ORIGINAL ARTICLE

AL-ANBAR MEDICAL JOURNAL
Anb. Med. J. 19(2): 155–161, 2023

Is There a Correlation between Serum SFRP5 and Wnt5a Proteins and Insulin Resistance in Iraqi Infertile Females Undergoing ICSI?

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(Received : 21 July 2023; Accepted : 10 September 2023; First published online: 24 November 2023)

ABSTRACT

Background: Secreted frizzled-related protein 5 (SFRP5) is an adipokine that inhibits the noncanonical Wingless Type (Wnt) signalling pathway. It may have anti-inflammatory and insulin-sensitizing effects, but the evidence is conflicting.
Objectives: This study examined the influence of serum and follicular fluid levels of SFRP5 and Wnt5a on intracytoplasmic sperm injection (ICSI) outcomes in infertile women, considering their relationship to insulin sensitivity.
Materials and methods: Ninety infertile women aged 18 to 45 years undergoing ICSI for various reasons were enrolled in this prospective cross-sectional study at the infertility centre of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, from September 2021 to July 2023. They received antagonist ovarian hyperstimulation treatment and had their blood fasting sugar, serum fasting insulin, serum and follicular fluid SFRP5, and serum and follicular fluid Wnt5a measured on ovum pickup. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated.
Results: Serum and follicular fluid SFRP5 levels were higher in normal-weight women (P-value < 0.001), while serum and follicular fluid Wnt5a levels were insignificantly higher in obese women (P-value > 0.05). HOMA-IR was higher in the obese group (P-value < 0.001). Insulin resistance markers were lower in pregnant women (P-value < 0.05), while serum and follicular fluid SFRP5 levels were higher in pregnant women (P-value < 0.01). Serum and follicular fluid SFRP5 levels were negatively correlated with insulin resistance markers and positively correlated with oocyte quality, quantity, fertilization rate, and embryo grade (P-value < 0.05).
Conclusion: SFRP5 and Wnt5a were detected in infertile women's serum and follicular fluid. Serum and follicular fluid SFRP5 levels were associated with lower insulin resistance, a lower body mass index, and better ICSI outcomes.
Keywords: Obesity; Secreted frizzled-related protein 5; Wingless Type Mouse Mammary tumor virus integration site; Homeostatic Model Assessment; Insulin Resistance.

DOI: 10.33091/amj.2023.141909.1273

INTRODUCTION

Obesity has been defined as an abnormal or excessive accumulation of body fat, which is a prevalent metabolic disorder. There is strong confirmation that female and male obesity increases the chance of subfecundity and infertility [1]. The global epidemic of overweight and obesity is growing and has become a serious public health concern. Obesity-related white adipose tissue disturbances are associated with developing insulin resistance (IR) and a chronic state of low-grade systemic inflammation. The secreted glycoprotein Wingless-type mouse mammary tumour virus integration site (Wnt) family plays important roles in cell differentiation and function in various tissues, including white adipose tissue [2]. Wnt signaling is essential in cell fate and differentiation, cell proliferation, cell motility, apoptosis, stem cell maintenance, beta-cell differentiation, pancre-

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http://doi.org/10.33091/amj.2023.141909.1273

atic development and function, adipogenesis regulation, IR, placental vascularization, and inflammation [3].

In mammals, 19 Wnt family members frequently overlap or have redundant roles. Wnts are generally categorized as either canonical (b-catenin dependent) or noncanonical (independent of b-catenin). Most Wnt proteins (e.g., Wnt1, Wnt3a, Wnt10b) are thought to preferably activate b-catenin-dependent pathways, whereas a few Wnts (mostly Wnt5a and Wnt11) predominantly stimulate b-catenin-independent pathways [4]. Wnt5a is a novel pro-inflammatory adipokine that can be expressed in adipose tissue, macrophages, and CD14+ monocytes. It is an especially unique Wnt protein because it establishes inflammation and the innate immune response [5]. Wnt5a ablation in animal models enhances systemic IR, while Wnt5a overexpression leads to glucose homeostasis abnormalities and adipose tissue inflammation [4]. Human data on Wnt5a serum concentrations related to obesity have been investigated in various research studies, with contradictory results [6].

SFRP5 is an anti-inflammatory and insulin-sensitizing adipokine in insulin-target tissues such as visceral and subcutaneous adipose tissue, skeletal muscle, the liver, and beta cells. It is associated with glucose metabolism and obesity. The effect of SFRP5 on insulin sensitivity in humans is less well understood and more debatable. Furthermore, the effect of obesity on circulating SFRP5 is unclear. Some studies show no differences in circulating SFRP5 between lean and obese subjects. Other studies, on the other hand, found that circulating SFRP5 concentrations increased or decreased in obese patients [2].

These inconsistencies imply that the roles of SFRP5 in the pathology of obesity are still not clear. To our knowledge, no study has yet been conducted on the relationship between serum and follicular fluid SFRP5 and serum and follicular fluid Wnt5a with intra cytoplasmic sperm injection (ICSI) outcomes in patients with different body mass indexes. Therefore, this study hypothesises whether serum and follicular fluid levels of SFRP5 and Wnt5a impact oocyte maturity, embryo quality, and biochemical pregnancy rate through their effect on insulin sensitivity.

MATERIALS AND METHODS

This is a prospective cross-sectional study performed on ninety infertile females undergoing ICSI at the infertility center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq, during the period from September 2021 until July 2023. Ethical approval of the current study was issued by the local medical ethical committee of this Institute, and written informed consent was obtained from each participant.

The sample size was calculated with this equation; (N = p (1-p)z2/me2), where P is the prevalence rate of infertile women (12%) according to a previous study [7], Z = 1.96, Me = 0.05. The sample size was 162. Seventy-two out of 162 female patients who were not eligible for the study were excluded (three empty follicles, 17 freezes in all, five poor responses, two abnormal embryos, and three atretic embryos), and 42 refused to participate. The remaining 90 female patients were categorized into three groups according to body mass index (BMI) ranking, as follows: Group 1: twenty-one (23%) normal-weighted females, Group 2: forty-two (47%) over-weighted females; and Group 3: twenty-seven (30%) obese females.

The women, ranging in age from 18 to 45 years, with various causes of infertility (tubal blockage, un-ovulatory cycle, unexplained infertility, and mild cases of male factor), were included. However, patients with low ovarian reserve, chronic diseases (such as hypertension, diabetes, and chronic renal illness), hyperprolactinemia, female patients with pelvic inflammatory disease, and all cases of endometriosis, except mild-type, as well as male partners with azoospermia, were excluded from the study. Complete medical, surgical, and obstetrical histories were obtained from the infertile couples.

The infertile women were thoroughly examined, including a general and gynaecological examination. For each participating woman, BMI is calculated by measuring the female's height and weight (BMI = weight (kg)/[height (m)]^2). The waist circumference (cm), hip circumference (cm), and waist-to-hip ratio were measured for them also.

Intracytoplasmic Sperm Injection Program

On day 2 of the spontaneous menstrual cycle, the baseline hormonal analysis [follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol E2, prolactin, and thyroid stimulating hormone (TSH)] was done. In addition, on day 2 of the menstrual cycle, transvaginal scanning was performed to assess the ovarian morphology and pathology (like polycystic ovary and ovarian cysts), antral follicle count (AFC), and ovarian accessibility. The women's BMI, age, AFC, and previous response to ovarian stimulation ICSI cycle were determined by the gonadotropin starting dose [8]. A flexible gonadotropin-releasing hormone (GnRH) antagonist regimen was used for all female participants according to the guidelines of the institute. The regimen included regular subcutaneous recombinant follicular stimulating hormone (r-FSH) (Gonaf -F®, Merk-Serono/ Switzerland). The r-FSH administration commenced on the second day of the menstrual cycle, with or without human menopausal gonadotropin (HMG) (Menogone®, Ferring, GmbH/ Germany). A transvaginal ultrasound was done on day 5 of stimulation, and subsequent scans were carried out on a two- to three-day basis as indicated.

As soon as the leading follicles reached a diameter of 13-14 mm, a daily subcutaneous injection of 0.25 mg of cetrorelix acetate (Cetrotide®, Merck Serono, Geneva, Switzerland) was administered with a multiple dose regimen, which was given daily until the trigger day. Follicular growth was tracked using E2 levels and trans-vaginal ultrasonography until the trigger day. When two to three leading follicles have an average diameter of 17-18 mm, final oocytes maturation is established by the administration of subcutaneous recombinant human chorionic gonadotropin (r-hCG) 500 μg (Ovitrelle® Merk-Serono/ Switzerland) or r-hCG 250 μg and Triptorelin 0.1 mg (Decapeptyl® Ferring / Germany) (dual triggering). Oocyte retrieval was performed under general anesthesia with transvaginal ultrasound guidance 34–36 hours after the trigger injection. The follicular fluid was immediately transported to an embryologist to collect the retrieved "cumulus-oocyte complexes (COC)". Each oocyte was carefully examined following denudation, looking for the presence or absence of the first polar body or germinal vesicle. Oocytes that had been morphologically intact and had extruded the first polar body (metaphase II) were chosen for microinjection. ICSI was performed, and about 16–18 hours after ICSI, injected oocytes were examined to confirm fertilization by the presence of two pronuclei (2PN) and 2PB.

http://doi.org/10.33091/amj.2023.141909.1273

Both maturation rate (MR) (number of mature oocytes/number of oocytes retrieved) and Fertilization rate (FR) (number of fertilized oocytes/total number of injected mature oocytes) were calculated [9]. The same embryologist performed morphological embryo grading. Based on the Istanbul consensus workshop, embryos were classified into grades I, II, and III [10]. Grade I: less than 10% fragmentation, stage-specific cell size, no evidence of multinucleation; grade II: 10–25% fragmentation, stage-specific cell size for the majority of cells, no evidence of multinucleation; and Grade III: severe fragmentation (> 25%), cell size not stage-specific, evidence of multinucleation.

Cleavage stage (day 3) embryo transfer was done for all participants using a flexible catheter (Gynetics®, Belgium) passing into the uterine cavity under trans-abdominal ultrasound guidance through the vagina without anesthesia. Luteal support was started on the day of oocyte retrieval by progesterone injection depot 250 mg twice weekly and daily vaginal progesterone (Cyclogest®400mg; Actavis, UK) or (Crinone, ® 8% progesterone gel, MERK, Switzerland). On the 14th day after embryo transfer, serum B-hCG levels were tested [11].

Collection of blood serum and follicular fluid samples and biochemical assay

On the day of oocyte retrieval, venous blood samples were drawn from the antecubital veins of all female patients for SFRP5, Wnt5a, fasting sugar (FS), and fasting insulin (FI) levels measurements. These blood samples (3 ml) were placed in a serum-separating tube (gel and clot activator) with a disposable syringe and left to coagulate for 10-20 minutes at room temperature, then centrifuged for 20 minutes at 3000 rpm. The serum was extracted, transferred to a labelled Eppendorf tube, and stored at – 20°C.

The acquired follicular fluid on the day of oocyte retrieval was used to measure SFRP5 and Wnt5a levels. After the cumulus-oocyte complex was taken out, the rest of the follicular fluid was put in a plain tube and spun at 3000 rpm for 20 minutes to separate debris and cellular contents. The resultant follicular fluid supernatant was transferred into a labelled collecting tube before being stored at – 20°C. The enzyme-linked immune assay (ELISA) technique used in the current study for the measurement of SFRP5, wnt5a, and FI levels utilized a detection kit (YL Biotech Co., Ltd., Shanghai, China) and microplate reader capable of measuring absorbance at 450 nm (Huma reader HS, Human, USA) in this study. For FS level measurement, the GOD-PAP enzymatic colorimetric test method using the glucose liquicolor kit was used. The semi-automatic microprocessor-controlled photometer (Humalyzer Primus, Human, USA) was used for FS level measurement. Insulin resistance was determined using the homeostatic model assessment of IR (HOMA-IR).

HOMA-IR = FI (IU/ml) × FS (mg/dL) /405 [12].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft Office 2010 were used to analyze the data. Descriptive statistics such as standard deviation, frequency, range, and mean were measured to describe the data. Analysis of variance ANOVA (comparison of more than two groups), independent sample *t*-test (comparison of two groups), and Chi-square (comparison of percentages and non-continuous variables) were used to compare the groups. Pearson's correlation coefficient (r) was used to calculate the degree of relationship between continuous variables, and the results were considered statistically significant when the P-value was less than 0.05.

RESULTS

The response rate (number of females who accepted to participate; 122/sample size; 162) was 75.3%. The mean female age was 32.99 ± 5.57 years, and the mean BMI was 28.13 ± 4.14, with a duration of infertility equal to 6.23 ± 4.19 years. Seventy-five females (83.3%) presented with primary infertility. The causes of infertility were distributed as follows: male factor infertility (62.2%, n = 56), polycystic ovary syndrome (15.6%, n =14), unexplained infertility (15.6%), n =14), and tubal factor infertility (6.7%, n = 6) (Table 1).

The comparison of SFRP5 and Wnt5a levels between the studied groups is demonstrated in Table 2. Serum and follicular fluid SFRP5 levels were significantly higher (P-value < 0.001) in normal-weight, females. Serum SFRP55 levels in normal weight, overweight, and obese females were 22.22 ± 5.90 versus 16.92 ± 5.43 and 14.44 ± 4.36, respectively; follicular fluid SFRP5 levels were 18.95 ± 5.26 versus 13.47 ± 4.93 and 11.44 ± 3.99, respectively. Contrary to SFRP5, serum and follicular fluid Wnt5a levels were insignificantly higher in obese females, with their lowest levels in normal-weight females (P-value > 0.05). Serum Wnt5a levels in normal weight, overweight, and obese females were 2.09 ± 1.41 versus 2.17 ± 1.29 and 2.99 ± 1.89, respectively. The follicular fluid Wnt5a levels in normal-weight females were 2.00 ± 1.58 versus 2.21 ± 1.08 in overweight females and 2.62 ± 1.68 in obese females.

IR markers were significantly lower in pregnant females (P-value < 0.05) (Table 3). However, there were significantly higher serum (P-value = 0 .002) and follicular fluid (P-value = 0.005) SFRP5 levels in pregnant females (16.52 ± 8.91 versus 11.14 ± 4.02), and (14.86 ± 8.98 versus 10.60 ± 4.59),

Table 1. Baseline characteristics of patients involved in the current study*.

Parameters		Range	Mean±SD
Age (years)		18-45	32.99±5.57
BMI (Kg/m^2)		21.05-40.68	28.13±4.14
Duration of infertility (years)		1–19	6.33±4.19
Type of infertility n(%)	Primary	75 (83.3 %)	
	Secondary	15 (16.7%)	
Cause of infertility n(%)	Male causes	56 (62.2%)	
	PCOS	14 (15.6%)	
	Tubal causes	6 (6.7%)	
	Unexplained	14 (15.6%)	
Parameters		Range	Mean±SD
Waist circumference (cm)		76–118	93.70±9.14
Waist-to-hip ratio		0.74–1.04	0.86±0.06
HOMA-IR		0.54–3.30	1.65±0.66
S. SFRP5		5.90–30.10	17.41±5.93
FF. SFRP5		5.40–27.00	14.14±5.47
S. Wnt5a		1.04–6.54	2.40±1.55
FF. Wnt5a		0.75–2.24	2.24±0.41

* SD: standard deviation; n: number of patients; PCOS: polycystic ovarian syndrome; HOMA-IR: homeostasis model analysis insulin resistance; S: serum; F.F: follicular fluid; SFRP5: secreted frizzle-related protein 5; Wnt5a: wingless-type mouse mammary tumour virus integration site 5a.

http://doi.org/10.33091/amj.2023.141909.1273

Table 2. Comparison of SFRP5 and Wnt5 levels between the studied groups*.

Parameter (Mean±SD) Range	Normal weight group n = 21	Overweight group n = 42	Obese group n = 27	P-value
Serum SFRP5	22.22±5.90 8.00–30.10	16.92±5.43 6.40–29.00	14.44±4.36 5.90–22.40	<0.001 ∀ S
Follicular fluid SFRP5	18.95±5.26 7.00–27.00	13.47±4.93 6.00–27.00	11.44±3.99 5.40–20.8	<0.001 ∀ S
FSerum Wnt5	2.09±1.41 1.08–5.98	2.17±1.29 1.04–6.42	2.99±1.89 1.04–6.54	<0.054 ∀ NS
Follicular fluid Wnt5	2.00±1.58 0.75–6.54	2.21±1.08 0.76–5.54	2.62±1.68 0.79–6.34	<0.211 ∀ NS

* SFRP5: "Secreted SFRP5 related protein 5"; Wnt5a: "wingless related integration site5a"; SD: Standard deviation; n: number; S: Significant at P-value < 0.05; NS: Non-significant at P-value > 0.05; ∀: ANOVA test.

Table 3. Comparison of insulin resistance markers between pregnant and-non pregnant females.*.

Parameter (Mean±SD)	Pregnant females n=27	Non-Pregnant females N=63	P-value
Waist circumference (cm)	90.30±7.90	95.16±9.31	0.020 ₸ S
Waist / Hip ratio	0.81±0.06	0.85±0.06	0.035 ₸ S
HOMA-IR	1.25±0.46	1.81±0.67	0.008 ₸ HS

* HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; SD: standard deviation; n: number; NS: non-significant at P-value >0.05; S: significant at P-value <0.05 ₸: independent sample *t*-test.

respectively. On the other hand, there were insignificantly (P-value > 0.05) lower serum Wnt5 levels (2.05 ± 1.53 versus 2.55 ± 1.54) and follicular fluid Wnt5 levels (1.94 ± 1.58 versus 2.36 ± 1.33), as illustrated in Table 4.

Table 4. Comparison of serum and follicular fluids SFRP5 and Wnt5 levels between pregnant and non-pregnant females*.

Parameter (Mean±SD)(ng/ml)	Pregnant females n=27	Non-Pregnant females N=63	P-value
Serum SFRP5	16.52±8.91	11.14±4.02	0.002 ₸ S
Follicular fluid SFRP5	14.86±8.98	10.60±4.59	0.005 ₸ S
Serum Wnt5	2.05±1.53	2.55±1.54	0.167 ₸ NS
Follicular fluid Wnt5	1.94±1.58	2.36±1.33	0.200 ₸ NS

* SFRP5: "secreted frizzle-related protein 5"; Wnt5a: "wingless-type mouse mammary tumour virus integration site5a"; SD: standard deviation; NS: son-significant at P-value > 0.05; S: significant at P-value < 0.05; ₸: independent sample *t*-test.

Also, this study found significant negative correlations between HOMA-IR, waist circumference, hip circumference, waist-to-hip ratio, serum, and follicular SFRP5 (P-value < 0.05). However, serum and follicular fluid of SFRP5 were significantly positively correlated to total oocyte count, metaphase II oocytes, maturation rate, fertilization rate, grade I embryos, and pregnancy rate (P-value < 0.05), as shown in Table 5.

DISCUSSION

This study looked at how insulin sensitivity affected the levels of SFRP5 and Wnt5a in the serum and follicular fluid of infertile women with different BMIs who had ICSI treatment. The study also looked at how these women's ICSI treatments turned out. The study also looked at how these women's ICSI treatments turned out. Based on what we know and what we found after a thorough search of published articles, this is the first study to look at the levels of SFRP5 and Wnt5a in serum and follicular fluid among different groups of women going through ICSI cycles in relation to IR based on their BMI and ICSI results. The points of originality in our study are the synchronous measurements of serum and follicular fluid (reflecting the oocyte microenvironment) SFRP5 and Wnt5a levels. This study shows that SFRP5 and Wnt5a were detected in infertile women's serum and follicular fluid. Serum and follicular fluid SFRP5 levels were associated with lower IR, lower BMI, and a better ICSI outcome.

In this study, a significant proportion of enrolled infertile women are either overweight or obese since they are in com-

Table 5. Correlations between HOMA-IR, SFRP5, and IR Markers Plus ICSI Outcome*.

Parameter		HOMA-IR	Serum SFRP5	F.F SFRP5
HOMA-IR	R	.....	– 0.377	– 0.385
	P-value	.....	< 0.001***	< 0.001***
Waist circumference	R	0.467	– 0.358	– 0.377
	P-value	< 0.001***	< 0.001***	< 0.001***
Hip circumference	R	0.502	– 0.298	– 0.303
	P-value	< 0.001***	0.004**	0.004**
Waist/Hip ratio	R	0.092	– 0.298	– 0.303
	P-value	0.390	0.004**	0.004**
Total oocytes count	R	– 0.051	0.272*	0.310*
	P-value	0.634	0.010	0.003
Metaphase II oocytes	r	0.047	0.216*	0.233*
	P-value	0.659	0.040	0.027
Maturation rate	r	0.049	0.289	0.054
	P-value	0.647	0.006**	0.615
Maturation rate	r	0.049	0.289	0.054
	P-value	0.647	0.006**	0.615
Fertilization rate	r	0.014	0.261	0.173
	P-value	0.895	0.013*	0.106
Grade 1 embryos	r	0.003	0.219	0.122
	P-value	0.978	0.038*	0.250
Pregnancy	r	– 0.390	0.304	0.336
	P-value	<0.001***	0.004**	0.001***

* SFRP5: secreted frizzle-related protein 5; FF: follicular fluid; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; *: Significant correlation; r: Pearsons correlation coefficient.

http://doi.org/10.33091/amj.2023.141909.1273

bination, accounting for 77%. Two suggestions can explain this finding: The first one is that in our community, being overweight or obese is common, based on data from various Iraqi reports [13]. The second possibility is the association between high BMI (overweight and obesity) and infertility in women [14].

This study found that serum and follicular fluid levels of SFRP5 were significantly higher in women with a normal BMI compared to overweight or obese women. On the other hand, Wnt5a levels, serum, and follicular fluid were lower in normal-weight women compared to overweight and obese women. Still, the difference in mean levels was statistically insignificant. This could be explained by the fact that Wnt signaling is important in regulating adipogenesis. Adipogenesis is inhibited by the canonical Wnt pathway [2]. It has been found that Wnt5a has a major correlation with obesity via the anti-Canonical Wnt and mitogen-activated protein kinase (MAPK)-independent pathway [15].

Peroxisome proliferator–activated receptor-γ (PPARγ) is a transcription factor that plays an important role in the differentiation and function of mature adipocytes and adipogenesis. Obesity has been linked to a decrease in activity and quantity of PPARγ. This association appears to be substantially linked to the etiology of obesity [16]. According to previous studies, SFRP5 act as a mature adipocyte marker, and stimulates PPARγ [15, 17].

Obesity is an excess of body fat, particularly visceral adipose tissue accumulation. It is regarded as a condition of chronic low-grade inflammation that occurs in the adipose tissue [1, 2]. It was linked to decreased concentrations of SFRP5 expression, greater concentrations of Wnt5a expression, and a spike in the ratio of proinflammatory Wnt5a to anti-inflammatory SFRP5 in wild-type mice, leptin-deficient (ob/ob) mice, and Zucker diabetic fatty mice given a high-fat, high-sugar diet for twenty-four weeks. Additionally, the expression of SFRP5 in biopsy samples taken from obese people's visceral fat was assessed. Compared to obese individuals without adipose tissue inflammation, those with adipose tissue inflammation showed reduced SFRP5 transcript expression [5].

These findings suggest that changes in the level of SFRP5 expression are linked to obesity. Also SFRP5 is negatively associated with obesity indices such as waist-to-hip ratio (WHR), BMI, and proportion of body fat in multiple studies [5, 18, 19]. Furthermore, weight loss altered Wnt5 and SFRP5 expression, resulting in elevated SFRP5 and reduced Wnt5a expression levels [5]. A large observational study of 1128 participants revealed that a low intake of fruits and vegetables and a high consumption of sugar-sweetened drinks reduced SFRP5 concentrations, indicating a dietary alteration of SFRP5 [20]. So, both proinflammatory Wnt5a and anti-inflammatory SFRP5 play critical roles in the development of obesity. Thus, it has been proposed that SFRP5 could have a protective role in the pathophysiology of adipose tissue inflammation and obesity by blocking the non-canonical Wnt5a signaling pathway [5].

There was a significant negative correlation between HOMA-IR, waist circumference, hip circumference, as well as waist - hip ratio, serum, and follicular fluid SFRP5 (P-value < 0.05). These findings are consistent with prior observations that SFRP5 may play a protective role as an anti-inflammatory marker in the progression of type 2 diabetes (T2D) [2, 21]. Further, other researchers showed in vitro that upregulating SFRP5 expression reduced the inflammatory IR state by inhibiting Wnt5a activity in 3T3-L1 adipocytes. In addition, the authors contend that decreased insulin and glucose signaling is linked to an increase in the Wnt5a to SFRP5 ratio [2]. However, additional cross-sectional investigations involving individuals with and/or without T2D discovered that circulating SFRP5 did not correlate with IR or did so in a neutral manner [22, 23].

Non-canonical Wnt5a signalling has been shown to be a major cause of inflammation and metabolic dysfunction in white adipose tissue caused by obesity. This is enough to cause IR in overfed people. It acts by activating c-Jun N-terminal kinase 1 (JNK1) in macrophages and adipocytes. When JNK1 is turned on, it stops insulin receptor substrate-1 (IRS-1) from working and makes adipocytes release more of the pro-inflammatory cytokine IL-6. As a result, insulin signaling is decreased, and IR develops [5, 18].

Additionally, it has been shown that Wnt5a secreted by adipose tissue reduces its ability to store fat due to its restricted expansion. So, ectopic accumulation of lipids in the liver and skeletal muscle occurs, which plays a crucial role in the development of IR [5]. Wnt5a has also been linked to oxidative stress and increased production of reactive oxygen species (ROS) through the potentiation of NADPH oxidase (nicotinamide adenine dinucleotide phosphate oxidase). As a result, it may increase endoplasmic reticulum (ER) stress and, in turn, induce IR [5, 19].

On the other hand, SFRP5 levels were significantly lower in obese subjects than in normal-weight subjects. It has been proposed that SFRP5 acts as an anti-inflammatory adipokine by inhibiting activated macrophage accumulation in adipose tissue via regulation of the non-canonical JNK signaling pathway, hence ameliorating glucose intolerance [18].

Thus, SFRP5 exhibits a defence mechanism evolved throughout human evolution to protect healthy adipocytes against proinflammatory wnt5a, revealing the glycoprotein's biological role in metabolic inflammation. Unfortunately, with obesity, SFRP5 expression is reduced in lipid-overloaded fat cells. At the same time, Wnt5a concentrations increase due to increased macrophages within adipose tissue, leading to an imbalance of Wnt5a/SFRP5 towards a proinflammatory phenotype and developing IR [6].

In the present study, there were significantly lower HOMA-IR levels, waist circumference, and waist/hip ratio in pregnant females compared with normal-weight women; this finding is consistent with previous studies [24, 25]. Song et al. [26] evaluated the value of HOMA-IR in women undergoing ICSI cycles. They found that clinical pregnancy rates were considerably lower in non-PCOS women with HOMA-IR levels that ranged from 2.2 to 3.15 (OR, 0.188, 95% CI, 0.084-0.42; P-value < 0.05) and at those greater than 3.15 (OR, 0.018, 95% CI, 0.004-0.081; P-value < 0.05). Indeed, the current study agrees with these later results. To explain these findings, we suggest that the higher expression of Wnt5a in visceral fat compared with subcutaneous fat [27] is the leading cause of such lower pregnancy rates in obese women with greater waist circumference, as it will lead to inflammation and metabolic dysfunction manifested as IR. Following ICSI, the oocytes of women with IR typically have a low fertilization rate, and a high percentage of the resultant embryos fail to implant, resulting in a reduced pregnancy rate [28].

The current study found a negative correlation between HOMA-IR and serum and follicular fluid SFRP5 levels. This finding can be explained as follows: high BMI and a lower pregnancy rate were associated with lower SFRP5 levels and

http://doi.org/10.33091/amj.2023.141909.1273

higher HOMA-IR, indicating that obese women with IR have lower pregnancy rates and SFRP5 levels. So, this molecule may play a role in the pathogenesis of obesity and metabolic abnormalities related to obesity through its action as an anti-inflammatory marker, as explained previously [6]. Lower levels of this marker will be associated with higher rates of obesity and its related metabolic and fertility adverse outcomes.

We also found a link between the levels of SFRP5 in serum and follicular fluid, the number of total oocytes and metaphase oocytes. This is explained at least partly by the finding that higher levels of this marker are associated with an anti-inflammatory role and reduced obesity through the reduction of adipogenesis caused by the wnt5a signaling pathway [5]. Thus, when this pathway is suppressed, all associated adverse fertility outcomes resulting from being overweight or obese will be reduced.

The authors are aware of the small sample size due to different factors. One is that cultural beliefs limit the response rate. Second, a probability of sampling bias is not uncommon because there is no documented data related to the size of the required population.

CONCLUSION

Both SFRP5 and Wnt5a glycoproteins were detected in infertile women's serum and follicular fluid. Serum and follicular fluid SFRP5 levels are negatively associated with IR and high BMI, resulting in a positive ICSI outcome.

ETHICAL DECLARATIONS

Acknoweldgements

None.

Ethics Approval and Consent to Participate

Ethical approval of the current study was issued by the Local Medical Ethical Committee of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad, Iraq (0701-PF-2021H4R on September 9, 2021). Informed consent was obtained from each participant.

Consent for Publication

Not applicable (no individual personal data included).

Availability of Data and Material

Data generated during this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that there is no conflict of interest.

Funding

No funding.

Authors' Contributions

All stated authors contributed significantly, directly, and intellectually to the work and consented it to be published.

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