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The Role Of Inhibin B, Kisspeptein ,Testosterone And Vitamin E In Male Infertility : A Study In AL-Najaf Provence

Muntadher Jihad Jahil[1] , Mohamed Abdulrazzaq Assi[2,3]
[1]Department of Medical Analytical Techniques , College of Health and Medical Techniques / Kufa,-Al_Furat Al_Awsat Technical Unuversity, 31003 Al-Kufa, Iraq .[1]
[2]Department of Anesthesia Techniques Techniques, College of Health and Medical Techniques/ Baghdad, Middle Technical University, Iraq.
[3]Department of Medical Laboratory Techniques, Institute of Medical Technology Al-Mansour, Middle Technical University, Iraq.

Abstract :
infertility in male is affected by many factor influenced by hormonal imbalance , environmental condition , diet ,smoking and genetic , one of these factor is hormone kisspeptein , inhibin B ,testosterone hormone and vitamin E play critical role in regulating spermatogenesis and oxidative stress ,the aim of this study is investigate the association between serum kisspeptein , inhibin B ,testosterone , vitamin E level in fertility and non fertile group also to evaluate semen parameter such as concentration ,motility and morphology . this study involved 60 case infertility men group and 30 case fertile men group as control in AL- najaf province the level of all markers measure by using ELIZA device while semen parameter conducted according to WHO 2021 . The result in this study demonstrated reduced the level of both inhibin B , kisspeptein and testosterone in infertile group compared to fertile group (P=< 0.005) ,and elevated level of vitamin E in infertile group also the markers show positive correlated with semen parameter such as concentration ,morphology and motility in conclusion Inhibin –B , kisspeptein and testosterone are used as a marker in investigation infertility man and impaired semen parameter while Vitamin E no correlation with fertility .
***Keyword** : Hormone , Infertility , Kisspeptein , Inhipin-B , Testosterone ,Vitamin E*

INTRODUCTION

Male infertility which can be defined as inability to achieved pregnancy after one year from regular sexual intercourse , infertility is one of more common disease distribution around the world affected both male and female , according to WHO the male factor contribute in 50% from infertility around the world (1) Recent year the infertility begin in progressive according to study between 1990 to 2019 demonstrated that 76 % increase in number of infertility , there are several factor affected on infertility in male such as hormonal imbalance varicosel, chemotherapy , life style , genetic ,smoking and alcoholic intake (2) In Iraq infertility still one of most healthy issue according to study in Arbil we found that there are difference variation in sperm parameter such as concentration , motility and morphology between infertility group (3). One of the most marker for prognosis and diagnosis infertility is inhibin-B defined as hormones secreted from sertoli cell that located in testes that impact important role in regulation of spermatogenesis pathway in male this hormone secreted mainly from pituitary gland , this hormone control in function and proliferation of sertoli cell which are essential for maturation and development sperm in semenifeurans tubal , in male several study indicate there are strong correlation between inhibin-B and sperm concentration , sperm movement and motility regarded as a marker for evaluation male infertility especially in idiopathic causes(4,5). Low level of inhibin –B seen in azoospermia ,oligozoospermia and sertoli cell syndrome when compared with high level in fertility male group confirming that good for sperm count than FSH making it novel marker for assessment infertility in male , also another role for inhibin -B in predicative sucsessful sperm intracytoplasmic(ICSE) injection where low level for this hormone is less success for fertilization(6)

The mechanism action for work this hormone by binding activin receptor for inhibition activin A and suppress Follicular stimulating Hormone (FSH) . despite many growing study on inhibin B in Iraq country but this study measure the level of this hormone only that related with infertility male but not measure the correlation with semen parameter such as concentration , motility and morphology

moreover increased prevalence consanguinity , genetic factor and dietary may affected on stimulation and secretion of this hormone lead to defect in spermatogenesis .
Another hormone founded the relative with reproductive organ is KISS -1 that found in tissue for epididymis and testes(7), the role of KISS-1 for development of germ cell and regulation of spermatogenesis as well as the role of this hormone in evaluation semen concentration ,semen motility and semen morphology , new research found this hormone affected on hyper activation for semen through measurement intracellular calcium level within the sperm cell Sharma et al.,2020 . in vitro intracellular administration of KISS-1 can stimulate spermatogenesis(8). the aim of this study to evaluate concentration the level of inhibin B and KISS-1 in infertility group and compared with fertile group also evaluate the correlation of this hormone with semen parameter such as concentration ,motility and morphology . The hormonal and oxidative stress also have role in pathophysiological in male infertility , testosterone prominent role for regulation spermatogenesis, libido and overall male reproductive function the normal level of testosterone associated with testicular health and sperm production(9) on the other hand vitamin E act as lipid soluble that protect sperm membrane from oxidative stress caused by reactive oxygen species where oxidative stress is one factor for male infertility , supplementation with vitamin E improve semen quality and reduced oxidative stress (10).

METHODOLOGY

The study carry out in Al-najaf provenance located in center- southern region of Iraq the study were conducted in fertility and infertility center Al-Sadr hospital regard one of the important hospital in the Iraq that accepted many infertility male from other provenance and near area the center have strong data previous diagnosed with infertile such as primary and secondary infertility and measure the semen analysis according to (WHO,2010) guideline for semen evaluation , the ethical accepted from Al-najaf health directorate ethical committee as will as the agreement accepted from all participated patient for study research The participated of patient that attenuated at this center divided into two group , first fertility (control) and the infertility (patient) the sample collection continues six month from (may 2024 to November 2024) . 90 sample was collected classified into two group 30 sample control group and 60 sample infertility group. The complete information is taken from the patient from any related disease and thein recorded the age , type of fertility ,any sperm cytoplasmic injection period of absences , smoking The sample of semen is collected from patient after 3 to 5day from absence in the inside sterile and clean disposable continuer and this container is labeled with name of patient and day of absence and time of collection, Then the sample is incubated to allow the semen for liquefied through 30-60 minuet thein the next steps examine both macroscopic and microscopic where macroscopic examination for see (appearance ,PH , ejaculate volume) on the other hand the microscopic examination for see the (concentration , motility , morphology ,grade , agglutination , germ cell and white blood cell) after finishing from diagnosis classify the sample into three group (normozooospermia ,oloigozoospermia and asthenozoospermia) the next step after collected semen sample thein collected blood sample Five milliliters of blood were obtained from infertile men and healthy controls, then collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. After that, the blood samples were centrifuged at 1000–2000 xg for approximately 10 minutes. The sera were aspirated and stored in deep freez at (–70C) until time of use after diagnosed the semen macroscopically and microscopically in the center of fertility and infertility part of study carry in Al-amen center for research study in AL-Najaf province for complete the measurement the level concentration of inhibin –B , kisspeptein, testosterone and vitamin E in serum sample were determine by ELIZA reader using the sandwich method according to manufacture instructional for Bio assay . finally the result descriptive statistic in SBSS version 26 and using T-test ,mann – wittney T test and person correlation test .

RESULT

According to the present study the mean age for infertility patient (32.83±7.70) while the fertility is (33.4±8.61) when compared two group found there are any statistically differences between two group (

0.752) . The percentage of age 22-31 year group is 51.7% for infertility patient and 50.0% for fertility patient , also the percentage age for (32-41)years represent as 33.3% equally for the corresponding group in fertile and the last group > 41 year was 15.0% for fertility and 16.7 fertility male so there is no significant correlation in age between fertility and infertility group P=(0.977) . On the other hand the distribution of male fertility and infertility is show in table that 90% of infertility group and 10% for fertility group was smoking while 10.0% for infertility group and 16.7 % for fertility group the result clarify also that there are not significant in smoking between infertility and fertility P=(0.363) as seen in table (1).

Table (1): Distribution of male infertility and fertility group according to age and smoking

		Groups		Total	p-value
		Infertile n=60	Fertile n=30		
Age (year)	Mean ± SD	32.83±7.70	33.4±8.61	33.02±7.96	0.752 ns
Age group N%	22-31 yr.	31 51.7%	15 50.0%	46 51.1%	X^2= 0.047 0.977 ns
	32-41 yr.	20 33.3%	10 33.3%	30 33.3%	
	> 41 yr.	9 15.0%	5 16.7%	14 15.6%	
Smoking N%	No	54 90.0%	25 83.3%	79 87.8%	X^2= 0.829 0.363 ns
	Yes	6 10.0%	5 16.7%	11 12.2%	

When compared 90 samples 60 for infertility and 30 fertility the result seen as the Serum Inhibin B According to the present study show the mean for the INH-B infertile group (287.04ng/l)while the mean for fertility groups (346.09ng/l) the fertility group show spared for value ranging from 200 to 600 ng/l has higher mean from infertility group 287.04 ng/l as illustrated in the figure(1) P = 0.001. The fertility group show spared for value ranging from 200 to 600 ng/l has higher mean from infertility group 287.04 ng/l however inhibin B was a direct marker of Sertoli cell function and spermatogenesis, also the result show The mean of KISS-1 in fertile group 184.79 is slightly higher than mean of KISS-1 in infertility group 172.49 , both group have similar range with value approximately 100 to 250 ng/l as illustrated in figure (2)

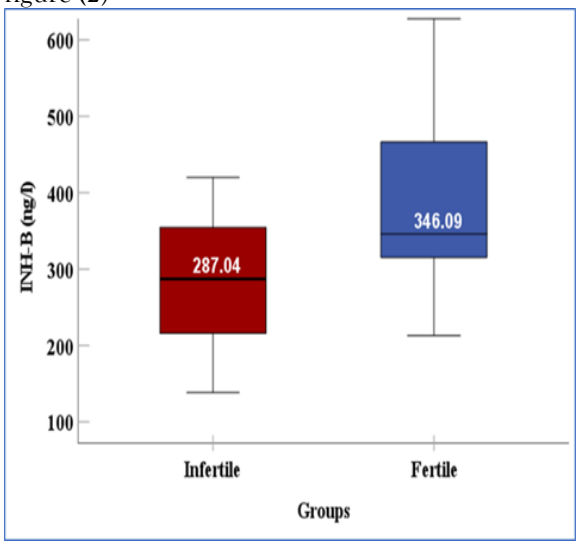


Figure (1): Mean level for INHB-1

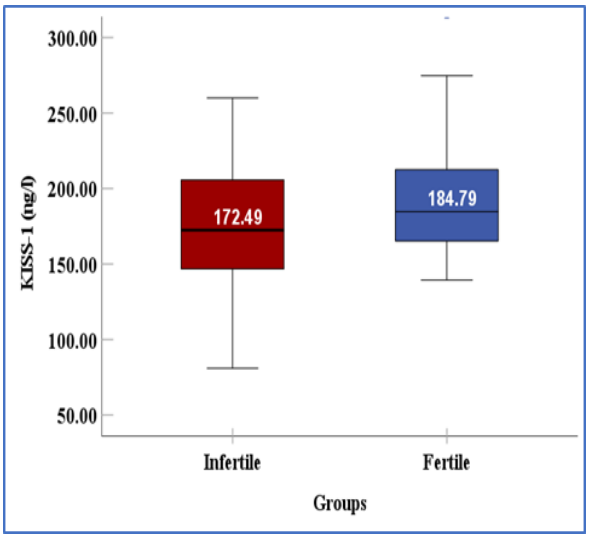
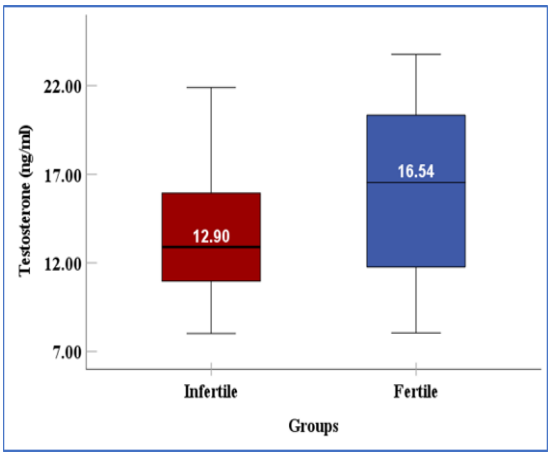
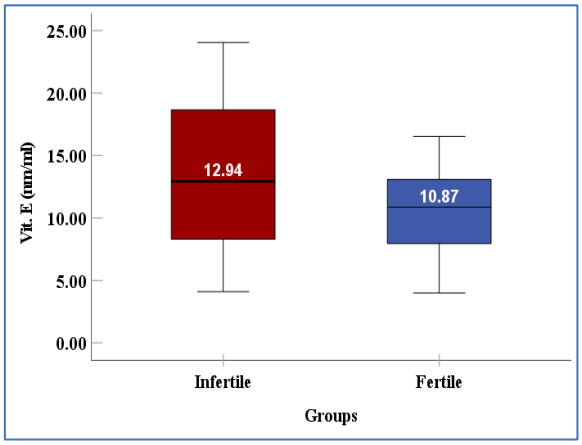


Figure (2) : Mean level for KISS-1

The figure (3) clarify the men level for testosterone between fertility and infertility where there are approximately similar in rang but the fertility group has higher mean (16.54) while the mean level of

infertility group (12.9). on the corresponding side figure (4) serum vitamin E level the mean in fertile group (10.87) compared with infertile group (12.94)





The majority of sperm sample in the current study was classified into three group Normozoospermia where 36 out of 60 represent as 60% and 22 out of 60 represent 37% was Oligozoospermia while 2 out of 60 show Asthenoaoospermia , statistically strong association between sperm abnormality p<0.001 ** as reported in figure (5)

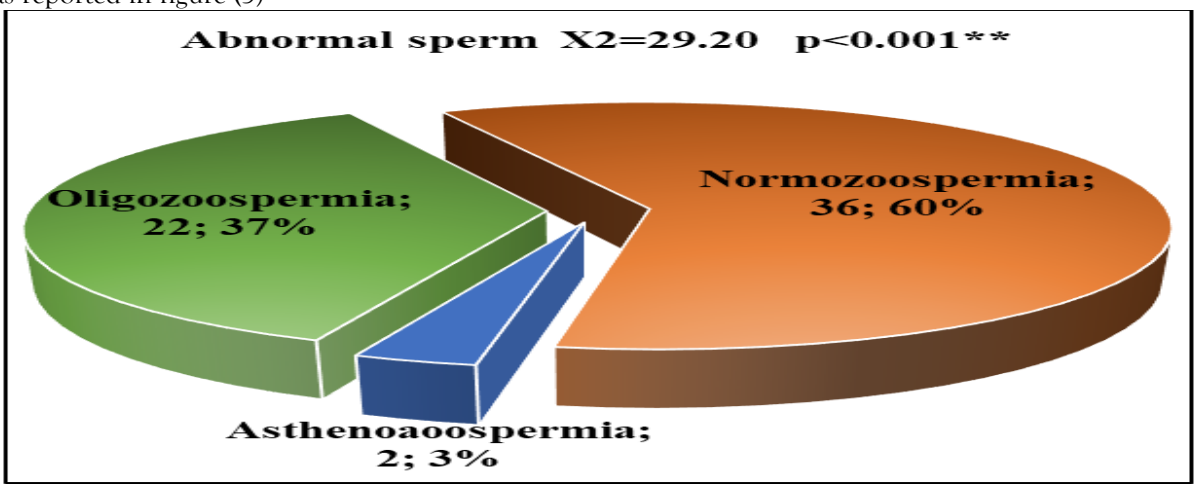


Figure (5) sperm sample classification in infertility group

The current study for the biochemical marker and hormonal for the for the three group Asthenozoospermia , Oligozoospermia , Normozoospermia , Inhibin B also show significant statistically variation value 0.011* founded that association between this hormone with abnormal sperm classification , the highest level appear in the Normozoospermia 307.62±317.97 while the lower level appear in the Oligozoospermia 248.45 ± 227.99 where the P =0.011* as seen in table (2). Kisspeptein appear significant variation statesticalley P = 0.002* where the highest mean in Normozoospermia 188.12±38.41 compared with Oligozoospermia 147±45.31 and Asthenozoosperm 160.53±9.23 , . testosterone level hormone increase in Normozoospermia mean 15.37 ± 3.59 and 10.8 ±0.54 ,11.44±2.15 for both Asthenozoospermia and Oligozooospermia so there are statistically difference between the three group for infertility 0.001* , There are no statistically difference in vitamin E concentration p =0.085 however VE is slightly higher in Normozospermia 14.72±5.16 compared with other group as illustrated in the table(2)

Table (2): Association between INH-B , KISS-1, testosterone and vitamin E with abnormal sperm

Marker	Abnormal sperm classification	Mean	SD	p- value
INH-B (ng/l)	Normozoospermia	307.62	317.97	0.011*
	Oligozoospermia	248.45	227.99	
	Asthenozoospermia	270.43	270.43	
KISS-1	Normozoospermia	188.12	38.41	0.002*
	Oligozoospermia	147	45.31	
	Asthenozoospermia	160.53	9.23	
Testosterone	Normozoospermia	15.37	3.59	0.001*
	Oligozoospermia	11.44	2.15	
	Asthenozoospermia	10.8	0.54	
Vitamin E	Normozoospermia	14.72	5.16	0.085
	Oligozoospermia	11.28	6	
	Asthenozoospermia	14.2	10.37	

Also The current study show significant statistically appear between hormonal and semen parameter in infertile man , the sperm concentration appear strong positive correlation (0.393**) with inhibin –B there are no statistically correlation between INH-B (ng/l) with age ,ejaculate volume and morphology parameter and strong association between kisspeptein hormone and sperm concentration (0.384**) and also with sperm morphology (0.559**) both hormone appear no statesticalley variation with age , motility and ejaculate volume .testosterone appear strong associated with both concentration (0.404**) and morphology of the sperm (0.371**). Vitamin E strong correlation with concentration and motility (0.315* , 0.266*) respectively .

Table (2) A association between testosterone, INH-B, Vit E and KISS-1 with age and semen parameter for infertility group

		Testosterone (ng/ml)	INH-B (ng/l)	Vit. E (nm/ml)	KISS-1 (ng/l)
Age (year)	**R**	**-0.148**	**-0.054**	**-0.061**	**-0.104**
	P	**0.260**	**0.684**	**0.642**	**0.429**
Ejaculate volume (ml)	**R**	**-0.026**	**-0.072**	**-0.121**	**-0.128**
	P	**0.841**	**0.585**	**0.356**	**0.328**
Sperm Conc. (10^6/ml)	**R**	**0.404****	**0.393****	**0.315***	**0.384****
	P	**0.001**	**0.002**	**0.014**	**0.002**
All progressive %	**R**	**-0.001**	**0.100**	**0.176**	**0.199**
	P	**0.992**	**0.447**	**0.178**	**0.127**
Non progressive	**R**	**-0.186**	**-0.251**	**0.268***	**0.049**
	P	**0.155**	**0.053**	**0.039**	**0.707**

Immotile	R	0.072	0.040	-0.269*	-0.248
	P	0.585	0.764	0.038	0.056
Total motility %	R	-0.074	-0.040	0.266*	0.244
	P	0.573	0.763	0.040	0.060
Grade activity	R	-0.074	0.075	0.219	0.166
	P	0.577	0.570	0.092	0.204
Normal morphology %	R	0.371**	0.045	0.195	0.559**
	P	0.004	0.732	0.135	0.000
Abnormal morphology %	R	-0.371**	-0.045	-0.195	-0.559**
	P	0.004	0.732	0.135	0.0001

DISCUSSION

The overall distribution of inhibin B levels in this study showed a significantly lower concentrations in infertile group compared to control group , This finding corresponds to Kajal et al.,2024 indicating that inhibin B functions as an accurate biomarker for spermatogenesis and Sertoli cell function both of which are important for development and nourishment of spermatozoa . reduced levels of inhibin B are frequently linked to testicular dysfunction and decreased spermatogenic activity. Inhipin –B regard as more specific and target than other hormone for early detection on testicular damage Olumide et al.,. (2023) .interestingly, obesity ,chronic stress and exposure to toxic substance participated to decline in the level of INHB-B. Jankowska et al. (2022) dis agarmented with my finding result this authors found there are no association between INHB-B and infertility. The level of INH-B show high level in Normozoospermia and decrees the level in both Oligozoospermia and Asthenozoospermia this result show this hormone role not only in compression between fertility and infertility group but also founded in distinguish between abnormal sperm

Additionally The current study revealed that decline the level of KISS-1 in infertility group compared with fertility group, possible explanation is that KISS-1 play important role in reproductive organ particularly stimulate secretion gonadotrophic releasing hormone (GnRH) , this result is agreement with the result finding by Lehmanw et al.,2024found decreases KISS-1 impaired spermatogenesis and reduce in GnRH also found that decreases of FSH and LH hormone is affected by KISS-1. support this study we found Kisspeptin is a potent peptide that initiates luteinizing hormone (LH) release in male in both prepreportal and adult male Kisspeptins were reported to have a predominately autocrine and paracrine action in the testes Prasath et al.,2025 . decrees the level of KISS-1 noted correlation with impaired concentration and sperm motility .

The low testosterone level lead to impaired spermatogenesis and reduced maturation of the sperm by disturbance hypothalamic pituitary gonad (HPG) that lead to reduced stimulation of sertoli cell , where the feed back mechanism regulate secretion of follicular stimulating hormone and leutinizing hormone, reduced this hormone impaired hormonal balance causes testicular dis function and impair leyding cell resulting decrees spermatogenesis Basar et al., (2020).

The result disagreement with many study that indicated interestingly which commonly reduced vitamin E associated with infertility because increase oxidative stress Mostafa, T. 2023 the possible explanation for increase vitamin E in infertility group due to supplementation and dietary factor . in conclusion noted that the three hormone INHB-B , KISS-1 and testosterone used for compression between fertility group and non fertility group as well as in identification on semen parameter such as concentration , motility and morphology while Vitamin E not associated with fertility group

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