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Evaluation the role of afamin , sperm antibodies and reproductive hormones in serum of infertility males in Al-Hawija city

Mohammed Ali Faris Ahmed1 Nagham Qasim Kadhim2
1, Technical institute- AL-Hawija, Northern Technical University, Iraq.
2, College of Science, Tikrit University, Iraq.
Email1: Mohammedchemist@ntu.edu.iq https://orcid.org/0000-0003-2081-4872
Email2: Naghamkassim@tu.edu.iq, https://orcid.org/0000-0002-7056-3314

Abstract :

Background: There is a scarcity of studies on the role of afamin and sperm antibodies in male infertility, as infertility is an escalating health and social issue with multiple causes. **Objectives**: To evaluate infertility by measuring testosterone levels, follicle-stimulating hormone, afamin, and sperm antibodies in participants from Hawija city. **Methods:** The study included 90 participants, comprising 30 healthy males as a control group and 60 males suffering from infertility. Levels of hormones and biochemical variables were measured, including testosterone, follicle-stimulating hormone (FSH), prolactin, afamin, and sperm antibodies. Infertility was categorized into primary and secondary types, and body mass index (BMI) was assessed across normal weight, overweight, and obese groups. **Results**: The results showed significant differences in hormone levels and biochemical variables (testosterone: 3.32 g/ml, FSH: 7.75 mIU/ml, prolactin: 13.98 ng/ml, afamin: 4.50 pg/ml, sperm antibodies: 37.56 pg/ml at $P \leq 0.05$. Additionally, afamin levels showed significant differences between primary infertility 40.04 pg/ml and secondary infertility 31.02 pg/ml at $P \leq 0.05$. **Conclusion**: The study found a clear correlation between reproductive hormones, sperm antibodies, and afamin with reduced fertility, indicating the role of these variables in evaluating infertility.

Keywords: Male infertility, primary infertility, secondary infertility, afamin, sperm antibodies.

دور الافامين والاجسام المضادة للحيوانات المنوية في مصل الذكور المصابين بالعقم في مدينة الحويجة في مدينة الحويجة

محمد علي فارس احمد[1] ، نغم قاسم كاظم[2]
[1]الجامعة التقنية الشمالية / المعهد التقني الحويجة /قسم تقنيات المختبرات الطبية
[2]جامعة تكريت / كلية العلوم / قسم الكيمياء

مستخلص:

هناك ندرة في الدراسات حول دور الأفامين والأجسام المضادة للحيوانات المنوية في العقم عند الذكور، حيث أن العقم يعد من القضايا الصحية والاجتماعية المعقدة والتي أصبحت في زيادة واضحة ذات أسباب متعددة. الهدف: تقييم العقم من خلال قياس مستويات هرمون التستوستيرون، والهرمون المنشط للحويصلة، والأفامين، والأجسام المضادة للحيوانات المنوية لدى المشاركين من مدينة الحويجة. طرائق العمل : شملت الدراسة 90 مشاركًا، منهم 30 من الذكور الأصحاء كمجموعة سيطرة و60 من الذكور المصابين بالعقم . تم قياس مستويات الهرمونات والمؤشرات الكيموحيوية ، بما في ذلك هرمون التستوستيرون والهرمون المنشط للحويصلة والافامين والاجسام المضادة للحيوانات المنوية تم تصنيف العقم إلى أنواع أولية وثانوية وتم تقييم مؤشر كتلة الجسم (BMI) الى مجاميع الوزن الطبيعي وزيادة الوزن والسمنة النتائج: أظهرت النتائج فروقًا معنوية في مستويات الهرمونات والمتغيرات الكيميائية الحيوية (التستوستيرون: 3.32 غم/ مل، FSH:7.75 ملي وحدة/ مل ، البرولاكتين: 13.98 نانوغرام/ مل، أفامين: 4.50 بيكو غرام/ مل، أجسام مضادة للحيوانات المنوية: 37.56 بيكو غرام/ مل عند $P \leq 0.05$. بالإضافة إلى ذلك، أظهرت مستويات أفامين فروقًا معنوية بين العقم الأولي 40.04 بيكو غرام/ مل والعقم الثانوي 31.02 بيكو غرام/ مل عند $P \leq 0.05$. الاستنتاجات: وجدت الدراسة وجود علاقة واضحة بين الهرمونات التكاثرية وألاجسام المضادة للحيوانات المنوية والأفامين مع انخفاض الخصوبة، مما يدل على دور هذه المتغيرات في تقييم العقم.

الكلمات المفتاحية: عقم الذكور، العقم الاولي ، العقم الثانوي ، الافامين ، الاجسام المضادة للحيوانات المنوية .

Introduction:

Infertility is defined based on reports from the International Committee for Monitoring of Assisted Reproductive Technologies (ICMART) and the World Health Organization (WHO) as the inability of a man to achieve pregnancy or the failure of pregnancy formation in the wife after at least 12 months of unprotected sexual intercourse(1)(2)(3).

To identify the causes of infertility, the condition of the infertile male is assessed through several semen analysis tests. Some causes may be congenital, while others may arise from diseases affecting the hypothalamus, pituitary gland, or gonads, which impact the hormones responsible for sperm production in the testes. Additionally, there are unknown causes believed to be related to oxidative stress and an increase in oxidants compared to antioxidants, which affect sperm production at the genetic level (4)..

Testosterone, a steroid hormone composed of 19 carbon atoms and belonging to androgens, is derived from cholesterol and is secreted in the testes by Leydig cells under the stimulation of LH. It contributes to various vital processes, including fertility, body morphology, secondary sexual characteristics, and aggressive behavior. Its effects are particularly evident during puberty when levels rise significantly and decline with age (5)..

The level of testosterone in the testes must be much higher than that in serum to support normal sperm formation. It increases indirectly within the testes through the maturation of germ cells due to its effect on Sertoli cells. Hormonal assessment is a critical component in evaluating male fertility, and endocrine disorders are contributing factors to infertility. Disruption of this delicate hormonal balance can lead to a range of endocrine disorders, resulting in reduced fertility or infertility (4).

FSH plays an important role in male fertility, as it stimulates the growth and development of sperm in the testes. It regulates the functions of the reproductive glands in men by increasing the production rates of Sertoli cells and accelerating sperm maturation. This hormone is produced by the anterior pituitary gland through stimulation by the hypothalamus for the gonadotropin-releasing hormone (GnRH) (6). Men

who have elevated or decreased levels of FSH tend to have a lower sperm count and poor sperm quality, which is why it is generally used to determine infertility (7).

Afamin is a complex protein with multiple functions, produced in the liver and secreted into the bloodstream. It plays an important role in transporting fat-soluble vitamin E, which helps regulate oxidative stress in all body cells and protects cells from damage (8)(9) It also has a significant role in neuroprotection, interacting with certain receptors and other proteins in the body. Researchers have found elevated levels of Afamin in males with infertility who have reduced sperm volume and motility, indicating a potential link between Afamin levels and semen quality (10).

Anti-Sperm Antibodies (ASAb) attack sperm and damage them, which can lead to sperm agglutination, a decrease in sperm count, reduced motility, or changes in their normal morphology (11). ASAb can also prevent sperm from fertilizing the egg due to the aforementioned reasons. ASAb are found in blood, lymphatic fluid, and semen, and they are also present on the surface of the sperm itself, meaning that these antibodies are part of the immune system that contains specialized cells that produce antibodies. Recently (12). ASAb have been considered a biomarker for infertility (13).

Prolactin plays a crucial role in regulating testosterone production in males. When its levels are elevated, it can lead to a decrease in testosterone production, causing a range of symptoms, such as reduced libido, erectile dysfunction, poor semen quality, fertility issues, mood changes, and headaches. This can potentially lead to male infertility, as it affects sperm production and function. Several factors can cause elevated levels of prolactin in the blood, such as tumors in the pituitary gland, certain medications (like antidepressants and antipsychotics), hypothyroidism, and kidney diseases. Estradiol is a key stimulant for prolactin secretion (14).

MATERIALS AND METHODS

The study included 90 individuals, comprising 60 males diagnosed with infertility and 30 males from the control group who were apparently healthy and had no medical history, with ages ranging from 20 to 50 years

in the Al-Hawija district. Consent was obtained from each participant before sample collection, which took place at the Beirut Specialized Laboratory in Al-Hawija. All tests were conducted from December 2023 to June 2024.

Studied Variables: Testosterone, follicle-stimulating hormone, prolactin, afamin, and sperm antibodies were measured using enzyme-linked colorimetric methods for both healthy individuals and patients.

Statistical Analysis: The results were statistically analyzed using the SPSS software (version V.27), with values represented in the tables as (Mean ± SD). The t-test was employed to compare the groups in analyzing the results between patients and healthy individuals, with significance levels set at ($P \leq 0.01$) and ($P \leq 0.05$). Graphical representations of the variables were created using Microsoft Office Excel .2020

RESULTS

Testosterone, Follicle-Stimulating Hormone, and Prolactin

The biological variables were distributed according to patients and healthy individuals.

Estimation of Testosterone, Follicle-Stimulating Hormone, and Prolactin Levels in the Serum of Infertile Patients Compared to Healthy Individuals.

The results in table (1) and figure (1) showed a significant decrease at a probability level of ($p \leq 0.05$) in testosterone levels, which were (3.32 ± 0.84) ng/mL in the serum of patients with infertility compared to the healthy group, where it was (5.07 ± 1.59) ng/mL. In contrast, the concentrations of follicle-stimulating hormone were higher in the patient group compared to the control group, with a significant increase at a probability level of ($p \leq 0.05$), where it reached (7.75 ± 1.75) mIU/mL in the serum of infertile patients compared to (4.98 ± 0.95) mIU/mL in healthy individuals. The results also indicated a significant increase at a probability level of ($p \leq 0.05$) in prolactin levels, which were (13.98 ± 3.79) ng/mL in the serum of infertile patients compared to (8.36 ± 1.20) ng/mL in healthy individuals.

Table 1: Levels of biochemical variables in the serum of male patients with infertility compared to male healthy individuals.

Groups / Parameter	Mean ± SD		P – value
	Control	Patients	
Testosterone (ml)	1.59±5.07	0.84±3.32	0.001≥
FSH (mIU/ml)	0.95±4.98	1.75±7.75	0.001≥
Prolactin (ng/ml)	8.36±1.20	13.98±3.79	0.001≥

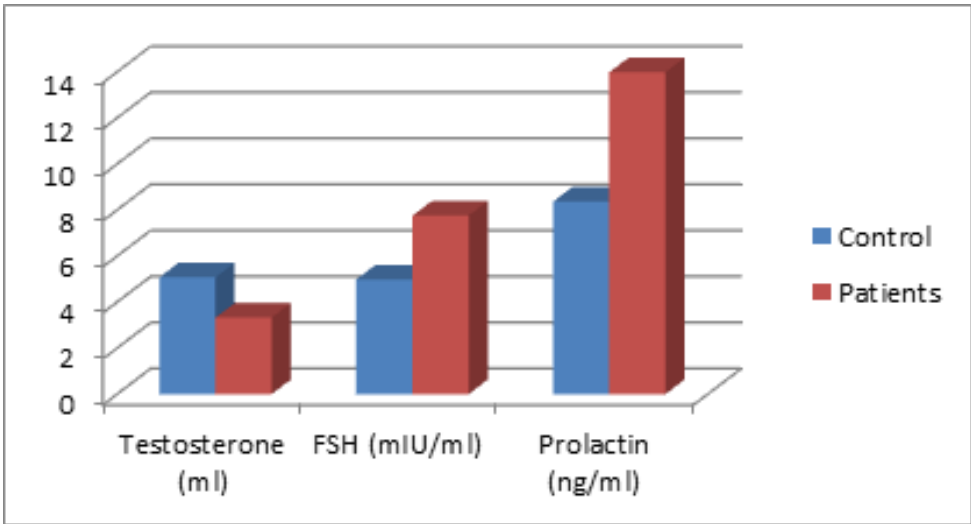

Figure 1: Levels of biochemical variables in the serum of male patients with infertility compared to male healthy individuals

The results (Table 1) are as shown in Table 1, and they align with the findings of researchers This confirms what Ibrahim Hadeel and Ramzi Z. found in their studies (15). This demonstrates that, in contrast to healthy people, infertile patients have lower testosterone (Testo) levels. Because of its essential function in sperm production—stimulating Sertoli cells—a decrease in Testo levels impacts male fertility and reproductive health. Oligospermia, a decrease in sperm count, may result from this. Gonadotropins like FSH, LH, and Testo play a key role in controlling how germ cells make DNA. A well-regulated connection along the HPG axis is essential for the full and proper development of male germ cells. The process starts with the stimulation of GnRH, which triggers the hypothalamus to release reproductive

hormones. These hormones, in turn, stimulate spermatogenesis, the process by which the reproductive system produces sperm, through Testo .(16). Alterations in gonadal activity and blood testosterone (Testo) levels are frequently linked to abnormal sperm production. Optimal reproductive hormone activity is essential for sperm formation (spermatogenesis), and fluctuations in Testo levels can affect metabolic processes, resulting to hyperlipidemia and infertility (hypofertility). Secondary hypogonadism, in which androgen levels fall as a consequence of problems with the gonadal axis or decreased production by Leydig cells as a result of inflammatory diseases, genetic abnormalities, or lifestyle factors, is associated with the highest risk of obesity among infertile patients, although most infertile patients experience weight gain. Reductions in sperm production caused by these conditions have an effect on fertility (17). This study agrees with previous work by Gowri V., Venkateswaran K.P., and others (18). with relation to secondary infertility and basic infertility, demonstrating no discernible distinction between the two. Infertility caused by secondary factors is not different from primary in terms of length or semen parameters such sperm count, motility, or morphology. People who suffer from secondary infertility and hypogonadism are more affected by fat (17).Findings from this study are in agreement with those of Gangwar PK (19). in accordance with what Hashem Mohammed O. and Sabri Hasan R. discovered (20). in relation to the increased prolactin (PRL) levels in infertile men. In a healthy man, the endocrine glands work in harmony to regulate the HPG axis, which allows for the full maturation of male germ cells and the creation of viable sperm. A small percentage of infertile males have PRL levels that are too high, preventing the hypothalamus from secreting GnRH. Because of this, FSH and LH levels drop, which means testosterone levels drop and sperm quality drops. Furthermore, decreased libido, impotence, changed sperm quality, impaired motility, decreased semen parameters, and raised PRL levels are all factors in these issues (21).

Serum PRL levels are inversely proportional to sperm motility; conversely, those whose sperm quality is compromised will have lower PRL levels. This

can have an adverse effect on fertility and is closely associated with hypogonadism, sexual dysfunction, and infertility. If a man's PRL level is high, his sperm count, and motility will be lower than if their PRL level is normal. Additionally, compared to individuals with normal PRL levels, their risk of infertility is 20% higher (22) .(23) .. Contrary to what Nada Habeeb found, the present study's findings are correct (24). that there are no discernible variations in PRL levels between healthy people and males experiencing infertility. But this study's results are in line with what Corinne TM and her colleagues found (25). demonstrating increased FSH levels in patients who are unable to conceive. Fertilizing hormone (FSH) triggers spermatogenesis by activating Sertoli cells within the seminiferous tubules. Reproductive hormones have an effect on male fertility because of their connection to sperm quality. High levels of FSH are associated with defective sperm production and testicular dysfunction; conversely, low levels of FSH are associated with normal sperm production. Hormonal imbalances (26) . which affect the HPG axis, are a leading cause of male infertility (27). When FSH levels are significantly elevated, particularly when they are more than twice the normal level, it suggests a problem with sperm production and is compatible with obstructive azoospermia (28). When sperm count is low or nonexistent, FSH levels rise because of the inverse relationship between the two values (29). As a result of disturbances in reproductive hormones, male fertility starts to deteriorate in people with a high body mass index (BMI), which might worsen the risk of infertility in obese males. Fertility and sperm production could fall at a very alarming rate (30). Extra weight raises the risk of sperm DNA damage and other issues, including an increase in scrotal temperature, which in turn reduces sperm production and alters semen properties. Adipose tissue secretion and reactive oxygen species (ROS) levels that are too high may damage Leydig cells, which in turn affect androgens and spermatogenesis (31).

Sperm Antibody Levels

The results in table (2) and figure (2) showed a significant decrease at a probability level of ($p \leq 0.05$) in levels of ASAb, which were (4.50 ± 0.87)

picograms/mL in the serum of infertile patients compared to (4.67 ± 1.03) picograms/mL in the healthy group. The results for sperm antibody levels, when dividing male infertility into primary and secondary types, and body mass index into three categories (normal weight, overweight, and obesity), as well as age into two categories, indicated an effect but did not reach significance.

Table 2: Levels of ASAb in the Serum of male Patients with Infertility Compared to male Healthy Individuals.

Groups		Mean ± SD of antisperm antibodies (pg/ml)		
		Control	Patients	
Total		1.05 **4.67**±	0.87 4.50±	
P value		0.001≥		
Types of infertilities	Primary	4.56±0.68		
	Secondary	4.39±0.75		
P value		**0.05>**		
Groups		Control	Primary	Secondary
BMI	B1(20-24.9)	4.45±1.308	4.68±0.58	4.701±0.536
	B2(25-29.9)	4.71±1.21	4.57±1.02	4.189±0.462
	B3(30-35)	-------	4.41±0.68	4.32±1.17
ANOVA/ P value		**0.05>**	**0.05>**	**0.05>**
Groups		Control	Primary	Secondary
Age (year)	A1 (20-34)	4.96±1.29	4.50±0.865	4.16±0.59
	A2 (35-50)	5.29±1.08	4.69±0.677	4.57±0.82
ANOVA / P value		**0.05>**	**0.05>**	**0.05>**

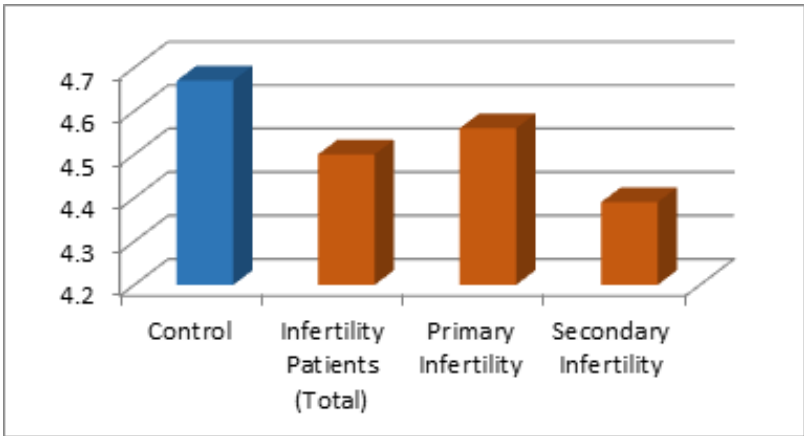

Figure 2: Levels of ASAb in the Serum of male Patients with Infertility Compared to male Healthy Individuals.

The study results in table 2 aligns with researcher Findings from this study agree with those of Alkhafaji and colleagues (32). demonstrating that ASAb levels are higher in men with infertility when contrasted with healthy controls. Significant disturbances to reproductive health can occur when ASAb levels are high, as they impact fertility and can cause male infertility in certain situations. In men, this is known as immunological infertility. Sometimes, men with hormone abnormalities have lower ASAb levels. Nevertheless, the present study's conclusions differ from those of Khatoon M. and colleagues (33). They did not find any statistically significant changes in the frequency of ASAb among infertile individuals, suggesting that ASAb's function in predicting infertility is still not well understood. A large number of people are impacted by immune infertility, which is seen as a serious issue. Researchers have looked into the possibility of an immune-based reason for infertility after finding ASAb in infertile males (34). There is no link between ASAb levels and infertility, according to another study (35). A primary infertility disorder is one that affects the production of sperm, the number of sperm, or the motility of the sperm. Immune responses can arise from these illnesses, which are among the most common causes of infertility. Alterations to a man's reproductive health brought on by outside forces, such as increased ASAb levels, are known as secondary infertility. Fertilization is hindered because sperm are unable to adhere to the egg's outer layer due to these increased levels (36). Significant hormonal imbalances and inflammation brought on by obesity and overweight can affect reproductive health in a number of ways, including an increase in ASAb levels and a decrease in sperm production. Obesity also increases oxidative stress and reactive oxygen species (ROS) since the body's oxidative agents are more numerous than its antioxidants. OS is a potent component that might diminish the quality and quantity of sperm. One of the possible causes of male infertility is obesity, which lowers testosterone levels and increases ASAb (37). Cofactors, both enzymatic and non-enzymatic, help to protect cells against oxidative stress. OS diminishes sperm motility, damages sperm DNA,

inhibits proteins, and stimulates lipid peroxidation, all of which impact male fertility. The imbalance between antioxidants and the high quantities of reactive nitrogen species (RNS) and ROS in seminal plasma may be disrupted by OS in males, and this imbalance has been linked to male infertility. In a healthy male reproductive system, the formation of reactive oxygen species (ROS) and the elimination of these ROS by antioxidants are in equilibrium. On the other hand, OS can occur when antioxidant defense mechanisms are overwhelmed by an overabundance of reactive oxygen species (ROS) created by immature sperm and white blood cells (38).Male infertility results from changes in seminal fluid characteristics brought about by increased oxidative destruction of sperm plasma membrane lipids, oxidization of sperm proteins, and fragmentation of sperm DNA, all of which are caused by greater OS levels, which are themselves caused by increased metabolic activity (39).In addition, sperm might be born with defects, and ASAb levels tend to rise with age (40).

Afamin levels

The results showed a significant increase at a probability level ($p \leq 0.05$) in afamin levels, which reached (37.56± 8.56) picograms/ml in the serum of infertile patients compared to the healthy group, which was (8.73±1.98) picograms/ml. The results for afamin levels, when dividing male infertility into two types (primary and secondary), showed significant differences indicating higher afamin levels in patients with primary infertility compared to those with secondary infertility. The body mass index was categorized into three categories: normal weight, overweight, and obesity, while age was divided into two sections. It was found that there was an effect, but it did not reach significance. The results are as shown in Table 3.

Table 3: Afamin levels in the serum of male patients with infertility compared to male healthy individuals.

Groups		Mean ± SD of Afamin (pg/ml)		
		Control	Patients	
Total		8.73±1.98	8.56 37.56±	
P value		0.001≥		
Types of infertilities	Primary	40.04±9.55		
	Secondary	6.65 31.02±		
P value		**0.05<**		
Groups		Control	Primary	Secondary
BMI	B1(20-24.9)	8.568±1.169	41.57±12.40	33.203±7.58
	B2(25-29.9)	8.96±2.88	38.21±7.32	30.072±0.94
	B3(30-35)	--------	43.48±7.51	37.26±8.81
ANOVA/ P value		**0.05>**	**0.05>**	**0.05>**
Groups		Control	Primary	Secondary
Age (year)	A1 (20-34)	7.87±2.19	40.07±8.533	30.88±7.92
	A2 (35-50)	7.53±2.80	42.48±7.90	31.13±9.11
ANOVA / P value		**0.05>**	**0.05>**	**0.05>**

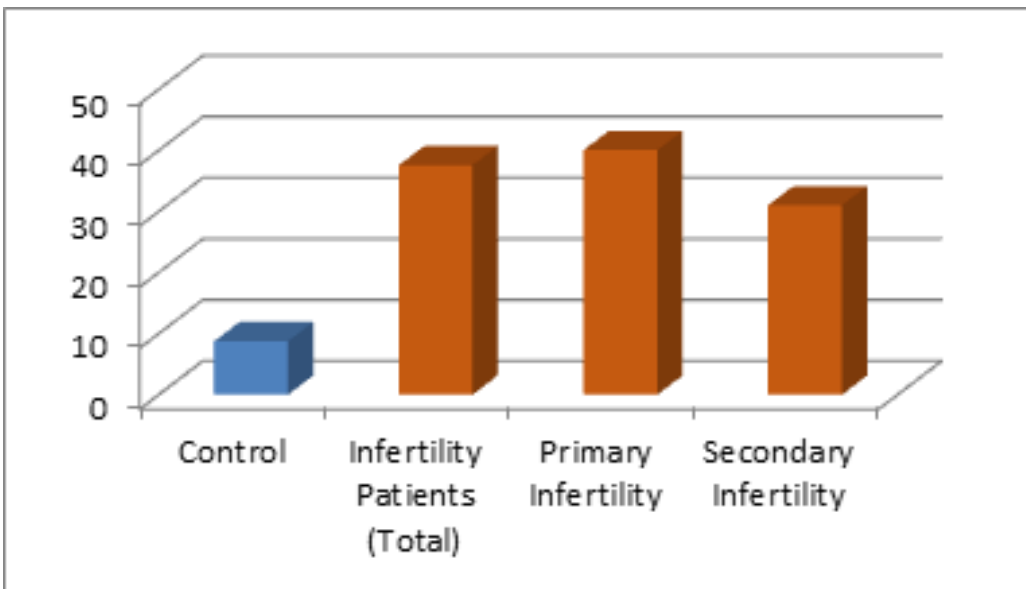

Table 3: Afamin levels in the serum of male patients with infertility compared to male healthy individuals.

Table (4) shows the criteria of diagnosis validity (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Accuracy tests) of Afamin level in patients compared with healthy control by using 18.94 pg/ml as cut-off value. The optimal cut-off value for copeptin in all groups estimated from ROC curves, according to these results , test is positive if test > threshold value (cut off values).

Table 4: Predictive values of Afamin

Sensitivity	Specificity	PPV	NPV	Accuracy	AUC
98%	93%	93%	95%	97%	0.997

Sensitivity = positive in disease , expressed as percent.

Specificity = negative in healthy , or absence of particular disease , expressed as percent.

Predictive value of positive test = percent of patients with positive test results that are diseased.

Predictive value of negative test = percent of patients with negative test results that are non diseased.

accuracy test = percent of patients correctly classified as diseased and non diseased.

The results result in table 3 are consistent with those reached by Rosio et al. (41), indicating that elevated afamin levels in males with infertility have an impact on fertility. Afamin is a complex protein that plays an important role in the transport of fat-soluble vitamin E, which is an antioxidant that protects against free radicals. It is considered an indicator of oxidative stress, which is one of the causes of infertility due to its negative effect on sperm quality and function.

The quality of semen in males has gradually declined worldwide. Certain components and nutrients in the diet have been identified as important factors affecting sperm function, male fertility, or the normal development of sperm. An imbalance in programmed cell death has a critical effect on sperm count and function, thus influencing reproductive capability. Male infertility is associated with increased expression of biomarkers for programmed cell death. There are many factors that

cause reproductive cell death, including hormonal disorders, exposure to toxins, increased oxidants at the expense of antioxidants, elevated scrotal temperature, and orchitis. AFM is believed to be a new protein marker for ovarian cancer and polycystic ovary syndrome, with elevated levels in serum (42). Male infertility may result from several factors, including genetic changes, environmental factors, and chronic diseases. It has been found that there is an increase in AFM levels in males with infertility who have reduced sperm volume and motility, indicating a potential link between AFM levels and semen quality (10).

There are two types of male infertility classified based on the ability to achieve pregnancy: primary infertility, where pregnancy is never achieved, and chronic infertility, which occurs when the testes do not produce enough sperm or produce sperm of insufficient quality, due to various issues that may be hormonal, anatomical (such as obstruction of ducts), environmental, or genetic (43). Secondary infertility is acquired, where pregnancy and the birth of one or two children occur, but then the male is unable to achieve further pregnancies. Secondary infertility in males occurs when there are difficulties in producing viable sperm or when sperm quality decreases due to health issues, lifestyle changes, weight gain, excessive smoking, or tumors, which prevents fertilization of the egg (44).. Generally, about 67-71% of males suffer from primary infertility and 29-33% from secondary infertility (45).

Obesity is a serious disease that involves risk factors for complications that develop with obesity and metabolic syndrome, with the risk of complications increasing with the severity of obesity. Recent studies have shown that there may be a causal relationship between afamin and obesity. Research by Helmi et al. (46) demonstrated that afamin levels decrease after weight loss, confirming that obesity increases afamin levels. Afamin is closely associated with all components of metabolic syndrome. Regarding age, oxidative stress increases with aging, leading to cell damage and affecting reproductive hormone levels, resulting in metabolic changes. All of these factors can contribute to decreased fertility, as afamin is affected by oxidants, playing a significant role in regulating reproductive functions.

4- Pearson Correlation Coefficient between biochemical indicators

The correlation coefficient (r) was measured, calculating the correlation between two variables from the same sample to describe the relationship and degree of correlation between reproductive hormones and the studied biochemical indicators. At a correlation coefficient "r" ($r \geq \pm 0.38$), Tables (4, 5) present the results of the Pearson correlation analysis between afamin and sperm antibodies, along with other studied variables for both infertility patients and healthy individuals. It shows that for both the patient and healthy groups, afamin and sperm antibodies did not exhibit statistically significant correlations.

Table 5: Analysis of the correlation coefficient between biochemical indicators in male healthy individuals.

Variables	Afamin	Antisperm antibodies
Afamin	1	0.16
FSH	0.14	0.12
LH	0.04	0.02
PROLACTIN	0.16	0.19
Testosterone	0.2	0.09

Table 6: Analysis of the correlation coefficient between biochemical indicators in male infertility patients.

Variables	Afamin	Antisperm antibodies
Afamin	1	0.14
FSH	0.14	0.16
LH	0.04	0.09
PROLACTIN	0.06	0.10
Testosterone	0.13	0.02

For results of correlations showed in tables 5 and **6**, sex hormones play a crucial role in the functioning of the male reproductive system, and their impact on semen quality is evident. Hormones can be used as indicators to predict semen quality. The roles of FSH and LH hormones synergistically contribute to the growth and protection of testicular function in males. FSH is one of the key hormones in the male reproductive system and aids in the function of Sertoli cells and the production of male gametes. The initiation of sperm production at puberty and the maintenance of healthy sperm production in males depend on FSH concentration. Higher levels of prolactin directly affect the reproductive system through the central nervous system and inhibit androgen production, which impacts libido when an individual has elevated prolactin levels. Testosterone production begins in Leydig cells through luteinizing hormone, which plays an important role in sperm production. It has been observed that the concentration of reproductive hormones in serum is abnormally altered in infertile males. Thus, increased concentrations of FSH, LH, and prolactin in the blood serum, along with decreased testosterone levels, disrupt the spermatogenesis process, leading to male infertility (47). Elevated FSH and LH levels in men with azoospermia can be considered significant indicators, demonstrating a negative correlation with semen quality. There is a strong relationship between testicular steroid hormones and ASAB, which affects sperm motility and causes morphological abnormalities in sperm, such as damaged sperm, tail and head defects, and infection of the reproductive system, leading to loss of motility. Sperm antibodies directly affect fertilization both in vivo and in vitro (49) (48).

Conclusion:

This study concluded that there is an increase in protein levels and a decrease in androgens, with an expected rise in follicle-stimulating hormone, and we found no effect from sperm antibodies. This indicates oxidative stress and its impact on fertility. Therefore, we recommend continuing biochemical, clinical, and molecular studies for infertility patients across different communities and investigating the serious implications of the ongoing in-

crease in infertility rates, as well as raising awareness about this condition and its causes.

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