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Effect of BMI on chemerin in serum on females Undergoing ICSI.

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ABSTRACT

Serum chemerin levels are associated with body fat percentage and BMI, and elevated chemerin levels are associated with metabolic problems and inflammatory shifts. This study aimed to evaluate the effect of BMI in serum and follicular fluid chemerin and Leptin levels on patients undergoing ICSI. This study was performed by 44 women who underwent ICSI procedures and divided into two groups based on BMI: the non-obese group, with a BMI<30 and the obese group, with a BMI>30. The result in the non-obese group showed a significant positive correlation was found between Serum chemerin levels, indicating a strong effect size (p< .001). This suggests that as Serum chemerin levels rise, so will FF chemerin. A significant positive correlation of r =0.92 was found between the oocytes retrieved and the Number of metaphases II, indicating a strong effect size (p<.001, This shows that the number of metaphase II oocytes increases as the number of retrieved oocytes increases. A significant positive correlation of 0.66 was found between the number of embryos and the G1 embryo, indicating a large effect size.

Conclusion: Serum chemerin had a significant correlation with oocyte maturity and embryo grading, and there was no significant difference between obese and non-obese patients regarding serum chemerin pregnancy.

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KEYWORDs

BMI, ICSI; Chemerin; Infertility.

1. Introduction

Failure to become pregnant after at least 12 months of regular, unprotected sexual contact is a sign of infertility for both sexes. It is estimated that infertility affects more than a billion people of reproductive age around the world. Infertility affects 48 million couples and 186 million individuals worldwide, according to current estimates. (WHO) (Zivari-Ghader et al, 2018 [1]). Infertility in women can be caused by a variety of conditions, including problems with the ovaries, uterus, fallopian tubes, or endocrine system. There are two types of infertility: primary and secondary. In the case of primary infertility, a woman has never been able to conceive; in the case of secondary infertility, she has had at least one previous pregnancy. (Ghaffari and Arabipoo, 2018 [2]). Preventing, diagnosing, and treating infertility all fall under the umbrella of fertility care. Most countries, particularly those with low and middle incomes, still struggle to provide equal and equitable access to reproductive health care. National universal health insurance benefit packages rarely priorities reproductive health care. Chemerin is a tiny chemotactic protein involved in the early stages of the immune response. Chemerin is an adipokine that has a role in energy homeostasis and reproductive function regulation. When chemerin was first identified in 1997, it was thought to be merely a protein linked to healthy skin function that starkly contrasted psoriasis. A global regulator of chemotactic, adipokine, autocrine/paracrine, adaptogenic, angiogenic, and reproductive functions, chemerin has recently come into the spotlight due to the discovery of multiple receptors for the chemerin protein and a large number of associations with various pathologies. (Fischer and Beck-Sickinger, 2022 [3]). Chemerin levels in the body and the blood are linked to BMI and obesity-related indicators. In previous research, Chemerin has been linked to adipogenesis, glucose homeostasis, food intake, and body weight, including gain or loss of function. (Marti, et al., 2021 [4]), Their nutritional status and body composition influence female reproductive functions in mammals. The aim of the study was to evaluate whether there is any relation between BMI on chemerin level in serum and follicular fluid with oocyte and Embryo quality in women with infertility candidates for fresh intracytoplasmic sperm injection.

2. Patients and Methods

This study was done on 44 infertile females who were undergoing intracytoplasmic sperm injection (ICSI) in the infertility centre for High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University/ Baghdad/ Iraq regardless of the presence or absence of previous trials. The morphological assessment of the oocytes aspirated from infertile females and their resulting embryos was done in the ICSI laboratory of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, while the measurement of serum and follicular fluid chemerin was done in a private laboratory. The present study was done on forty-four infertile females who were subjected to intracytoplasmic sperm injection (ICSI) cycles. Their ages ranged from 20 to 40 years old. The infertility duration ranges from 1 to 20 years, with both primary and secondary types of infertility. Some of these infertile females were having their first IVF/ ICSI attempt, while others had already undergone at least one cycle before. The infertile females who were enrolled in this study underwent flexible antagonist ovarian stimulation protocols. The duration of stimulation and gonadotropin dose depended on the female's age, markers of ovarian reserve (such as antral follicle count (AFC), Antimullerian hormone (AMH) levels, and/ or early Follicle stimulating hormone (FSH), Luteinizing hormone (LH), and Estradiol levels) in addition to the type of protocol used in previous IVF trials if present.

All the forty-four infertile females had a normal ovarian reserve, based on their serum AMH level and antral follicle count (AFC), evaluated on day 1 or 2 of the menstrual cycle (follicular phase), which is done routinely in the infertility clinic. Some have a history of ovulation disorders like polycystic ovarian syndrome (PCOS), and this was decided according to the Rotterdam criteria (oligo-and/or anovulation, hyperandrogenism signs, and polycystic ovary morphology on ultrasound examination). Others were having a history of tubal blockage, male factor, or unexplained infertility. Basal serum levels of Follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and.

The number of dominant follicles for each patient was documented by ultrasound assessment on the day of hCG triggering, and a total number of retrieved oocytes was recorded during the day of ova pick-up (OPU). At the same time, the follicular fluid was collected and stored in a deep freezer for later assessment of serum and follicular fluid chemerin and Leptin levels after denudation. The nuclear maturity of the retrieved oocytes was evaluated, and these oocytes were classified according to their nuclear maturity (depending on presence or absence of 1st polar body) into germinal vesicle (GV), metaphase I (MI), and metaphase II (MII).

Only normal mature oocytes were injected with morphologically normal sperm (ICSI). On day three, following sperm injection (ICSI), embryo quality was assessed, and the results were based on morphological criteria (Ozturk, 2020) .

The patients who were included in the present study were selected according to the following criteria.

Inclusion criteria:

-Their age ranged from 20-40 years old .These patients had normal ovarian reserve documented by normal anti-Mullarian hormone (AMH), Fsh, and Afc, which should be at least within the satisfactory or normal ovarian reserve. They have normal thyroid function tests. Patients with patent or non-patent tubes (except those with endometriosis).Couples with unexplained infertility, Male factor infertility, except those with non-obstructive azoospermia.

Exclusion criteria:

-Age less than 20 and more than 40. Patients with poor ovarian reserve diagnosed by high FSH >12 IU/ml low antral follicle count) and low AMH, Heavy smokers, and Uncontrolled endocrinological disorders such as thyroid dysfunction. Male partner with non-obstructive azoospermia., intrauterine pathology. In all forty infertile females, serum FSH, LH and estradiol (E2) were measured on day 1 or 2 of the cycle just before starting ovarian stimulation

Gonadotropin releasing hormone (GnRH) antagonist protocol:

The flexible GnRH antagonist protocol was used in this study in which gonadotropins were initiated on the second day of the menstrual cycle in the doses of (Gonal- F 150-300IU) and when the leading follicle reached a diameter of 12- 14 mm, GnRH antagonist (Cetrotide) was administered in a dose of 0.25 mg daily until the day of HCG injection

The ovarian response to stimulation was monitored by measuring the serum E2 concentration and ultrasound assessment of follicular growth. Ovulation was triggered with an injection of 250 µg human chorionic gonadotropin (hCG) (Ovitrelle) when at least two follicles reached a diameter of 17 mm or more on ultrasound examination. At ovulation triggering day, the hormonal ovarian response was also evaluated by quantifying serum estradiol (E2) level.

-Oocyte retrieval and follicular fluid collection:

Oocytes were retrieved by transvaginal ultrasound-guided aspiration 34-36 hours after HCG injection. For each patient, all follicles were aspirated without flushing; cumulus-oocyte complexes were isolated. Oocyte retrieval was done under general or sometimes spinal anaesthesia according to the patient's condition. Normal saline solution was used to clean the vagina thoroughly. Aspiration was achieved by a single-lumen needle using aspiration media as a flushing medium. Aspiration pump pressure was set around 120 mm Hg.

In- vitro fertilization/ Intracytoplasmic sperm injection (IVF- ICSI procedure:

- After oocyte retrieval, the cumulus was denuded 38 to 42 hours after hCG administration. Stripping was accomplished by placing the cumulus-oocyte complex in 80 IU/mL of hyaluronidase in Ferti Cult flushing media, which contains 4g/L human serum albumin (HSA) and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) for 30 to 60 seconds, rinsing five to six times in ferticult flushing media which consists of HSA, HEPES, phenol red and 10 mg/L. gentamycin, and then mechanically removing the cumulus from the oocyte using an EZ-Grip micropipettor170 and 135 µ
- The sperm was first visualized under 40 magnification and then was immobilized in 10% polyvinylpyrrolidone, which consists of HSA, HEPES, PVP, MEA≥80%, endotoxin<1IU/ml using the tip of the injection needle. It was then aspirated tail-first into the injection needle.
- The oocyte was grasped with a holding pipette at 9 o'clock using gentle suction and then was rotated such that the first polar body was located at either the 6 o'clock or 12 o'clock position.
- injection pipette was pushed through the zona at the 3 o'clock position and advanced to the outer surface of the oolemma. The oolemma

was penetrated by direct penetration technique. Entry into the oocyte was confirmed by the free flow of cytoplasm into the injection pipette.

The cytoplasm was then slowly injected back into the oocyte until the sperm was seen to pass the tip of the injection pipette and slide into the cytoplasm. The procedure was concluded by gently removing the injection pipette.

3. Results

All women aged below 40 years old. The most frequently observed age category within the non-obese group was (20- 30) years old (n = 17, 53.12%). While the most frequently observed age group within the Obese group was (31- 40) (n = 11, 91.67%). For Non-Obese, the average age was 31.34±5.60 (SEM = 0.99, Median = 30.00). For Obese, age had an average of 34.50 ± 3.83 (SEM = 1.10, Median = 34.50). The result of the t-test comparison was not significant, t (40) = -1.79, p = .080, which suggests the mean of females' age was not significantly different between the Non-obese and Obese groups. The results are presented in Table 4-1. A bar plot of the means is presented in Figure 1. The absence of PCOS was the most frequently seen Within the non-obese group (n = 28, 73.7%). As well as within the obese group (n = 10, 26.3%). The findings of the Fisher exact test were inconclusive, with an OR of 0.93 and a p-value of 1.000. This indicates that PCO and obesity might not be related to one another in the study subjects. In another ward, there was not a significant difference between the expected frequencies and the frequencies that were actually observed. The outcomes of Fisher's exact test are summarized in Table 2. The most frequently observed Type of infertility within the non-obese group was primary (n = 21, 65.62%). While the most frequently observed Type of infertility within Obese patients was secondary infertility (n = 6, 54.55%).

The Fisher exact test results were insignificant, with an OR = 2.24 and p =.295, To put it another way, this means that the observed frequencies did not differ from the expected frequencies. The Frequency, percentage, and exact test findings of Fisher are shown in Table 3 below.

Fisher's exact test P = 0.295450074 An absence of a previous IVF cycle had the frequency of (n = 23, 71.88%) within the non-obese women. The same observation was noticed within the Obese group (n = 6, 50.00%). Frequencies and percentages are presented in Table 4. The most frequently observed category of Previous IVF results within the non-obese group was failed (n = 8, 88.89%). The most frequently observed category of Previous IVF status within the Obese group was failed (n = 5, 83.33%). Frequencies and percentages are presented in Table 5. FSH levels in non-obese people averaged 5.23 ± 5.01, (Median = 3.00, SEM = 0.91). FSH observations for Obese averaged 3.07 ± 0.58, (Median = 3.32, SEM = 0.22). Statistical analysis found no statistically significant difference between groups, t (42) = 1.75, p =.087. This finding implies that the mean FSH did not differ significantly between the Non-obese and Obese groups Table 6. S. chemerin had an average of 302.40± 199.51 for non-obese (Median =217.64, SEM = 35.27). S. Chemerin had an average of 365.80± 264.48 (Median =253.98, SEM = 79.74) for Obese. The statistical analysis indicated no statistically significant difference between the groups, t (40) = -0.67, p =.509, This finding implies that the mean of S. Chemerin did not differ significantly between the Non-obese and Obese groups Table 7

Table (1): The age-wise distribution of the infertile females included in the study

Age in years	Non-Obese N= 32	Obese N=12	Total N=44	P- value
	N (%)	N (%)	N (%)	
(20- 30)	17 (53.125%)	1 (8.3%)	18 (40.91%)	0.0078[a]
(31- 40)	15 (46.87%)	11 (91.66%)	26 (59.1%)	
Total	32 (72.73%)	12 (27.27%)	44 (100%)	
Mean± SD	31.34± 5.60	34.50± 3.83	N =32	0.0800[b]
Median ± SE_M	30.00±0.99	34.50±1.10	N = 12	

a chi-squared, b T-test

Table (2): Comparison of causes of infertility among study groups.

	PCOS		total
Obesity	No	Yes	
Non-obese	28 (73.7%)	3 7 (5.0%	31 (73.8%)
Obese	10 (26.3%)	1 (25.0%)	11 (26.2%)
	38 (90.5%)	4 (9.5%)	42
Fisher's exact test	P = 1.000		

Table (3): Frequency table for Association of type of infertility with obesity

	Type of infertility		
Obesity	primary	Secondary	
Non-obese	21 65.6%	11 34.4%	32 (74.4%)
Obese	5 45.5%	6 54.5%	11 (25.6%)
	26 (60.5%)	17 (39.5%)	43
Fisher's exact test P = 0.295450074			

Table (4): Frequency table of Association between the number of previous IVF cycles with obesity

	Number of a previous IVF cycle				
Obesity	No previous IVF	one	two	three	
Non-obese	23	7	2	0	32 (72.7%)
Obese	6	5	0	1	12 (27.3%)
	29 (65.9%)	12 (27.3%)	2 (4.5%)	1 (2.3%)	44
Fisher's exact test	P = 0.1509				

Table (5): Frequency table of Association between previous IVF status and obesity.

	Previous IVF status			
Obesity	Failed	Na	Succeed	
Non-obese	8	23	1	32 (72.7%)
Obese	5	6	1	12 (27.3%)
	13 (29.5%)	29 (65.9%)	2 (4.5%)	44
Chi-squared	1.975			
DF	2			
Significance level	P = 0.3725			

Table (1): Hormonal levels in women undergoing ICSI by obesity level

Variable	Non-obese		Obese		
	n	Mean± SD	n	Mean± SD	P [a]
FSH mIU/ml	32	5.17± 4.86	12	2.69± 0.72	0.0874
LH mIU/ml	32	3.83± 2.38	12	3.24± 0.95	0.4143
AMH ng/ml	30	1.99± 0.94	7	2.06± 0.65	0.8512

a Two-Tailed Independent Samples t-Test

Table (7): Mean values comparison of the serum and follicular fluids study parameters by obesity

Variable	Non-obese		Obese		
	n	Mean± SD	n	Mean± SD	P [a]
Serum Chemerin	32	302.40± 199.51	12	351.11± 257.26	0.5092

4-Discussion

Obesity can have a negative impact on fertility. Early onset obesity in women favours the development of oligo anovulation, chronic menstrual abnormalities, and adult-onset infertility. When the body mass index of a woman is greater than 30 kg/m2, obesity can potentially worsen the results of assisted reproductive technologies and pregnancy. Insulin resistance and overproduction may be the key reasons for the connection. Particularly noticeable are these negative effects of obesity in polycystic ovary syndrome. So much more attention should be paid to the impact of obesity on uncertainty in both women and men. (Gambineri, et al., 2019 [5]). Age has already been identified as one of the most predictive factors for fertility assessment. The decline in fertility with age is caused by a decrease in the amount and quality of oocytes (Jaswa et al., 2021 [6]), which also leads to a lower success rate in achieving pregnancy through in vitro fertilization. In the present study, it was found that there was no significant difference in the mean age between the obese and non-obese groups; this finding indicated that both groups had comparable ages, which is important to eliminate any variable that may affect the results of the study and the effects on IVF results. (ULRICH and MARSH, 2019 [7]). In the present study, the results within the non-obese group were that the absence of PCO was the most frequently seen, which was the same observation in the obese group in another ward; there was a difference between the expected frequencies and the frequencies that were actually observed. Here, several methodological issues must be discussed, but the present study has several limitations. For instance, the sample size was quite small.

Additionally, the lifestyles (such as their eating habits or how often they exercise), which could have affected their weight regardless of PCOS, are not asked. Moreover, some studies have indicated that BMI is not an entirely accurate measure of obesity. (Alfahl et al., 2020 [8]) who agree with our results. Also, the similarity in the PCO between obese and non-obese groups since it has been reported that only one-third of PCOS patients are obese (Toosy, et al., 2018 [9]; Glueck and Goldenberg, 2019 [10]). Basal hormones (FSH, LH and E2) were measured for the females shared in the present study because the determination of 'ovarian reserve' is important before any expensive IVF trial is undertaken. Detection of both low and high responders before starting protocol may decrease cycle cancellation rate and side effects, such as ovarian hyperstimulation syndrome (OHSS). The baseline FSH level is among the most often utilized tests for predicting success in IVF studies. Previous research found that women with an increased cycle basal FSH had a lower ovarian reserve. Several studies since then have found that women with elevated FSH levels, regardless of age, had a poor response to ovarian stimulation protocols, resulting in a reduced pregnancy rate with ART. (Xu et al., 2020 [11]). Some studies argued that young age does not protect against the negative effects of decreased ovarian reserve, implying that an elevated basal FSH level is associated with a low response, poor oocyte quality, and a rise in miscarriage rates, leading not only to a decrease in pregnancy rates but also to a rise in miscarriage rates.

(Siddiqui, et al, 2019 [12]). Other studies, however, have demonstrated that women with elevated baseline FSH levels can still achieve reasonable pregnancy rates with ART, particularly in younger women (Yang et al., 2022 [13]). It was suggested that basal serum LH could predict ovarian response to controlled ovarian hyperstimulation.

(COH) (Jiao et al., 2021 [14]). In this study, statistical analysis found no statistically significant difference between groups; this finding implies that the mean AMH ng ml was not statistically different between the non-obese and obese groups, and this indicated that the samples which were chosen to compare in the two tested groups were identical in their ovarian reserve. Also, in this study, a significant positive correlation between AMH and a number of retrieved oocytes was observed with a correlation coefficient of =0.44, indicating a moderate effect size of p=0.12, and these results were in agreement with (Neha et al., 2022 [15]), who found that AMH level was strongly associated with the number of retrieved oocyte and the availability of embryos suitable for cryopreservation. In this study, the statistical analysis indicated no statistically significant difference between the groups; this result reveals that the mean of serum chemerin did not differ significantly between the Obese and Non-obese groups; these results could be due to the fact that Chemerin has been suggested as a potential link between obesity and associated comorbidities in humans. Since chemerin was related to parameters of central fat mass and metabolic syndrome, these findings suggest an impact of chemerin on metabolic syndrome factors in obese persons (Niklowitz et al., 2019 [16]). Also, the relationship between serum chemerin and PCO has been studied by many researchers (Mansoori et al., 2021 [17]). Who's found that there is a strong correlation between serum chemerin and PCO, and in this study, there is an insignificant correlation between obese and non-obese groups regarding PCO that may be leading to the insignificant correlation of serum chemerin between obese and non-obese. The current study doesn't include the relation of this therapy on chemerin levels and overall metabolic status. There is research that found exercise training may decrease serum concentrations of chemerin in individuals who are overweight and/or obese (Ashtary-Larky et al., 2021 [18])

6- Conclusion

The present study concluded that the evaluation of serum chemerin may be a promising parameter for predicting ICSI cycle success. Serum chemerin had a significant correlation with oocyte maturity and embryo grading.

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Author Contribution

Tawadud E. Kadhim performed the study, and Muayad S. Abbood supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

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