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The Relationship between Osteocalcin, Leptin and Testosterone Hormones and Infertility in Syrian Males

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The Relationship between Osteocalcin, Leptin, and Testosterone Hormones and Infertility in Syrian Males

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Abstract:

Infertility is a complex and multifactorial issue affecting numerous individuals worldwide. While numerous studies have explored the etiology of infertility, precise mechanisms underlying the condition remains elusive, particularly in specific population such as Syrian males. This comprehensive study aims to investigate the relationship between three key hormones, namely osteocalcin, leptin and testosterone and their potential impact on Syrian males with infertility.

Osteocalcin is a bone-derived hormone that stimulates testosterone production in the testes. Leptin is an adipose-derived hormone that inhibits testosterone synthesis. Testosterone is the main androgen that supports spermatogenesis and male reproductive functions.

The dysregulation of osteocalcin, leptin and testosterone has been associated with impaired spermatogenesis, decreased sperm motility, altered semen parameters and various other reproductive abnormalities. Furthermore, factors such as stress, malnutrition and exposure to toxins, may further disrupt the delicate hormonal balance, exacerbating infertility issues. Understanding the intricate relationship between osteocalcin, leptin and testosterone in the context of infertility is of paramount importance in developing strategies for diagnosis, treatment and prevention of male infertility.

Key Words: Osteocalcin, Uncarboxylated osteocalcin, Leptin, Testosterone, BMI, Sperm count, Infertility.

Introduction:

Previously, bone was considered a supportive tissue with mechanical properties, but recent studies have shown that bone acts as an endocrine gland through producing uncarboxylated osteocalcin which in turn affects the testes, pancreas, brain, placenta, and many other organs.(1)

Adipocytes of white adipose tissue mainly produce leptin hormone, and it has a role in reducing appetite and preventing hepatic glucose production, increasing energy expenditure, stimulating insulin, and glucose uptake, and increasing the secretion of inflammatory cytokines.(2)

Infertility is defined as the inability to conceive after one year of unprotected intercourse, and almost half of all infertility cases are related to masculine factors such as: (sperm count, sperm shape, testosterone), and recent studies have shown there is a positive correlation between body mass index and leptin level. Excessive leptin production may contribute to low testosterone and decreased reproductive function (infertility) in obese men.(3)

In this study, we will discuss our assay that were performed on a group of men with azoospermia and oligospermia, and the statistical associations that we found between leptin hormone and BMI, leptin hormone and uncarboxylated osteocalcin, leptin hormone and testosterone, leptin hormone and sperm count.

Materials and Methods:

6-2- Materials and Methods:

This was a case control study conducted in 2019. The data and archived serum samples used in this analysis were retrospectively selected from a cohort of patients initially recruited for a broader study on male infertility at the Reproductive Health Clinic, Damascus University Hospital, Syria.

The samples were taken from a study conducted at Damascus University, Faculty of Pharmacy, entitled (Association between Undercarboxylated Osteocalcin and single Nucleotide polymorphism in Osteocalcin Receptor Gene with Testosterone in Idiopathic Infertile males in Syria), after acquiring the approval of the Ethical committee at the University of Damascus, No.18p. dated 2019. And it has a case-control design.

The study included 190 men who attended Al-Sharq Hospital for assisted reproduction by artificial insemination and IVF. Informed consent was provided and participants' signatures and written permission were obtained after the patients underwent clinical examination by the hospital’s medical team and were nominated to participate in the study.

The inclusion criteria was as follow: men with idiopathic primary infertility presenting with non-obstructive azoospermia or oligospermia associated with low levels of testosterone and high levels of the gonadotropins LH and FSH.

While the exclusion criteria was obstructive azoospermia and known infertility deficiency such as testicular cancer, testicular trauma, orchitis, patients undergoing drug treatment that has a toxic effect on the gonads, patients with Klinefelter syndrome, patients with ascites disease.

In addition to secondary testicular failure represented by low testosterone concentration and low gonadotropin concentration, patients treated with drugs that affect bone metabolism and vitamin K such as warfarin were excluded.

The results of the semen examination of the participants were approved in the Andrology Laboratory at Al Sharq Hospital for Assisted reproduction which was applied according to WHO guidelines (World Health Organization) in 2010 (WHO, 2010) (34).

In this study, 82 samples were taken from the previous samples and divided them into groups

according to the total number of sperm, as follows:

1. **Abnormozoospermia**: It includes Azoospermia number of sperm 0 (million/ejaculate), and Oligozoospermia with a number of sperm < 39 (million/ ejaculate). and The number of samples was 62 men.
2. **Normozoospermia**: As a control group age compatible with the diseased group, they have a sperm count > 39 (million / ejaculate) and The number of samples was 20 men.5 ml of venous blood was taken from each participant in the study after fasting for an entire night and it was divided into two parts:

- 1 ml of Full blood was collected in EDTA tubes and kept at -20 °C until the LEPTIN is performed.

- 4ml was collected into dry tubes, and for 20 minutes, the clot was separated and the centrifugal process was at the speed of 1680Xg for 10 minutes to obtain the serum.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site of the leptin molecule. During the first incubation, leptin in the added sample binds to the immobilized antibody.

The unbound sample material is washed off. In a second incubation added antiserum, which contains a biotinylated anti-leptin antibody, forms a sandwich complex with the bound leptin on the microtiter wells. Afterward, the unbound antiserum is washed off and streptavidin peroxidase complex (Enzyme Complex) is added for the detection of the biotin molecules. After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution.

The colorimetric reaction is stopped by the addition of a stop solution, and the optical density

(OD) of the resulting yellow product is measured. The intensity of the color is proportional to

the concentration of the analyte in the sample. A calibration curve is constructed by plotting

OD values against concentrations of calibrators and concentrations of unknown samples are

determined using this calibration curve.

Before starting the procedure, and to ensure accurate results, the samples were centrifuged

(using an electric centrifuge) because some of them contained high percentages of protein

and this may hinder accurate sampling causing inaccurate results.

The remaining steps were as follows:

1. The desired number of Microtiter wells were secured in the frame holder.
2. Pipette 15 µL of each Calibrator, Control, and sample with new disposable tips into appropriate wells.
3. 100 µL Assay Buffer was added into each well and thoroughly mixed for 10 seconds.
4. The samples were incubated for 120 minutes at room temperature.
5. The wells were washed using an automated well washer and at the end of the washing step, we struck the wells sharply on absorbent paper to remove residual droplets.
6. 100 µL Antiserum was dispensed to each well and incubated for 30 minutes at room temperature.
7. Washing has been performed again as described in step 5.
8. 100 µL Enzyme Conjugate was dispensed into each well and incubated for 30 minutes at room temperature.
9. Washing has been performed again as described in step 5.
10. 100 µL of Substrate Solution was added to each well and incubated it for 15 minutes at room temperature.
11. The enzymatic reaction was stopped by adding 50 µL of Stop Solution to each well.
12. In the end, the optical density of the solution was measured in each well at 450 nm (reading) and 620 nm to 630 nm (background subtraction, recommended) with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

6-3- Results:

The results have been calculated automatically using a 4-Parameter curve fit. (4- Parameter Rodbard or 4-Parameter Marquardt is the preferred method.) Other data reduction functions may give slightly different results.

The concentration of the samples can be read directly from this calibration curve. Samples with concentrations higher than that of the highest calibrator can be further diluted or must be reported as > 100 ng/mL. For the calculation of the concentrations, its dilution factor must be considered. For duplicate determinations, the mean of the two values must be taken. If the two values deviate substantially from one another, DIA source recommends retesting the samples.

6-3-1- Descriptive Statistics for Quantitative Variables:

Descriptive Statistics								
						Percentiles		
	N	Mean	Std. Deviation	Minimum	Maximum	25th	50th (Median)	75th
Un-OC	82	4.71249	1.477128	1.408	7.704	3.53500	4.60000	5.95175
Leptin	82	3.18050	4.360522	.000	19.090	.17250	1.46200	4.37525
Testosterone	82	7.9305	3.87154	1.40	19.70	5.1000	7.8500	9.5250
BMI	82	22.5852	4.91582	15.23	34.80	19.0250	20.9000	24.9000
Sperm count	82	16.0159	18.52530	.00	54.00	.0000	11.7500	28.5000

Table (1): Descriptive Statistics for Quantitative Variables. (SPSS Software)

Table (1) shows the below:

- Where the arithmetic means for Un-OC was 4.712 with a standard deviation of 1.477, the highest value was 7.704 and the lowest was 1.408.

- The arithmetic mean of the leptin hormone for the studied sample was 3.180 with a standard deviation of 4.360, the highest value was 19.090, and the lowest value was zero.

- The arithmetic mean of Testosterone (T) was 7.930 with a standard deviation of 3.871 The highest value was 19.70, and the lowest value was 1.40.

- The arithmetic mean of BMI was 22.585 with a standard deviation of 4.915, with the highest value of 34.80 and the lowest value of 15.23.

- The arithmetic mean of Sperm count was 16.015 with a standard deviation of 18.525, the highest value was 54 and the lowest value was 0.

- Finally, the arithmetic mean of Sperm count was 16.015 with a standard deviation of 18.525, the highest value was 54 and the lowest value was 0.

6-3-2- Descriptive Statistics for Qualitative Variables:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Azo	30	36.6	36.6	36.6
	Oligo	32	39.0	39.0	75.6
	Normo	20	24.4	24.4	100.0
	Total	82	100.0	100.0	
Valid	Normozoospermia	20	24.4	24.4	24.4
	Abnormozoospermia	62	75.6	75.6	100.0
	Total	82	100.0	100.0	

Table (2): Descriptive Statistics for Qualitative Variables. (SPSS Software)

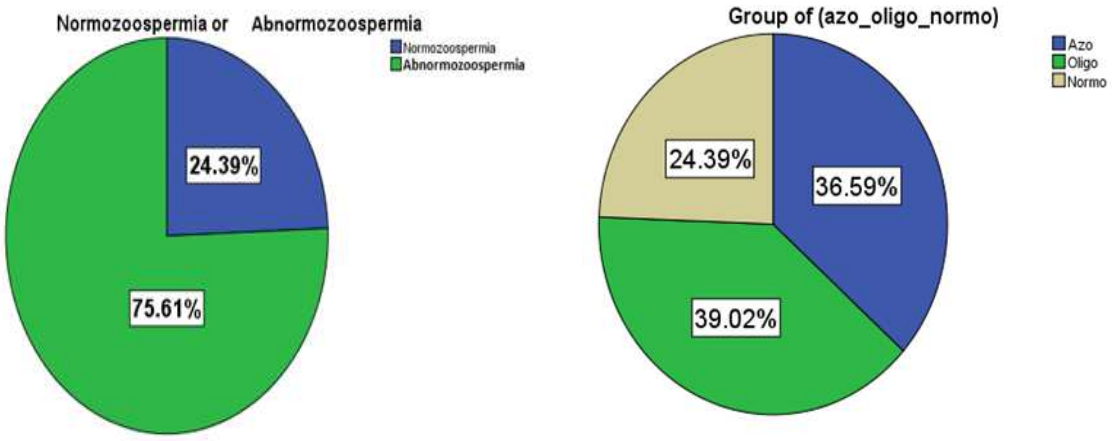


Figure (1): Graphic Presentation for Descriptive Statistics of Qualitative Variables. (SPSS)

Table (2) and figure (1) show that 20 men out of 82 of the studied sample recorded the

result on their medical examination as Normozoospermia or 24.4%. In comparison, the result for 62 men out of 82 of the studied sample was Abnormozoospermia or 75.6%.

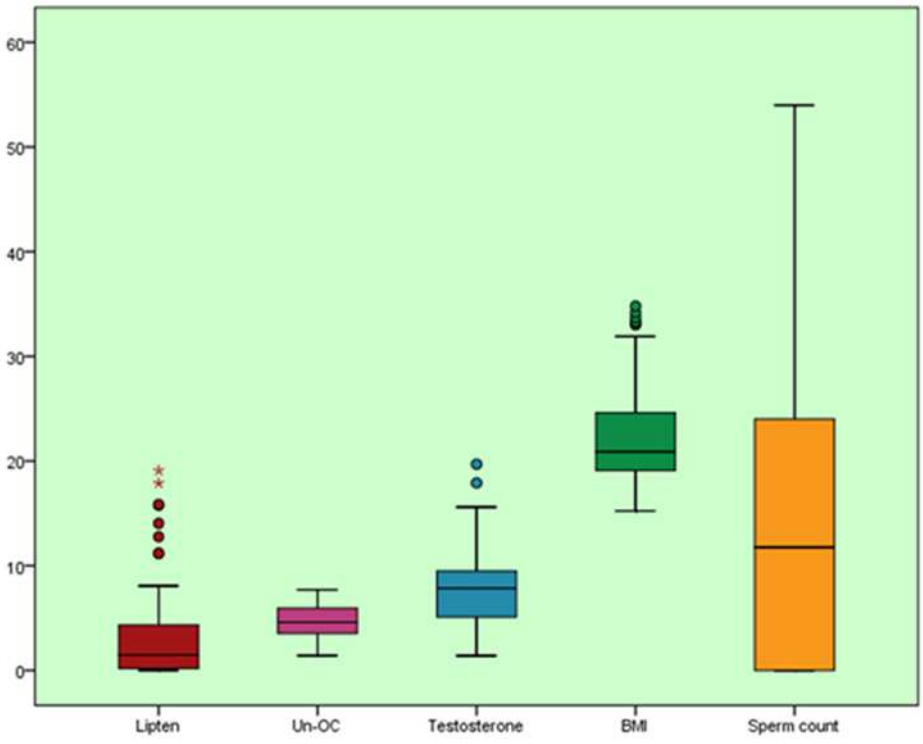
6-3-3- Analytical Statistics:

To conduct an in-depth analysis of the data, it was necessary to know the form or type of data distribution to determine the type of tests that will be applied. Therefore, the normal distribution test for the data was carried out using the Kolmogorov-Smirnov test.

One-Sample Kolmogorov-Smirnov Test								
		Un-OC	Leptin	Testosterone	BMI	Sperm count	Group of (azo-oligo-normo)	Normo/ Abnormo
N		**82**	**82**	**82**	**82**	**82**	**82**	**82**
Normal Parameters[a,b]	Mean	4.71249	3.18050	7.9305	22.5852	16.0159	1.88	1.76
	Std. Deviation	1.477128	4.360522	3.87154	4.91582	18.52530	.776	.432
Most Extreme Differences	Absolute	.065	.233	.106	.168	.198	.237	.470
	Positive	.065	.194	.106	.168	.198	.237	.286
	Negative	-.065-	-.233-	-.046-	-.105-	-.194-	-.197-	-.470-
Test Statistic		.065	.233	.106	.168	.198	.237	.470
Asymp. Sig. (2-tailed)		.200[c,d]	.000[c]	.024[c]	.000[c]	.000[c]	.000[c]	.000[c]
a. Test distribution is Normal.								
b. Calculated from data.								
c. Lilliefors Significance Correction.								
d. This is a lower bound of the true significance.								

Table (3): Study Data Analysis with Kolmogorov-Smirnov Test. (SPSS Software)

Through the results (Table 3), it was found that all variables were not subject to the normal distribution except for the variable (Un_OC), and therefore the null hypothesis for the other variables was rejected, and the data didn't follow the normal distribution, while it follows the normal distribution for the variable (Un_OC), where the test value is greater than 0.05, and here we accept The null hypothesis that the distribution is normal for Un_OC. As shown in the following figure:



6-3-4- Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia & Abnormozoospermia):

A comparison for the quantitative variables (Sperm count, BMI, Testosterone, Un-OC, Leptin) has been performed according to the qualitative variables (Normozoospermia, Abnormozoospermia).

Descriptives							
Sperm count	BMI	Testosterone	Un-OC	Leptin	Statistic		
46.3900	22.9885	13.1730	5.84084	3.30140	Mean		Normozoospermia
44.5419	20.6913	11.9441	5.30030	1.12578	Lower Bound	95% Confidence Interval for Mean	
48.2381	25.2857	14.4019	6.38138	5.47702	Upper Bound		
46.2111	22.7094	13.0478	5.89293	2.78767	5% Trimmed Mean		
45.0000	22.0500	12.6500	6.23250	1.66250	Median		
15.593	24.092	6.894	1.334	21.610	Variance		
3.94874	4.90834	2.62567	1.154959	4.648607	Std. Deviation		
42.00	17.30	8.90	3.137	0.000	Minimum		
54.00	33.70	19.70	7.607	15.850	Maximum		
12.00	16.40	10.80	4.470	15.850	Range		
6.75	8.25	2.93	1.417	4.082	Interquartile Range		
0.686	0.792	0.777	-0.904	2.156	Skewness		
-0.974	-0.338	1.018	0.842	4.161	Kurtosis		
6.2177	22.4551	6.2394	4.34850	3.14150	Mean		Abnormozoospermia
4.4080	21.1977	5.6258	3.99572	2.04888	Lower Bound	95% Confidence Interval for Mean	
8.0275	23.7124	6.8529	4.70128	4.23412	Upper Bound		
5.7670	22.1873	6.3051	4.32643	2.55333	5% Trimmed Mean		
2.1000	20.4100	6.7500	4.13300	1.37100	Median		
50.785	24.514	5.838	1.930	18.511	Variance		
7.12639	4.95114	2.41617	1.389138	4.302455	Std. Deviation		
0.00	15.23	1.40	1.408	0.000	Minimum		
24.00	34.80	9.80	7.704	19.090	Maximum		
24.00	19.57	8.40	6.296	19.090	Range		
13.00	5.05	4.23	2.038	4.203	Interquartile Range		
0.593	1.099	-0.462	0.229	2.142	Skewness		
-1.104	0.342	-1.015	-0.473	4.560	Kurtosis		

Table (4): Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia & Abnormozoospermia). (SPSS Software)

Table (4) demonstrates the following:

- The Mean Sperm count in the Normozoospermia was (46.390) greater than the Mean Sperm counts in the Abnormozoospermia (6.217). While the standard deviation of sperm count in the Normozoospermia was (3.948) smaller than the standard deviation of Sperm count in the Abnormozoospermia (7.126).
- The Mean BMI in the Normozoospermia was (22.988) greater than the Mean BMI in the Abnormozoospermia (22.455). While the standard deviation of BMI in the Normozoospermia was (4.908) smaller than the standard deviation of BMI in the Abnormozoospermia (4.951).
- The Mean Testosterone in the Normozoospermia was (13.173) greater than the Mean Testosterone in the Abnormozoospermia (6.239). While the standard deviation of Testosterone in the Normozoospermia was (2.625) greater than the standard deviation of Testosterone in the Abnormozoospermia (2.416).
- The Mean Un-OC in the Normozoospermia was (5.840) greater than the Mean Un-OC in the Abnormozoospermia (4.348). While the standard deviation of Un-OC in the Normozoospermia was (1.154) smaller than the standard deviation of Un-OC in the Abnormozoospermia (1.389).
- The Mean Leptin in the Normozoospermia was (3.301) greater than the Mean Leptin in the Abnormozoospermia (3.141). While the standard deviation of Leptin in the Normozoospermia was (4.648) greater than the standard deviation of Leptin in the Abnormozoospermia (4.302). and this can be seen in the following Figure (3).

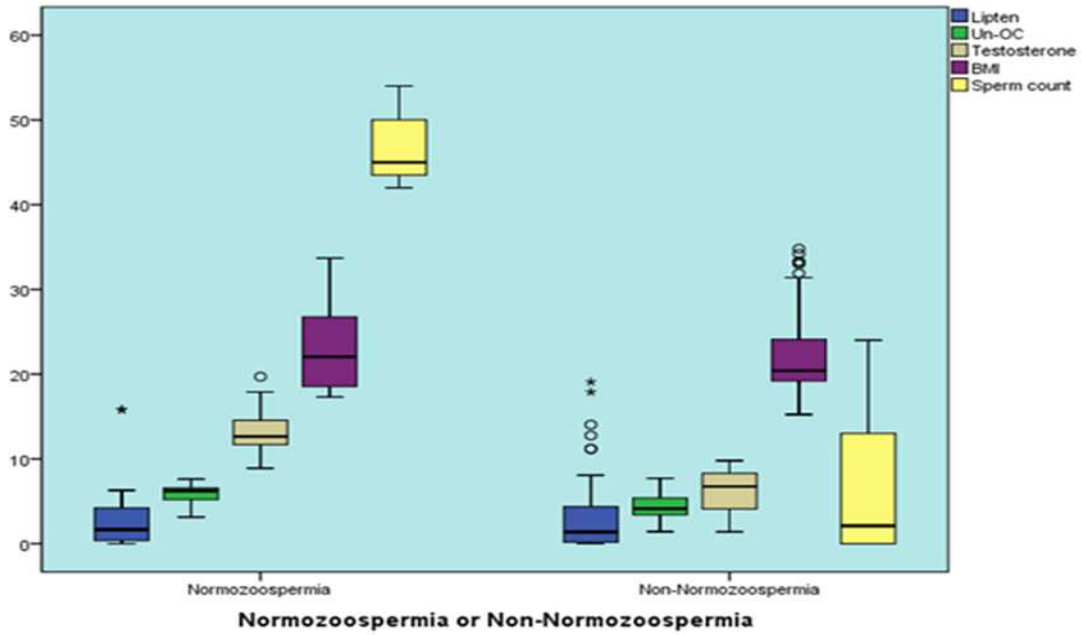


Figure (3): Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia & Abnormozoospermia). (SPSS Software)

6-3-5- Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia, Azoospermia, Oligospermia):

A comparison for the quantitative variables (Sperm count, BMI, Testosterone, Un-OC, Leptin) has been performed according to the qualitative variables (Normozoospermia, Azoospermia, Oligospermia) (Table 5).

Spem count	BMI	Testosterone	Un-Oc	Leptin	Statistic		
0.000	22.253	6.174	4.524	3.045	Mean		Azo
0.000	20.322	5.281	4.026	1.518	Lower Bound	95% Confidence Interval for Mean	
0.000	24.184	7.067	5.021	4.572	Upper Bound		
0.000	22.025	6.202	4.489	2.513	5% Trimmed Mean		
0.000	20.400	6.450	4.132	2.036	Median		
0.000	26.745	5.718	1.776	16.721	Variance		
0.000	5.172	2.391	1.333	4.089	Std. Deviation		
0.000	15.234	2.130	2.229	0.000	Minimum		
0.000	33.300	9.800	7.704	17.890	Maximum		
0.000	18.066	7.670	5.475	17.890	Range		
0.000	5.281	4.100	1.591	3.333	Interquartile Range		
0.000	0.987	-0.242	0.549	2.259	Skewness		
0.000	0.073	-1.220	-0.084	5.548	Kurtosis		
12.047	22.644	6.301	4.184	3.232	Mean		Oligo
10.158	20.910	5.408	3.664	1.589	Lower Bound	95% Confidence Interval for Mean	
13.936	24.379	7.193	4.704	4.875	Upper Bound		
12.022	22.256	6.394	4.174	2.636	5% Trimmed Mean		
12.500	20.410	6.950	4.212	1.355	Median		
27.445	23.141	6.130	2.078	20.765	Variance		
5.239	4.811	2.476	1.442	4.557	Std. Deviation		
1.900	17.600	1.400	1.408	0.000	Minimum		
24.000	34.800	9.600	6.900	19.090	Maximum		
22.100	17.200	8.200	5.492	19.090	Range		
4.825	5.150	3.600	2.543	4.525	Interquartile Range		
-0.317	1.310	-0.675	0.066	2.129	Skewness		
0.217	0.931	-0.774	-0.875	4.585	Kurtosis		
46.390	22.989	13.173	5.841	3.301	Mean		Normo
44.542	20.691	11.944	5.300	1.126	Lower Bound	95% Confidence Interval for Mean	
48.238	25.286	14.402	6.381	5.477	Upper Bound		
46.211	22.709	13.048	5.893	2.788	5% Trimmed Mean		
45.000	22.050	12.650	6.233	1.663	Median		
15.593	24.092	6.894	1.334	21.610	Variance		
3.949	4.908	2.626	1.155	4.649	Std. Deviation		
42.000	17.300	8.900	3.137	0.000	Minimum		
54.000	33.700	19.700	7.607	15.850	Maximum		
12.000	16.400	10.800	4.470	15.850	Range		
6.750	8.250	2.925	1.417	4.082	Interquartile Range		
0.686	0.792	0.777	-0.904	2.156	Skewness		
-0.974	-0.338	1.018	0.842	4.161	Kurtosis		

Table (5): Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia, Azoospermia, Oligospermia). (SPSS Software)

Table (5) demonstrates that:

- The Mean Sperm count in the Normozoospermia was (46.390) greater than the Mean Sperm counts in the Oligospermia (12.047), and the Mean Sperm counts in the Azoospermia was (0.000). While the standard deviation of sperm count in the Normozoospermia was (3.948) smaller than the standard deviation of Sperm count in the Oligospermia (4.811), and the standard deviation of Sperm count in the Azoospermia was (0.000).
- The Mean BMI in the Normozoospermia was (2.989) greater than the Mean BMI in the Oligospermia (2.644), and the Mean BMI in the Azoospermia (2.253). While the standard deviation of BMI in the Normozoospermia was (4.908) greater than the standard deviation of BMI in the Oligospermia (4.811), and the smaller standard deviation of BMI in the Azoospermia (5.172).
- The Mean Testosterone in the Normozoospermia was (13.173) greater than the Mean Testosterone in the Oligospermia (6.301), and the mean Testosterone in the Azoospermia (6.174). While the standard deviation of Testosterone in the Normozoospermia was (2.626) greater than the standard deviation of Testosterone in the Oligospermia (2.476), and the standard deviation of Testosterone in the Azoospermia (2.391).
- The Mean Un-OC in the Normozoospermia was (5.841) greater than the Mean Un-OC in the Oligospermia (4.184), and the mean Un-OC in the Azoospermia (4.524). While the standard deviation of Un-OC in the Normozoospermia was (1.155) smaller than the standard deviation of Un-OC in the Oligospermia (1.442), and the standard deviation of Un-OC in the Azoospermia (1.333).
- The Mean leptin in the Normozoospermia was (3.301) greater than the Mean leptin in the Oligospermia (3.232), and the mean leptin in the Azoospermia (4.649). While the standard deviation of leptin in the Normozoospermia was (4.649) smaller than the standard deviation of leptin in the Oligospermia (4.557), and the standard deviation of leptin in the Azoospermia (4.089). This indicates a decrease in the leptin hormone for both Azoospermia and Oligospermia, and this can be seen in the following figure (4).

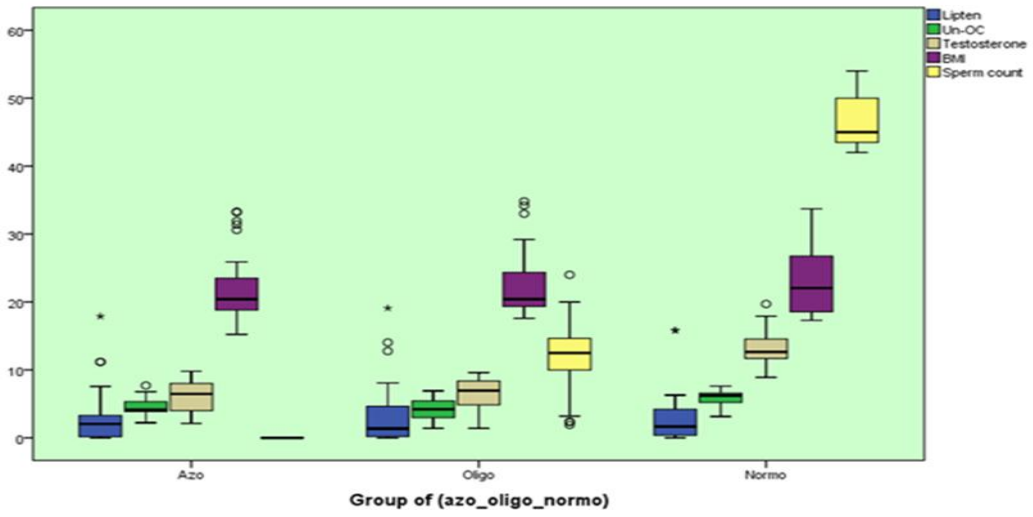


Figure (4): Observation of Changes in Quantitative Variables According to Qualitative Variables (Normozoospermia, Azoospermia, Oligospermia). (SPSS Software)

It was found that there was a difference in the values (Sperm count, BMI, Testosterone, Un-OC, Leptin) in Normozoospermia compared to Abnormozoospermia, but it was necessary to know the significance of this difference, by conducting a Spearman's rho correlation coefficient and then Friedman Test.

6-3-6- Spearman's rho Correlation Coefficient on Quantitative Variables in the Sample:

Due to the presence of quantitative variables, Spearman's rho correlation coefficient was studied through the following matrix:

Correlations " Spearman's rho "								
Normozoospermia or Non	Group of (azo_oligo_normo)	Sperm count	BMI	Testosterone	Un-OC	Liptin	Statistic	
-0.023	0.023	0.039	.588**	-0.087	-0.081	1.000	Correlation Coefficient	Liptin
0.839	0.837	0.726	0.000	0.438	0.467		Sig. (2-tailed)	
-.436-**	.301**	.299**	-.223-*	.361**	1.000		Correlation Coefficient	Un-OC
0.000	0.006	0.006	0.044	0.001			Sig. (2-tailed)	
-.732-**	.594**	.625**	0.037	1.000			Correlation Coefficient	Testosterone
0.000	0.000	0.000	0.745				Sig. (2-tailed)	
-0.040	0.067	0.052	1.000				Correlation Coefficient	BMI
0.720	0.547	0.645					Sig. (2-tailed)	
-.763-**	.961**	1.000					Correlation Coefficient	Sperm count
0.000	0.000						Sig. (2-tailed)	
-.794-**	1.000						Correlation Coefficient	Group of (azo_oligo_normo)
0.000							Sig. (2-tailed)	
1.000							Correlation Coefficient	Normozoospermia or Non
							Sig. (2-tailed)	
82	82	82	82	82	82	82	N	

Figure (5): Spearman's rho Correlation Coefficient on Quantitative Variables in the Sample. (SPSS Software)

Based on Figure 5, when testing the relationship between variables, it was not possible to use parametric statistical tests, and here it was necessary to use non-parametric tests, therefore Friedman test was used, as it is an alternative to the analysis of variance in data that is not normally distributed.

6-3-7- Friedman's Test:

As a result of the Friedman test, which is conducted if the data is not distributed in a manner according to the natural curve, it was found that there is a relationship between the variables of the study at the level of significance of 0.05, which was statistically significant. As shown in Figure (6).

Friedman Test

Ranks

	Mean Rank
Lipten	2.70
Un-OC	4.40
Testosterone	4.99
BMI	6.74
Sperm count	4.32
Group of (azo_oligo_normo)	2.38
Normozoospermia or Non-Normozoospermia	2.48

Test Statistics[a]

N	82
Chi-Square	273.171
df	6
Asymp. Sig.	.000

a. Friedman Test

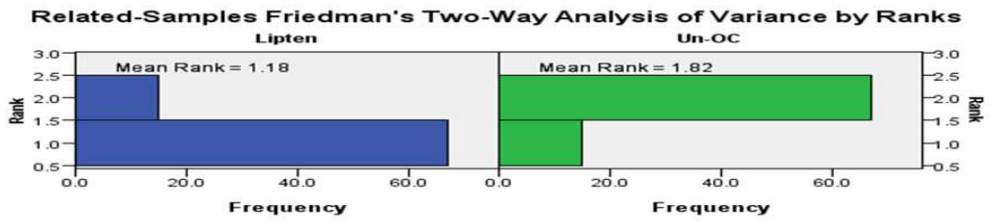
Figure (6): The emergence of a statistically significant relationship when conducting the Friedman test. (SPSS Software)

6-3-8- Investigation of the Relationship between Leptin and Uncarboxylated Osteocalcin in Syrian Men According to Friedman's Test:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Lipten and Un-OC are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



Total N	82
Test Statistic	32.976
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.000

1. Multiple comparisons are not performed because there are less than three test fields.

Figure (7): Hypothesis Test Summary & Evaluating the relationship between leptin and Un-Oc. (SPSS Software)

Friedman's test is conducted on samples with an abnormal distribution, and in our case, the null hypothesis states that the two variables (Leptin and Un-OC) have the same distribution was investigated and rejected.

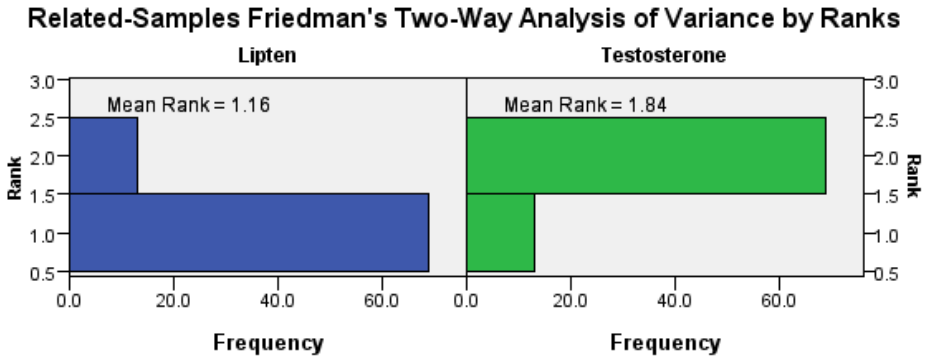
It was found that there is a statistically significant relationship (sig = .000) between Leptin and Un-Oc, and when serum leptin values were high, Un-Oc was low and vice versa. As shown in Figure (7).

6-3-9- Investigation of the Relationship between Leptin and Testosterone in Syrian Men According to Friedman's Test:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Lipten and Testosterone are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



Total N	82
Test Statistic	38.244
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.000

1. Multiple comparisons are not performed because there are less than three test fields.

Figure (8): Hypothesis Test Summary & Evaluating the Relationship between Leptin and Testosterone (T). (SPSS Software)

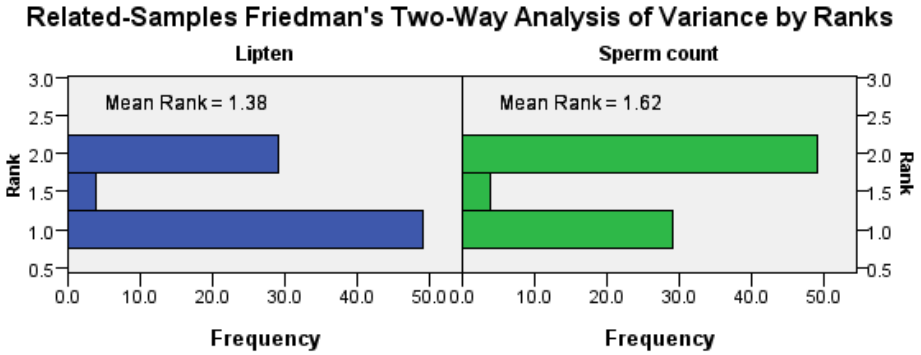
The null hypothesis was rejected and it was found that there is a statistically significant relationship (sig = .000) between Leptin and Testosterone, and when serum leptin values were high, Testosterone was low and vice versa. As shown in Figure (8).

3-6-10- Investigation of the Relationship between Leptin and Sperm Count in Syrian Men According to Friedman's Test:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Lipten and Sperm count are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.024	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



Total N	82
Test Statistic	5.128
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.024

1. Multiple comparisons are not performed because there are less than three test fields.

Figure (9): Hypothesis Test Summary & Evaluating the Relationship between Leptin and Sperm Count. (SPSS Software)

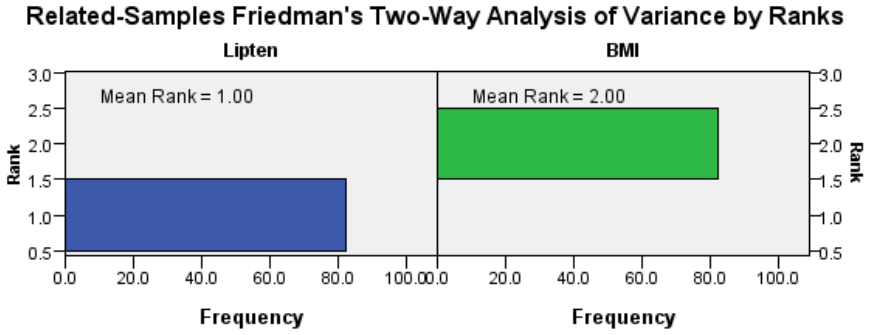
The null hypothesis was rejected, and it was found that there is a statistically significant relationship (sig = .024) between Leptin and sperm count, and when serum leptin values were high, sperm count was low and vice versa. As shown in figure (9).

3-6-11- Investigation of the Relationship between Leptin and Body Mass Index (BMI) in Syrian Men According to Friedman's Test:

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distributions of Lipten and BMI are the same.	Related-Samples Friedman's Two-Way Analysis of Variance by Ranks	.000	Reject the null hypothesis.

Asymptotic significances are displayed. The significance level is .05.



Total N	82
Test Statistic	82.000
Degrees of Freedom	1
Asymptotic Sig. (2-sided test)	.000

1. Multiple comparisons are not performed because there are less than three test fields.

Figure (10): Hypothesis Test Summary & Evaluating the Relationship between Leptin and BMI. (SPSS Software)

The null hypothesis was rejected, and it was found that there is a statistically significant relationship (sig = .000) between Leptin and BMI, and when serum leptin values were high, BMI was high. As shown in figure (10).

3-7- Discussion:

According to the results, it was demonstrated that there is a statistically significant inverse relationship between leptin and undercarboxylated osteocalcin. In a study conducted by Suh et al., 2013 of 199 healthy non-smoking Korean men after receiving weight 3 months of weight loss treatment. measured OC, leptin, ghrelin serum, and body mass index. It was found that the decrease in leptin was significantly associated with the increase in serum undercarboxylated osteocalcin concentration after weight loss. This means that leptin levels correlate with changes in serum OC (36), which is consistent with our study.

It was found that there is a statistically significant inverse relationship between leptin and testosterone, and this finding is consistent with a study conducted by Khodamoradi et al., 2022 that explained the association between leptin and low testosterone levels through imbalanced leptin levels leading to higher levels of estrogen levels, which leads to increased activity of aromatase, which suppresses testosterone levels and the hypothalamic-pituitary-genital axis (37).
In a study conducted by Luukkaa et al., 1998, blood samples were collected and serum leptin was measured using a human leptin assay kit, as well as testosterone, insulin, and body mass index were measured. A significant inverse relationship was found between leptin and testosterone ($r = -0.39$; $P < 0.001$), and a significant positive relationship between leptin and BMI (38), both outcomes were related to our study.

It was found that there is a statistically significant inverse correlation between leptin and sperm count in our study. And in a study conducted by Mo et al., 2022, 19 studies were included in the meta-analysis, which included 1138 infertile men and 756 controls, and demonstrated that leptin levels correlated inversely with sperm count and sperm motility in infertile males. This indicates that leptin is associated with male infertility (39).

In a study published in the International Journal of Impotence Research on a sample of 71 men, plasma leptin was measured using an ELISA, the same test we used in our study. The semen was analyzed by a series of tests. The results showed that leptin levels decreased in azoospermia and oligozoospermia (40), consistent with our research.

It was found that there is a statistically significant positive correlation between leptin and body mass index. In a study conducted on 35 overweight Omani men, and 20 healthy controls. After measuring leptin levels in the blood and calculating the body mass index, it was found that there is a statistically significant relationship between leptin and obesity (37). and this is consistent with our study.

4- Conclusion:

Leptin has been shown to have a large impact on male fertility and this study demonstrates the strong statistical relationship between leptin and body mass index, how high leptin levels are associated with weight gain, and the solid statistical inverse relationship between leptin and Uncarboxylated osteocalcin. We also found a strong inverse relationship between leptin and both testosterone and sperm count, which may indicate leptin's effect on infertility.

Broader research should be established in Syria with a larger sample size, and annual statistics should be extracted to provide sufficient information on the relationship between weight gain and infertility with taking in consideration the leptin concentrations in infertile men.

Therefore, it is strongly recommended to follow a healthy lifestyle, since multiple studies concluded that increased leptin and BMI may lead to male infertility. Besides spreading awareness among adults about the dangers of obesity and its effects on fertility.

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Ethics, Consent to Participate, and Consent to Publish declarations:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Data availability:

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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