prolactin. This suggests the Estrogens that stimulate the release of Prolactin. [51, 32].Prolactin hormone has a great effect on spermatogenesis, which controls on both LH and FSH hormones by regulating the release of GnRH.[43].The reduction of GnRH is done by rising prolactin level, and the most causes of infertility are hyperprolactinaemia in both sexes and can be associated with galactorrhoea, or the suitable flow of breast milk, in men and women.[7,51]Total protein: It is a complex blend of proteins which can be separate in different methods, plasma albumin, α1-globulin, α2-globulin, β-globulin, prothrombin, fibrinogen, and other clotting factor formed by liver cells, while γ- globulin created by plasma cell. Tain and other co-workers found that the number and volume of liver cells drop off with age, therefore serum total protein, which formed by liver cells will be reduced gradually with age. [48, 49]. In spite of proteins play important role in fertility, also consider essential substances for sperm morphology and physiology, but increase total protein (hyperproteinemia) may cause many different diseases such as infectious disease, rheumatoid, arthritis, nephritis, and diabetes that affect fertility and increase male infertility [19].

II. MATERIALS AND METHODS

Subject .This study was conducted in the Infertility Laboratory Unit of Babylon Hospital for women and children in the province of Babylon, and fertility center in Al- Sader Hospital in the province of AL-Najaf Al- Ashraf/Iraq during the period from 1/Sep./2017 to 30/Jan./2018. Semen and serum specimens were collected from Azoospermic, Oligospermic patients in addition to controlling group (Normozoospermia) attended to the fertility center. The mean age of infertility patients was (34 ± 1.63), (30 ± 7.11) and (30 ± 5.54) years in Azoospermia, Oligospermia and Normospermia respectively.Semen specimens and serum were collected from patients according to the WHO (2010) guidelines.The following hormones FSH, LH, AMH, Prolactin, Testosterone,Inhibin B and NAG had been measured depend on immunological method (Enzyme-Linked-Immuno-Sorbent –Assay) by using ELISA reader (Huma Germany origin), while Total protein had been measured by using spectrophotometer in the laboratories of Biology Department/ faculty of Sciences/ University of Kufa. The ELISA kits used in this study was Elabscience Company USA in Origin.

Statistical Analysis

The well known statistical system statistical Package for Social Science (SPSS ver. 20) was adopted, and the analysis of variance table (one – way ANOVA) with contrast coefficient test is used for inferring the significant statement of rate and Standard Deviation, and use post hoc for high significance which has less than 0.05, such as tukey, and Gabriel test for the specimens with variable size, and Spearman's coefficient for detecting a statistical correlation between two variable. T-Test with (paired samples statistics) used to determine difference significant between seminal plasma and serum for the same persons.

RESULTS

In Azoospermia, there is a significant decrease ($p<0.05$) of pH and volume. While the liquefaction time and round cell are significantly ($p<0.05$) higher in Azoospermia than Normospermia.In Oligospermia, sperm concentration and progressive motility of sperms are significantly ($p<0.05$) low, while there is significantly ($p<0.05$) increase of non-progressive motility, immotile and abnormal sperm compare with Normospermia[Table 1].Both seminal and serum concentration of FSH, LH, PRL, and Total protein are significantly ($p<0.05$) higher in Azoospermia and Oligospermia than Normospermia. While both seminal and serum concentration of Testosterone, Inhibin B, and α-glucosidase (NAG) are significantly ($P<0.05$) lower in Azoospermia and Oligospermia than Normospermia. [Table 2].There is a significantly different ($p<0.05$) in the main BMI between three groups, BMI has a significant increase ($p<0.05$) in both Azo (29.80± 3.68 Kg/m²), and Oligo (27.96± 2.42 Kg/m²) comparison with Nor (22.10± 3.09 Kg/m²). [Fig. 1]

BMI significantly correlated ($p<0.05$) negative with sperm concentration and progressive motility[Table 3]. Generally, BMI significantly correlated ($p<0.05$) a positive with serum PRL, and Total protein, and significantly correlated ($p<0.05$) a positive with seminal PRL. While there is a significant ($p<0.05$) negative correlation between BMI and levels of Inhibin B, NAG, and AMH in both serum and seminal plasma and with the serum concentration of Testosterone only. In spite of no relationship between BMI and levels of FSH ,LH and testosteron in both serum and seminal plasma[Table 4].

DISCUSSION

This is the only study done in Iraq, which aims to estimate the main fertility hormones and some biochemical markers in blood and in seminal plasma, as well as to determine its correlations with the semen parameters, in addition to compare BMI with serum, seminal plasma and semen parameters in three groups (Azoospermia, Oligospermia and Normospermia). Comparison of biochemical markers in serum and seminal plasma of infertility men was seldom and had directed to the different result [56]. This study purposes to evaluate whether biochemical analysis of seminal plasma could be used as the alternative as the serum to determine of male infertility. The results of the semen analysis are the bedrock for diagnosis of infertility, which is considered abnormal if sperm quality is below criteria according to WHO [53]. The pathologic process in mild Oligospermia such as decreased sperm motility or abnormal morphology originates within the testes rather than being secondary to extra testicular influences such as hypothalamic- pituitary-insufficiency [41] .

The concentrations of all hormones were slightly lower in the seminal plasma than serum except seminal LH, AMH, and Inhibin B were higher than those in serum. This finding is in agreement with Fossati et al. who reported that seminal FSH value was significantly lower than serum FSH value and the seminal of LH value was higher than serum LH value. [26] also this study is in agreement with Hample et al. who explains that the level of testosterone in seminal plasma is seven times lower than in serum, FSH and PRL levels in seminal plasma were close to those in serum. While the level of seminal LH was three times higher than serum, but differs with Hample reported that inhibin B and AMH concentrations were difficult to determine in seminal plasma because these hormones contain sets of active peptides and the seminal plasma is a very rich in protein so cannot separate peptide hormone from the protein matrix. [31] in contrast, the current study agrees with Drabovich et al. suggested that determination of protein biomarkers is more abundant in seminal plasma than serum so it is more easily estimated in semen by mass spectrometry or other ways. [21] As obtained by Fakhrildin reported that the levels of gonadotropin in serum were higher than in seminal plasma, in spite of the measurement of some biochemical markers in seminal plasma may be more accurate to estimate the extent of spermatogenic suppression. [24] also Duvilla et al. suggested that seminal biomarkers are weak indicators of testicular sperm extraction.[22] Determination of seminal testosterone did not bring an advantage over determination in serum, it is used as biomarkers for estimation of semen quality, and inhibin B used as an indicator for spermatozoa and activity of the seminiferous tubule.[31] While this study is in disagreement with wijeratna et al. which reported that seminal LH was lower and seminal PRL was higher than those in serum [52]. Moreno-escallon et al. showed that seminal testosterone and PRL concentrations were higher in the infertility men than their concentrations in serum. [40] At present, the available data cannot explain why this divergence occurred. In addition to this, semen ejaculates are more difficult to obtain than blood samples. The same finding was stated by [33]

One limitation of this study is that infertility men who had doses of fertility treatment drugs, or who suffer from chronic illnesses or abnormality in the urogenital, those cases were excluded to avoid overlap in the factors of infertility as possible. Low semen volume is another limitation to exclude many patients of Azoospermia, as well as the levels of sex hormones, are varied depending on age. This limitation was minimized because age did not significantly differ between three groups.

Duration of infertility was (10.1 ± 2.21), (5.2± 3.21) and (3.5 ± 4.33) years in Azoospermia, Oligospermia and Normospermia respectively. This result agreed with Gardi et al. who reported that duration of infertility was between (1-8) years [28] also with Shaya et al. reported that increase male age-related to decrease in sperm morphology and semen volume as reported by [46, 17] Bayer show that the fertility correlated inversely with an increase the main age that started in the late thirty and early forty. While this finding is disagreement with Abdella et al reported that increasing duration of infertility was between (1-4) years. [1]

In this study, a significant ($p<0.05$) decrease in pH and semen volume was noticed in Azoospermia as compared with Normospermia. This result is in agreement with Azenabor et al. showed that lower pH value was in Azoospermia. [15] Seminal fluid composed of the secretions of the prostate gland, testis, epididymis, bulbourethral glands (Cowper's glands), periurethral gland (glands of Littre) and seminal vesicles. The secretions of acidic prostate balance with alkaline vesicle secretions to compose alkaline semen pH (≥7.2). [2] the semen pH is significantly low less than 6.8 as a result of the absence of the seminal vesicles or dysplasia.The seminal plasma is formed mainly from the relative scanty and acidic prostatic secretion, when the fructose-rich alkaline secretion of the seminal vesicles is lost[16]. A Negative and significant ($p<0.05$) correlation was observed between low semen volume with high levels of LH, Inhibin B and total protein in both serum and seminal plasma and with serum FSH only. The same finding was reported by Fakhrildin. Low testosterone secreted by the Leydig cell under the influence of LH, which can explain decrease testosterone level with increasing LH level may be due to primary testicular failure or mutation in LH receptor which impacts secretions of semen volume. [24]

Sperm concentration has an inverse significant ($p<0.05$) correlation with both serum and seminal FSH, LH, inhibin B and total protein concentrations. This result is in agreement with [24,15] while is in disagreement with Govind et al. who reported that inhibin B and FSH correlated positively with sperm concentration, so it can be used as good markers for spermatogenesis. As well as seminal NAG correlated positively and significantly ($p<0.05$) with sperm concentration.[2] Duvilla et al. reported that a significant positive correlation between seminal inhibin B and sperm count. [22]

Low semen parameters with an increased level of LH and FSH and decreased levels of testosterone in non-obstruction Azoospermia and severe Oligospermia support the fact that abnormality of spermatogenesis induces production of FSH by the pituitary gland to stimulate testes to increase their sperm production. [42] Moreover the defect in the spermatogenesis may be also because disturbance between androgen and estrogen balance while another reason is due to hyperprolactinaemia that interacted with low level of testosterone which lead to disorder in spermatogenesis,where high levels of prolactin may be lead to death of spermatogonia before mitosis [7, 42] Alrekabe et al. reported that high level of serum FSH relates to increase seminiferous epithelial destruction because FSH affects directly on the seminiferous tubules while LH acts indirectly on spermatogenesis via testosterone. [8].There is a significant positive correlation between testosterone and each of the sperm concentration, motility and morphology this may support the fact that determination of testosterone is the better biomarker than Inhibin B and AMH in estimate spermatogenesis in Oligospermia.[46] While this result is in disagreement with [7].

Serum and seminal AMH and Inhibin B levels were significantly ($p<0.05$) lower in Azoospermia and Oligospermia. This study is in agreement with Vitku et al. in case of AMH, not inhibin B, who reported that AMH and inhibin B have positive correlation with sperm quality and negative correlation with the percentage of damage spermatozoa but disagreed with Vitku et al. in case of inhibin B. [50] the current study is in agreement with Jensen et al. suggested that increase level of seminal FSH with low level of seminal inhibin B were noticed in study between two groups of Danish men.[33] Moreover is in line with [44, 13] Andersen stated that seminal AMH positively correlated with sperm concentration and progressive sperm motility so can be used as a biomarker of spermatogenesis and Sertoli cell maturity. Masato et al. stated AMH secretes from apical aspect of Sertoli cells, toward the seminiferous tubular lumen may be associated in spermatogenesis and germ cell proliferation, so low seminal AMH appears to be related to spermatogenesis or could be due to immaturity of Sertoli cell which cannot secret AMH through its apical layer into seminiferous tubules .[27, 44] While Fenichel et al. reported that seminal plasma AMH in non - obstructed Azoospermia was less than other infertile men. [25] It is thought that AMH is a better biomarker of Sertoli cell growth and activity than FSH, and seminal AMH level is a good indicator to determine mature spermatozoa. Seminal AMH utilized as a marker in the case testicular spermatozoa, but it is absolutely absent in all types of obstructive Azoospermia[31]. In this study, a

significant positive ($p<0.05$) correlation was observed between sperm motility with serum and seminal levels of NAG. This result differs with Azenabor et al. reported that no correlation between it [15]. This result is may be due to obstruction in ejaculatory duct or low testosterone level.[33,53] Normal of sperm morphology is high in Normospermia as compared with Oligospermia, as obtained by other researchers [46,10]. Kret et al. found a negative correlation of NAG activity with seminal parameters. [34] There is a significant ($p<0.05$) positive correlation between sperm characteristics and NAG in both serum and seminal plasma. As obtained by Mahmoud et al.[35]

Seminal PRL has positive and significant ($p<0.05$) correlation with sperm concentration and its motility. The same finding was reported by Hampel et al. in case sperm motility only. PRL affects sperm morphology with no clear details about its mechanism, while PRL affects sperm motility may be via any physical perturbation of the membrane or receptors. [31] A significant ($o<0.05$) positive correlation between PRL and sperm concentration that because PRL acts synergistically with LH to regulate Leydig cells. This is in agreement with [7]. A significant positive correlation between PRL and sperm motility this is because PRL is polypeptides hormone-like GH and IGF-I (INSULIN-LIKE GROWTH HORMONE - 1) in the structure which produce by Sertoli cells to play an important role in spermatogenesis and acts on sperm motility through competitive inhibition of PRL receptors [7]. High concentrations of serum and seminal FSH, LH, PRL and total protein were found in Azoospermia and Oligospermia, as obtained by [40, 44, 42], this explained the fact that a significant ($p<0.05$) positive correlation between each of serum and seminal FSH, LH, PRL and Total protein, except the relationship between seminal FSH with both PRL and Total protein was not significant in seminal plasma, in contrast with Fakhrildin reported no significant relationship between FSH and LH[24]. In this study, high levels of LH with a decreased level of testosterone may be due to loss of germinal epithelial but Leydig of testes remain intact, or the mutations of LH receptor gene that impact its associated with receptors on Leydig cells or due to dysfunction in the Leydig cell.[8, 7, 6] While this finding is in disagreement with [25, 51] who reported that seminal FSH, LH, and PRL were lower in infertile men. High levels of FSH, LH with decrease levels of testosterone and AMH as obtained by Wisam et al. reported these results are indicated to be primary hypogonadism (primary testicular failure) leads to the defect in spermatogenesis [41, 6], where the levels of FSH and LH are high while testosterone values are low due to the negative feedback mechanism of hypothalamic-pituitary-gonadal axis. [41] Low level of serum and seminal testosterone was noticed in Azoospermia and Oligospermia as obtained by [7, 42, 43, 44, 46]. Mehta et al. reported that testosterone activates genes in Sertoli cells to stimulate differentiation of sperm production. [39] although other workers suggested there are no significant differences in the level of testosterone between healthy men and infertility men. [50,40] this explained the fact that There is a significant ($p<0.05$) positive correlation between each of Testosterone, AMH, Inhibin B, and NAG. The same result was reported by Govand in case of inhibin B with testosterone reinforcing the hypothesis that inhibin B secretion influenced by Leydig cells. Many authors were stated that unknown factors originated by Leydig cells may modify the inhibin B synthesis in the tubular compartment of the humane testis. [2]. However, there is a negative significant ($p<0.05$) correlation between AMH with LH and FSH in both serum and seminal plasma, As obtained by Saleh et al. who suggested that this relationship may explain a crucial contribution of AMH in the regulation of the reproductive system, especially in the gonadotropin hormones[44].In serum and seminal, a significant ($p<0.05$) negative correlation was noticed between inhibin B levels and FSH levels. This is in agreement with Saleh et al. who reported that serum inhibin B determination is a better biomarker to estimate male fertility and to provide accurate information on spermatogenesis than another biomarker. a negative correlation between inhibin B and FSH assist the fact that inhibin B is the testicular feedback signal for FSH[44].

Serum and seminal NAG concentrations were significantly ($p<0.05$) reduced in Azoospermia and Oligospermia as compared with Normospermia which is in agreement with other workers [34, 54, 56] The epididymis secrets NAG under the control of the testosterone hormone, NAG concentration in Azoospermia is very low maybe because of two reasons, firstly the present of obstruction of the first part of the ejaculatory duct and secondly low testosterone which induces epididymis to secrete low NAG. Kret et al. and Yassa et al. found a positive correlation between seminal NAG and progressive sperm motility, and determination of seminal NAG is a very sensitive noninvasive technique to estimate the status of the epididymis and the site of obstruction. [34,54] Low level of NAG may be associated with sperm maturation and inflammatory condition of the epididymis.[54] Zopfgen et al. explained this test is very important as a sensitive and non- invasive method for differentiating between a testicular and an obstruction Azoospermia. [56].Low concentrations of serum and seminal NAG < 0.13 ng/ml in infertile groups were noticed in 14 cases in each of serum and seminal samples. This result may be indicated bilateral obstruction of the vas deferens, as well as the values between 0.13 and 0. 2 ng/ml, were observed in 26 cases in each of serum and seminal plasma. This may be due to primary testicular Azoospermia. While other cases may be due to bilateral obstruction at the epididymis. In Oligospermia, low concentration

of NAG may be due to varying types of epididymis obstruction or epididymis dysfunction.This is in agreement with [35].

Serum and seminal testosterone, inhibin B and NAG were significantly ($p<0.05$) low in Azoospermia and Oligospermia. The same result was reported by [24,15]. Fakhrildin who stated that low concentration of seminal testosterone associated with abnormal sperm morphology [24].Azenabor et al. observed that seminal NAG in Azoospermia was lower than Azoospermia[15].This is simply explained that patients may be free of obstruction of the reproductive tract. Another study by Guerin et al. observed that the bilateral obstruction between the epididymis and ejaculatory duct in Azoospermia who have decrease NAG in their seminal plasma [29]. Low inhibin B levels and high FSH levels in the serum and seminal plasma were noticed in Azoospermia and Oligospermia. This finding is in agreement with Ahmed et al. that explained this case occurred in the formerly cryptorchid men. FSH and inhibin B are the best two serum markers for determining spermatogenesis. [2], also Al-Halabi suggested Inhibin B used as a direct biomarker of Sertoli cell function and an indirect biomarker of the spermatogenesis[4].The levels of total protein in serum and seminal are significantly ($p<0.05$) higher in Azoospermia and Oligospermia that have overweight than Normospermia. This finding is in agreement with Abdullatif et al. that show total protein significantly increased in obese than normal weight. Where obesity-related to chronic inflammation and a number of adipose-related pro-inflammatory cytokines[5]. High significant ($p<0.05$) levels of BMI were observed in Azoospermia (29.80 ± 3.68), Oligospermia (27.96 ± 2.42) as compared with Normospermia (22.10 ± 3.09). This finding is in agreement with [18,55].

BMI significantly ($p<0.05$) and inversely correlated with all seminal fluid parameters except semen pH and abnormal sperm morphology is not significant. This result is in agreement with [14,55,18]. Yenzeel reported that a possible correlation between male infertility and metabolic syndrome (MS) that correlated with hypogonadism. [55] Jorunn and co-workers found high of BMI levels increase DNA damage in overweight and obese men. [14].BMI acts directly and indirectly on male infertility by prompting alteration in sexual behavior sleep, scrotal temperature, hormonal profiles and semen parameters, high BMI level correlated with increasing Estradiol and prolactin levels, and increase conversion of testosterone to Estradiol, this support the fact that fat produce estrogen from testosterone by aromatase which catalyzing this process, lead to decrease the levels of testosterone which directly affect spermatogenesis [9,14].In the current study, BMI significantly ($p<0.05$) and negatively related with a concentration of inhibin B, AMH and NAG in both serum and seminal plasma and with serum concentrations of testosterone only, that proposing a possible correlation between Sertoli cell function and adiposity. In spite of high BMI acts on AMH which produced by Sertoli cells the mechanism of this action is still unknown, may be the effect of the inflammatory in obese patients suppresses the Sertoli cells and AMH production. BMI correlated positively and significantly ($p<0.05$) with prolactin and total protein.This finding agreed with [15]. No relationship was noticed between BMI and levels of FSH and LH in both serum and seminal plasma. This result is in agreement with [44,14] while is in disagreement with [9,55].

In conclusion, estimation of reproductive hormones in seminal plasma along with serum is a crucial tool in order to better identify and accurate treatment for male infertility disorders such as impaired spermatogenesis, especially in case of seminal LH, Inhibin B, and AMH were higher than those in serum. As well as high of BMI level has a mischievous effect on semen quality and concentrations of reproductive hormones. Finally, there is about 34% of infertility patients (Azoospermia and Oligospermia) are suffering from primary testicular failure (primary hypogonadism failure) this case accompanied with high FSH, LH, with low Testosterone and Inhibin B.

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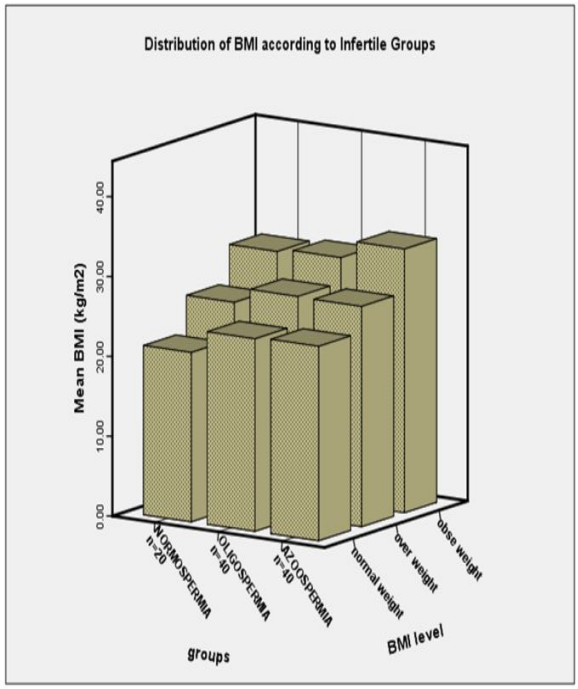

[Fig. 1] Comparison of main BMI in three groups

a : significant difference ($p<0.05$) between Azoospermia and Normospermia.

b : significant difference ($p<0.05$) between Oligospermia and Normospermia.

c : significant difference ($p<0.05$) between Azoospermia and Oligospermia.

Table[1] :Comparison of seminal parameters between three groups With WHO criteria 2010.

characteristic	AZOOSPERMIA N=40 Mean Std Deviation	OLIGOSPERMIA N=40 Mean Std Deviation	NORMOSPERMIA N=20 Mean Std Deviation
pH (Alkaline ≥7.2)	6.4553 ± 0.37 [a]	8.2588 ± 0.56 NS	7.6255 ± 0.49
Volume (ml)	1.061± 0.64 [a]	2.295 ± 0.58 NS	2.746 ± 0.30
Liquefaction time (within 60 minutes)	32.13± 1.785 [a]	30.41 ± 0.67 NS	30.05 ± 0.39
Sperm concentration ($\geq 15\times10^6$ sperm/ml)	0.00 ±0.0 NS	7.35 ± 1.03 [b]	50.84 ± 21.73
Progressive motile sperm (≥32% within 60 minutes)	0.00± 0.0 NS	11.77 ± 2.71 [b]	52.94 ± 17.83
Non-Progressive motile sperm (%)	0.00 ± 0.0 NS	14.93 ± 5.30 [b]	10.39 ± 3.41
Immotile sperm (%)	0.00 ± 0.0 NS	27.43 ± 7.64 [b]	20.83 ± 5.47
Abnormal sperm (%)	0.00 ± 0.0 NS	70.15 ±16.34 [b]	20.75 ± 11.61
Round Cell (10^6 cell/ ml/ H.P.F)	2.21 ± 0.82 [a]	2.41 ± 0.74 [b]	1.20± 0.89

a: significant difference ($p<0.05$) between .Azoospermia and Normospermia

b: significant difference ($p<0.05$) between Oligospermia and Normospermia

[Table 2] Comparison of Biochemical Markers in Three Groups

Biochemical markers		AZOOSPERMIA N=40 Mean Std. Deviation	OLIGOSPERMIA N=40 Mean Std Deviation	NORMOSPERMIA N=20 Mean Std Deviation
FSH (mIU/ML)	-Serum	21.51 ± 2.45 [a]	21.95 ± 2.59 [b]	10.96 ±2.28
	-Seminal plasma	8.96 ± 2.06 [a]	9.06± 2.11 [b]	7.56 ±2.32
LH (mIU/ML)	-Serum	12.41 ± 1.64 [a]	12.82 ±1.64 [b]	6.22 ±1.01
	-Seminal plasma	16.59 ± 1.87 [a]	16.60 ±1.81 [b]	2.67 ±0.95
T (ng/ml)	-serum	0.65± 0.23 [a c]	1.42± 0.29 [b]	7.71 ±1.55
	-seminal plasma	0.16± 0.14 [a c]	0.42± 0.22 [b]	3.47 ±1.05
NAG (ng/ml)	-Serum	0.08 ± 0.03 [a]	0.09 ± 0.05 [b]	8.21 ±1.05
	-Seminal plasma	0.14 ± 0.02 [a]	0.14 ± 0.02 [b]	6.55 ±2.29
PRL (ng/ml)	-Serum	19.62 ±1.10 [a]	19.74 ±1.20 [b]	12.62 ±2.91
	-Seminal plasma	15.62 ±1.74 [a]	15.62 ± 1.74 [b]	7.14 ±1.82
AMH (pg/ml)	-Serum	49.27 ±16.45 [a]	46.15 ±10.43 [b]	61.26 ±10.98
	-Seminal plasma	82.45 ±12.90 [a]	82.21 ±12.33 [b]	195.89±36.42
Inhibin B (pg/ml)	-Serum	2.16 ± 0.63 [a]	2.16 ± 0.63 [b]	27.96 ±7.00
	-Seminal plasma	4.95 ±1.00 [a]	4.95 ±1.00 [b]	80.85 ±34.01
Total Protein(mg/ml)	-Serum	90.03 ± 5.52 [a]	90.03 ± 5.52 [b]	68.86 ±5.55
	-Seminal plasma	72.66 ± 8.36 [a]	72.54 ± 7.95 [b]	51.81 ±10.44

[Table 3] correlation between BMI and seminal fluid parameters

Seminal fluid parameters	BMI Correlation coefficient-r	P value
Semen pH	-0.190	NS
Volume (ml)	-0.498	*
Liquefaction time	0.220	*
Sperm concentration(10^6/ml)	-0.606	*
Progressive motility (%)	-0.641	*
Non-progressive (%)	-0.297	*
Immotile sperm (%)	-0.330	*
Abnormal sperm morphology (%)	-0.075	NS

NS: not significant
*: significant ($p<0.05$)

Table 4] correlation between BMI and biochemical markers

Biochemical markers		BMI	P value
FSH	-Serum	0.062	NS
(mlU/ML)	-Seminal plasma	0.250	NS
LH (mlU/ML)	-serum	0.152	NS
	-Seminal plasma	0.132	NS
T (ng/ml)	-Serum	-0.677-	*
	-Seminal plasma	-0.639-	*
NAG (ng/ml)	-Serum	-0.207-	*
	-Seminal plasma	-0.384-	*
PRL (ng/ml)	-Serum	-0.604-	*
	-Seminal plasma	-0.598-	*
AMH (pg/ml)	-serum	-.660-	*
	-Seminal plasma	-0.603-	*
Inhibin B (pg/ml)	-Serum	0.579	*
	-Seminal plasma	0.610	*
Total Protein(mg/ml)	-Serum	0.520	*
	-Seminal plasma	0.394	*

*: significant ($p<0.05$) NS: not significant

★★★