

## Research article

**The impact of serum and follicular fluid secreted frizzled-related protein-5 on ICSI outcome in Iraqi infertile women with different body mass index**

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Corresponding author: **Hameedah Abdul Hussein**. Email: hameedah.mohsin@ierit.nahrainuniv.edu.iq, hameedah.abdul.h.mohsin@gmail.com**ABSTRACT**

**Introduction and Aim:** Adipose tissue adipokines and cytokines impede oocyte maturation and endometrial epithelial receptivity. Secreted frizzled-related protein 5 (Sfrp5), a novel antagonist adipokine secreted by adipocytes, may have anti-inflammatory and insulin-sensitizing actions, but data is conflicting. This study examined serum and follicular fluid Sfrp5 levels in Iraqi infertile women with varying BMIs undergoing IVF/ICSI, as well as ICSI outcomes.

**Materials and Methods:** This prospective cross-sectional study comprised 90 infertile females aged 18–45 who underwent IVF/ICSI at the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies Centre in Baghdad, Iraq. Participants received antagonist ovarian hyperstimulation. All individuals' blood fasting sugar, serum fasting insulin, serum, and follicular fluid SFRP5 were tested on oocyte retrieval day. HOMA-IR was computed. Oocyte maturity and embryo morphology were categorized. Serum B-hCG was measured 14 days after embryo transfer.

**Results:** A notable disparity was observed in the levels of serum and follicular Sfrp5, total oocyte count, mean metaphase II (MII), grade I, and pregnancy rate ( $p < 0.05$ ) among the participants categorized as normal weight, overweight, and obese. A notable disparity was observed in the average germinal vesicles (GV), grade III (GIII) embryos, and HOMA-IR ( $p < 0.05$ ) among patients with varying body mass index (BMI) values. The study found a positive correlation between the levels of Sfrp5 in both serum and follicular fluid and the total count of oocytes, MII oocytes, grade I embryos, and pregnancy rate. However, there was a negative correlation between Sfrp5 levels and HOMA-IR, with statistical significance at  $p < 0.05$ .

**Conclusion:** Increased BMI is associated with lower serum and follicular fluid Sfrp5, reduced total oocyte count, reduced metaphase II oocyte count, more germinal vesicle oocytes, reduced embryo quality and lower pregnancy rate. Serum and follicular fluid Sfrp5 levels are negatively correlated with HOMA-IR but positively associated with ICSI outcome.

**Keywords:** Obesity; BMI; Sfrp5; ICSI; HOMA-IR.

**INTRODUCTION**

Obesity is a metabolic condition characterized by abnormal fat buildup. Overweight, obese, and severely obese individuals are defined by the CDC and WHO as having a body mass index (BMI) of 25 kg/m<sup>2</sup>, 30 kg/m<sup>2</sup>, or 40 kg/m<sup>2</sup>. BMI can be used to estimate the healthy weight (1, 2). Obesity causes metabolic diseases, cardiovascular events, cancers, gastrointestinal disorders, and arthritis. In addition to the cardio-metabolic implications associated with obesity, substantial evidence supports the notion that both male and female obesity significantly elevate the risk of subfecundity and infertility (3,4). The presence of metabolic and reproductive abnormalities in individuals with obesity can lead to an adverse follicular fluid (FF) microenvironment. This unfavorable FF microenvironment can have detrimental effects on fertilization, potentially leading to infertility. It can impair the processes of ovulation and oocyte maturation, resulting in decreased quality of

oocytes. Additionally, it can negatively impact pregnancy outcomes (5).

Maintaining glucose homeostasis, synthesizing hormones, controlling inflammation, and promoting reproduction are just a few of the many important physiological roles played by adipose tissue, a highly dynamic endocrine organ. The multifaceted function of adipose tissue primarily stems from its capacity to produce and secrete numerous adipokines. Adipokines possess the ability to exert their effects both in a localized manner and as signaling molecules in distant organs. Hence, the release of adipokines from adipose tissue may be responsible for various aspects of disorders associated with obesity. The induction of the release of pro-inflammatory and immune-related molecules from adipose tissue leads to the emergence of a state characterized by low-grade chronic inflammation. This condition has been found to be associated with a decrease in fecundability (6). Moreover, alterations in the concentrations of adipokines in both the bloodstream and follicular fluid

(FF) during obesity have been found to be associated with infertility and a reduction in the efficacy of in vitro fertilization (IVF) (7). The Wnt signaling pathway, more specifically the wingless-type mouse mammary tumor virus integration site family member (Wnt), has been shown to have a vital role in a variety of cellular activities, including cell proliferation, differentiation, motility, and development, according to previous research that has been conducted in this area. Additionally, it has been suggested that this route is involved in beta-cell differentiation, pancreatic development, and function, as well as the regulation of adipogenesis, insulin resistance (IR), placental vascularization, and inflammation. All of these processes take place in the pancreas (8). Limited research has demonstrated that secreted frizzled-related protein 5 (Sfrp5), an emerging adipokine that acts as an antagonist of the Wnt signaling pathway, is secreted by adipocytes. Sfrp5 belongs to a family of five known members known as Sfrp. The Sfrp5 protein has been found to play a role in the regulation of lipid metabolism, exerting a negative influence on adipogenesis, insulin resistance, and metabolic dysfunction. Additionally, it functioned as an adipokine with anti-inflammatory properties and exerted regulatory effects on metabolic processes. The effects of Sfrp5 were observed to be mediated through the sequestration of Wnt proteins in the extracellular compartment, thereby inhibiting the binding of Wnt proteins to their respective receptors (9). Obesity, insulin resistance (IR), and secreted frizzled-related protein 5 (Sfrp5) have only been studied in a small number of human and animal research. These investigations have shown contradictory results, with some showing increased levels of Sfrp5 in the setting of obesity and insulin resistance and others showing decreased levels of Sfrp5 (10). Serum and FF Sfrp5 levels may predict intracytoplasmic sperm injection (ICSI) success, although studies examining this link in patients of varied BMIs are lacking. Therefore, the purpose of this study was to analyze the relationship between secreted frizzled-related protein 5 (Sfrp5) levels in serum and follicular fluid (FF) and the success of intracytoplasmic sperm injection (ICSI) in men of varied BMIs.

## **MATERIALS AND METHODS**

The research study conducted by the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University received ethical approval from the Human Research Ethics Committee (September, 2021). Furthermore, prior to their participation, each participant provided written informed consent. Women who underwent in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at Al-Nahrain University's High Institute for Infertility Diagnosis and Assisted Reproductive Technologies between September 2021 and July 2023 constituted the study cohort. Patients with poor ovarian

reserve, hyperprolactinemia, chronic disease like diabetes, hypertension, chronic renal disease, pelvic inflammatory disease, endometriosis and a male partner with azoospermia, were excluded from the study. Full history (medical, surgical, and obstetrical) was obtained from infertile couples and infertile women. Patients were completely examined, including general and gynecological examination. Assessment of height & weight was used to obtain BMI calculated as BMI = weight (kg)/height (m<sup>2</sup>).

## **Intracytoplasmic sperm injection program**

At day 2 of the menstrual cycle, a female participant's baseline hormonal analysis (FSH, LH, E2, Prolactin, and TSH) was measured using the enzyme-linked fluorescence assay technique (ELFA) using the Mini vids analytical equipment (BIOMRIEUX/Italy). All the patients went through controlled ovarian hyperstimulation with the help of a flexible protocol and a Gonadotropin Releasing Hormone (GnRH) antagonist. This was done in accordance with the institute's recommendations. On the fifth day of stimulation, a trans-vaginal ultrasound was performed, and further scans were performed at intervals of two to three days. The evaluation of E2 levels and trans-vaginal ultrasound scans were used up until the day of the trigger to monitor the growth of the follicles in preparation for the trigger. Within a timeframe of 34-36 hours after the administration of the trigger injection, the transvaginal ultrasound-guided aspiration that was used to retrieve the oocytes was performed. This was done before the occurrence of ovulation that occurs in a physiologically normal cycle. All follicles inside each ovary were recovered by a single lumen needle (Gynetics®, Belgium). Then, the follicular fluid (FF) was immediately sent to an embryology specialist to gather the retrieved cumulus-oocytes complexes (COC).

Categorization of oocytes with respect to its level of maturity was carried out following the denudation of its corona layers and cumulus. Nuclear maturity in oocytes was assessed by looking for the absence of a germinal vesicle (GV) and the presence of an extruded first polar body (PB I) in the perivitelline region. In this study, intracytoplasmic sperm injection (ICSI) was conducted on seemingly normal, fully developed metaphase II (MII) oocytes. The procedure took place approximately 16-18 hours following ICSI. The oocytes that were subjected to injection were analyzed to verify fertilization, which was determined by the presence of two pronuclei (2PN) and two polar bodies (2PB). The determination of embryo morphology in the advanced stage was conducted by assessing the quantity of blastomeres, the extent of fragmentation (categorized as follows: ≤10% = score 0; 11–20% = score 1; 21–30% = score 2; >30% = score 3), and the symmetry of blastomeres (scored as equal = score 1, different = score 2) (11). The number of embryos selected for transfer was determined by the patient's

age, quality of embryo and previous ICSI cycles history and embryo transfer was done. Luteal support was done by progesterone injection depot 250 mg twice weekly and vaginal progesterone (Cyclogest®400mg; Actavis, UK), or (Crinone, ® 8% progesterone gel, MERK, Switzerland), and daily continued. The supplementation was started on the day of oocyte retrieval. Assay of serum B-hCG was performed on day 14 following transfer of embryo (12).

### Blood serum and follicle fluid collection and biochemical analysis

The research conducted encompassed the collection of venous blood samples from the antecubital veins of patients for the purpose of quantifying the concentrations of Sfrp5, fasting sugar (FS), and fasting insulin (FI). The collection of blood samples took place concurrently with the oocyte retrieval procedure. The quantification of Sfrp5 and FI concentrations was performed utilizing the enzyme-linked immunosorbent assay (ELISA) methodology. For this purpose, a kit provided by YL Biotech Co., Ltd., situated in Shanghai, China, was employed. The experimental procedure adhered to the instructions provided by the company. The assessment of insulin resistance was conducted using the homeostatic model assessment of insulin resistance (HOMA-IR), which involved the application of a specific formula for its computation. The formula used for calculating HOMA-IR is as follows:  $HOMA-IR = FI (\mu IU/ml) \times FS(mg/dL) / 405$  (13).

### Statistical analysis

The information was analyzed using SPSS 23.0 and Microsoft Office 2010. Numbers were broken down into ranges and averages and standard deviations. The groups were compared using an ANOVA, a t test, and a chi-square test. The level of association between continuous variables was calculated using Pearson's r, and significance levels of p less than 0.05 were accepted.

## RESULTS

A total of 30 out of 120 female patients who did not meet the eligibility criteria were excluded from the study. The reasons for their exclusion were as follows: three patients had empty follicles, 17 patients were placed in the freeze-all protocol, five patients exhibited poor responses, and three patients had abnormal or atretic embryos. Body mass index (BMI) rankings were used to divide the remaining 90 female patients into three groups. Group 1 consisted of 21 females (23%) who were classified as having a normal weight. Group 2 included 42 females (47%) who were classified as being overweight. Group 3 comprised 27 females (30%) who were classified as being obese. The average age of the female participants was  $32.99 \pm 5.57$  years, while the average BMI was  $28.13 \pm 4.14$ . The duration of infertility for these participants was found to be  $6.23 \pm 4.19$  years. Eighty-three percent of the women in the study had primary infertility, while sixteen percent had

secondary infertility. These are the most common reasons for infertility: Infertility was caused by male factors in 62.2% of cases (n = 56), polycystic ovarian syndrome in 15.6% (n = 14), unexplained infertility in 15.6% (n = 14), and tubal factor infertility in 6.7% (n = 6) of cases.

Table 1 displays the comparative analysis of demographic data across the three examined cohorts, namely normal weight, overweight, and obese females. There were no statistically significant differences observed in the mean age of females ( $31.48 \pm 4.56$  versus  $32.93 \pm 5.02$  and  $34.22 \pm 6.87$ ) with a p-value of 0.240. Similarly, the p-value for the comparison of infertility durations was 0.768, finding no significant differences between the three groups ( $5.97$   $4.49$  vs.  $6.61$   $2.63$  and  $6.32$   $4.84$ ). The prevalence of primary infertility among females was 90.5%, 85.7%, and 74.1% in the respective groups. Conversely, the prevalence of secondary infertility among females was 9.5%, 14.3%, and 25.9% in the respective groups. While there were some noticeable differences, none of them were statistically significant (p=0.271). In addition, there was no discernible difference in infertility causes amongst the three groups studied (p=0.279).

Serum and follicular fluid (FF) Sfrp5 levels on the day of oocyte retrieval were significantly different between groups in relation to laboratory and clinical factors. The serum Sfrp5 levels were found to be  $522.22 \pm 5.90$  in normal weight females compared to  $16.92 \pm 5.43$  and  $14.44 \pm 4.36$  in overweight and obese females, respectively. Similarly, the FF Sfrp5 levels were  $18.95 \pm 5.26$  in normal weight females compared to  $13.47 \pm 4.93$  and  $11.44 \pm 3.99$  in overweight and obese females, respectively. It was determined that there was a statistically significant difference between these two groups with a p-value that was lower than 0.001. Notably, the levels of Sfrp5 were found to be higher in females who were of normal weight compared to those who were overweight or obese. There were statistically significant differences observed in the total count of oocytes (p=0.023), mean metaphase II (MII) (p=0.012), and grade I (p=0.01) among the three groups of patients. These differences indicated that the counts and quality of oocytes were higher in normal weight females compared to overweight and obese females. Moreover, notable disparities were observed in the average number of germinal vesicles (GV) (p=0.01), grade III (GIII) embryos (p=0.01), and HOMA-IR (p<0.001) across the three cohorts of patients (Tables 2, 3, 4 respectively).

Out of a sample of ninety women, twenty-seven experienced pregnancies, resulting in an overall pregnancy rate of 30%. An analysis of pregnancy rates categorized by BMI indicated that females with a normal weight exhibited a higher pregnancy rate (44.4%) in comparison to obese females (18.6%) and overweight females (37.0%; Table 5). The results of the

correlation analysis indicate a statistically significant negative correlation between serum and FF Sfrp5 and HOMA-IR, as well as a statistically significant positive

correlation with total oocyte count, MII oocytes, grade I embryos, and pregnancy rate ( $p < 0.05$ ), as presented in Table 6.

**Table 1:** Comparing the demographic characteristics of the examined groups

Parameter	Groups based on BMI			p value
	Normal (n=21)	Overweight (n=42)	Obese (n=27)	
Age (years)	31.48 ± 4.56	32.93 ± 5.02	34.22 ± 6.87	0.240
	24 - 40	23 - 45	18 - 40	NS
BMI (kg/m <sup>2</sup> )	23.13 ± 1.34	27.52 ± 1.47	32.96 ± 2.98	< 0.001
	21.05 – 24.91	25.00 – 29.97	30.00 – 40.68	HS
Duration of infertility (years)	5.97 ± 4.49	6.61 ± 3.63	6.32 ± 4.84	0.768
	1-19	1.5- 16	1.5-19	NS
<b>Type of infertility n (%)</b>				
Primary	19 (90.5 %)	36 (85.7 %)	20 (74.1 %)	0.271
Secondary	2 (9.5 %)	6 (14.3 %)	7 (25.9 %)	NS
<b>Causes of infertility n (%)</b>				
Male causes	15 (71.4 %)	27 (64.3 %)	14 (51.9 %)	0.279 NS
PCOS	2 (9.5 %)	9 (21.4 %)	3 (11.1 %)	
Tubal causes	2 (9.5 %)	1 (2.4 %)	3 (11.1 %)	
Unexplained infertility	2 (9.5 %)	5 (11.9 %)	7 (25.9 %)	

Values given as Mean ± SD, n: number; BMI: body mass index; PCOS: poly cystic ovarian syndrome; SD: standard deviation; HS: highly significance at  $p \leq 0.01$  NS: non-significant at  $p > 0.05$ ;

**Table 2:** Evaluation of insulin resistance using the homeostatic model and SFRP5

Parameter	Groups based on BMI			p value
	Normal (n=21)	Overweight (n=42)	Obese (n=27)	
Serum SFRP5	22.22 ± 5.90	16.92 ± 5.43	14.44 ± 4.36	< 0.001
	8.00 – 30.10	6.40 - 29.00	5.90 – 22.40	S
Follicular fluid SFRP5	18.95 ± 5.26	13.47 ± 4.93	11.44 ± 3.99	< 0.001
	7.00 - 27.00	6.00 – 27.00	5.40 – 20.80	S
HOMA-IR	1.15 ± 0.39	1.47 ± 0.49	2.30 ± 0.56	< 0.001
	0.54 – 1.90	0.80 – 2.83	1.26– 3.30	HS

Values given as Mean ± SD

**Table 3:** Comparison of oocytes characteristics, maturation rate and fertilization rate among the studied groups

Parameter	Groups based on BMI			p value
	Normal (n=21)	Overweight (n=42)	Obese (n=27)	
Total oocytes count	12.33 ± 2.98	10.79 ± 3.14	10.00 ± 2.28	0.023
	7 -18	6-16	7-15	S
Metaphase II (MII)	8.05 ± 2.53	6.38 ± 2.38	6.11 ± 2.13	0.012
	2-11	1-10	1-9	S
Metaphase I (MI)	1.14 ± 0.96	1.33 ± 0.97	1.48 ± 1.01	0.50
	0-3	0-3	0-4	NS
Germinal vesicle (GV)	0.81 ± 0.68	1.36 ± 1.03	1.63 ± 0.88	0.01
	0-2	0-3	0-3	S

Values given as Mean ± SD; BMI: Body mass index; n: number; S: Significant at  $p \leq 0.05$  chi square test

**Table 4:** Comparison of embryos characteristics among the study groups

Parameter	Groups based on BMI			p value
	Normal (n=21)	Overweight (n=42)	Obese (n=27)	
Grade I embryos	4.14 ± 1.76	2.85 ± 1.93	2.66 ± 1.98	<b>0.01</b>
	0-8	0-7	0-7	<b>S</b>
Grade II embryos	2.09 ± 1.26	1.73 ± 1.12	1.48 ± 0.80	0.15
	0-4	0-4	0-3	NS
Grade III embryos	1.0 ± 0.77	1.28 ± 0.89	1.74 ± 0.85	<b>0.01</b>
	0-2	0-3	0-3	<b>S</b>
Transferred embryos	2.76 ± 0.88	2.43 ± 0.83	2.59 ± 0.97	0.36
	1-4	1-4	1-4	NS

Values given as Mean ± SD; n: number; NS: non-significant at p > 0.05; S: significant at p ≤ 0.05

**Table 5:** Pregnancy rates according to body mass indexes ranking

BMI ranking	Positive pregnancy N= 27 (%)	Negative pregnancy n =63 (%)	p value
Normal weight	12 (44.4 %)	10 (15.9 %)	<b>0.030</b> <b>S</b>
Overweight	10 (37.0 %)	31 (49.2 %)	
Obese	5 (18.6 %)	22 (34.9 %)	

**Table 6:** Correlations between HOMA-IR, Sfrp5 with ICSI outcome

Parameters		HOMA-IR	Serum Sfrp5	F.F Sfrp5
HOMA-IR	r	----	-0.377	-0.385
	p value	----	< <b>0.001***</b>	< <b>0.001***</b>
Total oocytes count	r	-0.051	<b>0.272*</b>	<b>0.310*</b>
	p value	0.634	0.010	0.003
Metaphase II oocytes	r	0.047	<b>0.216*</b>	<b>0.233*</b>
	p value	0.659	0.040	0.027
GV oocyte	r	0.206	-0.133	-0.138
	p value	0.052	0.210	0.195
Grade 1 embryos	r	0.003	0.219	0.122
	p value	0.978	<b>0.038*</b>	0.250
Pregnancy	r	-0.390	0.304	0.336
	p value	< <b>0.001***</b>	<b>0.004**</b>	<b>0.001***</b>

Sfrp5: secreted frizzled related protein 5; FF: follicular fluid; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; \*: Significant correlation; r: Pearson’s correlation coefficient

**DISCUSSION**

This is the first investigation into secreted frizzled related protein 5 (Sfrp5) in Iraqi women with varying BMIs who were treated for infertility with in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Patients' BMIs were found to have a significant link with their rates of infertility in this investigation. The results showed that overweight and obesity were significantly more common in female individuals than in male ones. Obesity, as shown by prior study, is associated with reduced success rates in assisted reproduction programs (5). Patients who are overweight or obese have poorer oocyte quality, a lower preimplantation rate, and a less receptive uterus, all of which contribute to unsuccessful in vitro fertilization (IVF) results (4). However, the issue at stake is still contentious, calling for more research to reach an agreement among fertility healthcare

providers (14). In contrast to women who were overweight or obese, women who were classified as having a normal weight were found to have higher amounts of Sfrp5 in their serum and follicular fluid. This observation raises the possibility that changes in serum and follicular fluid Sfrp5 levels are related to body mass index. Women with a normal body mass index (BMI) had higher levels of Sfrp5 in serum and follicular fluid (FF) than women with an overweight or obese BMI, according to the current study.

Obesity is typified by the accumulation of excessive adipose tissue and the presence of persistent low-level inflammation. The Sfrp5 and Wnt5 pathway has been hypothesized to play a role in the development of obesity and its related diseases (9). The canonical Wnt signaling pathway primarily regulates adipogenesis, which is a key factor in the expansion of adipose tissue due to excessive caloric intake. Aberrant activation of

the Wnt signaling pathway, on the other hand, may be harmful and contribute to the onset of several chronic disorders, including obesity (10). The anti-inflammatory adipokine Sfrp5 may prevent the accumulation of activated macrophages in adipose tissue by modulating the JNK signaling cascade in a non-canonical manner (15). Therefore, it can be inferred that Sfrp5 exhibits a distinct mechanism of defense that has emerged over the course of human evolution. This mechanism serves to safeguard the well-being of adipocytes by shielding them from the detrimental effects of pro-inflammatory Wnt5a. Consequently, these findings underscore the biological significance of Sfrp5 in the context of metabolic inflammation. Regrettably, in the context of obesity, there is a decrease in the expression of Sfrp5 in adipocytes that are overloaded with lipids. Simultaneously, there is an increase in the concentrations of Wnt5a, which can be attributed to the higher presence of macrophages within adipose tissue. This imbalance in the Wnt5a/Sfrp5 ratio leads to the promotion of a proinflammatory phenotype (9,16).

Numerous clinical studies have examined Sfrp5 and Wnt5a's roles in obesity and their release in a weight-gaining setting. In this work, obese, leptin-deficient (ob/ob) mice and wild-type mice fed a high-fat/high-sucrose diet for 24 weeks showed decreased Sfrp5 expression and increased Wnt5a expression. These mice had a greater Wnt5a-Sfrp5 ratio (9). Furthermore, it was observed that in biopsy samples of visceral fat from obese individuals, the presence of tissue inflammation, as indicated by the presence of crown-like structures (CLSs), was correlated with decreased expression of Sfrp5 in comparison to obese individuals who did not exhibit CLSs. Collectively, these findings indicate a correlation between the regulation of Sfrp5 and obesity. Similarly, Sfrp5 has been shown to have a negative correlation with several obesity markers in cross-sectional studies. These variables include body mass index (BMI), waist-to-hip ratio (WHR), body fat percentage, glycolipid metabolism, and inflammation (15). In a comprehensive observational study involving a sample size of 1128 participants, an investigation was conducted to assess the dietary patterns of the volunteers. The results of this study showed that people who drank a lot of sugary drinks but ate little fruits and vegetables had lower levels of the protein Sfrp5. This result hints at the possibility that dietary changes can affect Sfrp5 levels (9). While there is ample evidence indicating the downregulation of Sfrp5 in obesity, it is important to note that several studies have reported contradictory findings (9). According to the report, there were no discernible differences observed between individuals classified as lean and those classified as obese. Conversely, certain studies have demonstrated that levels of circulating SFRP5 were found to be either elevated or decreased

in individuals with obesity (10). Additional investigation is necessary to elucidate the regulatory mechanisms through which these factors are influenced by excessive adiposity and how they are altered in response to changes in body mass (9).

This study found that obese females demonstrated elevated HOMA-IR levels, which were significantly different from those observed in females with normal weight. According to the findings of Yang *et al.* (17), In a study involving 3,615 women who had IVF/ICSI cycles, researchers found a significant link between the average body mass index (BMI) and the average homeostatic model assessment of insulin resistance (HOMA-IR). Research has demonstrated that the noncanonical Wnt5a signaling pathway plays a crucial role in the inflammation of white adipose tissue and metabolic dysfunction caused by obesity. Additionally, it has been observed that this signaling pathway alone is capable of inducing insulin resistance in situations of excessive nutrient intake. The activation of c-Jun N-terminal kinase 1 (JNK1) is the mechanism of action, and it occurs in macrophages as well as adipocytes. The activation of JNK1 in adipocytes inhibits the function of insulin receptor substrate-1 (IRS-1) and stimulates the production of proinflammatory cytokines, resulting in impaired insulin signaling and the onset of insulin resistance (18). Moreover, previous studies have demonstrated that the secretion of Wnt5a by adipose tissue leads to a decrease in the storage capacity of adipose tissue, primarily because of restricted expansion. Therefore, this ultimately results in the accumulation of ectopic lipids in the liver and skeletal muscle, a critical factor in the development of insulin resistance (9). Furthermore, previous studies have established a correlation between Wnt5a and oxidative stress, as well as an elevation in the production of reactive oxygen species (ROS) (18). This phenomenon has the potential to exacerbate endoplasmic reticulum (ER) stress and insulin resistance (IR).

The findings of our study align with prior research (19, 20) regarding the decrease in average total oocyte count and average mature oocytes. However, our results contradict the findings of Bahgat (21). The decrease in oocyte quality may be attributed to the detrimental impact of impaired systemic maternal endocrinology and metabolism, which can manifest as increased levels of glucose, insulin, or free fatty acids, as well as alterations in adipokine levels. These factors can contribute to cellular damage and the development of a persistent, low-level inflammatory condition (22). Broughton and Moley (23) discovered that obesity can lead to impaired meiotic spindle formation and mitochondrial dynamics, as well as induce endoplasmic reticulum (ER) stress. Wnt5a has been linked to oxidative stress. Wnt5a may directly cause ROS production by boosting nicotinamide adenine dinucleotide phosphate oxidase activity and

decreasing nitric oxide bioavailability (18). Additionally, the involvement of Wnt5a in the suppression of the canonical signaling pathway and the induction of the non-canonical pathway, along with a decrease in factors that promote cellular well-being, such as IGF1, should be considered. Therefore, it is plausible that this phenomenon could potentially play a role in the induction of granulosa cell apoptosis and subsequent follicle regression (24). The observed findings can be plausibly explained by the sequestration of Wnt5a by Sfp5, which may lead to a reduction in oxidative stress. Consequently, this could result in an overall increase in the counts of total oocytes and MII oocytes. A notable disparity was observed between obese and overweight women, as compared to women of normal weight, in terms of the distribution of grade I (GI) and grade III embryos. Specifically, a higher proportion of grade III embryos and a lower proportion of grade I embryos were observed in the former group. Based on the research conducted by Liu et al. (25), it was observed that there was a notable decrease in the quantity of high-quality embryos in individuals with a higher body mass index (BMI). Nevertheless, García-Ferreira *et al.*, (26) did not find any noteworthy differences in embryo quality in their investigation of the correlation between BMI and the outcome of intracytoplasmic sperm injection (ICSI). One possible explanation for the observed disparity in embryo quality is that obese female patients may possess inherited genomic instability, which could be attributed to telomere attrition. This phenomenon arises from heightened oxidative stress on oocytes. In the context of the cleavage stage embryo, it can be hypothesized that inadequate telomere reconstitution would likely result in anaphase lag, mosaicism, and copy number variants. These factors, in turn, would contribute to a slower and suboptimal development of the embryos (27). Moreover, the rationale can be extended to elucidate the correlation between wnt5a and Sfrp5 in the context of oocyte quality and subsequently, embryo quality.

They observed that overweight and obese women exhibited a significantly reduced pregnancy rate when compared to women with a normal weight. This observation exhibits similarities to the findings reported by Emre et al. (28). García-Ferreira *et al.*, (26) agree that obesity and excessive weight negatively affect pregnancy, implantation, and live birth rates in women undergoing in vitro fertilization (IVF). Prior research on overweight or obese females who underwent in vitro fertilization (IVF) did not show lower pregnancy rates (PR) than females with normal weight (29). Obesity is a systemic and tissue-specific condition that contributes to an increase in the synthesis of free fatty acids, leptin, cytokines, and unregulated secretion of adipokines. All these may negatively impact oocyte maturation and endometrial receptivity. Indeed, obesity is known to have a deleterious impact on oocyte quality via mitochondrial

dysfunction, lipotoxicity, and apoptotic responses (28,29). In view of our previous opinion regarding the interaction between wnt5a and sfrp5 in determining oocyte quality one can suggest that these 2 markers might affect pregnancy in overweight and obese patients. In our opinion, the dysregulation of adipokine (Sfrp5, Wnt5) in obesity may negatively impact on the quality of oocytes, embryo quality and pregnancy rate through lipotoxicity, mitochondrial dysfunction, and apoptosis response due to chronic inflammation and oxidative stress.

We observed an inverse relationship between HOMA-IR and both serum and follicular Sfrp5 levels in the present investigation. The following factors may account for this result: 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 above, and lower levels of this marker will be associated with higher rates of obesity and its related metabolic abnormalities in women with a high body mass index and a low pregnancy rate. Total oocyte and MII oocyte counts, as well as quality embryos and pregnancy rate, were positively correlated with serum and FF levels of Sfrp5. This can be partially explained by the fact that increased levels of this marker are linked to fewer health problems related to obesity and overweight because the wnt5a signaling pathway is suppressed, which has an anti-inflammatory effect and reduces adipogenesis.

After thorough search in the available published article, we did not find a study that is similar to our study in estimating serum and FF levels of Sfrp5 and Wnt5a among various groups of women undergoing ICSI cycles based on their corresponding BMI. This study is the first to examine the levels of Sfrp5 in both serum and follicular fluid (FF) among different groups of women undergoing in vitro fertilization (IVF) cycles using intracytoplasmic sperm injection (ICSI). The investigation specifically focuses on the relationship between Sfrp5 levels and the body mass index (BMI) of these women, as well as the outcomes of their IVF procedures. The key aspects of novelty in our research encompass the simultaneous assessment of serum and FF (representing the oocyte microenvironment) Sfrp5 levels, the investigation of correlations between Sfrp5 and other parameters, and the evaluation of ICSI outcomes across different cohorts of women undergoing ICSI cycles.

## CONCLUSION

Higher BMI is associated with lower serum and follicular fluid Sfrp5, reduced total oocyte count, reduced metaphase II oocyte count, more germinal vesicle oocytes, reduced embryo quality and lower pregnancy rate. Serum and follicular fluid Sfrp5 levels are negatively correlated to HOMA-IR but positively associated with ICSI outcome.

**CONFLICT OF INTEREST**

The authors have no conflicts of interest.

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