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Evaluation of SFRP5 Role in Relevance to BMI in Prediction of Pregnancy in a Sample of Iraqi Infertile Females Undergoing ICSI

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ABSTRACT

There is a relation between adipose tissue and macrophages. These macrophage cells influence adipose tissue by releasing many bioactive chemicals, including the wingless-type MMTV integration site family 5a (wnt5a) glycopeptides and the more traditional cytokines. Healthy adipocytes can release the wnt5a inhibitor Secreted frizzled-related protein 5(SFRP5). However, this protective effect was found to be reduced in obesity. This study aimed to assess the effect of follicular and serum levels of SFRP5 as well as serum and follicular levels of wnt5a on ICSI outcome and determine the potential roles of SFRP5 as a predictor of positive pregnancy in Iraqi infertile females undergoing ICSI with different body mass index .Ninety infertile females who received intracytoplasmic sperm injection and had various infertility factors between 18 and 45 years participated in this prospective clinical cross-sectional study. Follicular fluid, serum SFRP5, and serum and follicular fluid wnt5a levels were measured for all participants on the day of ovum pickup. In addition, ICSI outcomes were determined. There was a significant difference in the mean total gonadotrophin dose used among the three studied groups (p = 0.023), higher in overweight and obese females than normal-weight females. There was a positive significant correlation between serum plus follicular fluid wnt5a and total gonadotropin dose. Serum and follicular fluid SFRP5 levels were significantly higher in normal-weight females. There were higher serum (p-value=0.002) and follicular fluids (p-value=0.005) SFRP5 levels in pregnant females. Both SFRP5 and wnt5a glycoproteins were detected in infertile women's serum and follicular fluid. Serum and follicular fluid secreted frizzled-related protein 5 level positively associated with ICSI outcome. The cutoff values for serum and follicular fluid SFRP5 were ≥15.86 (ng/ml) and ≥ 11.3(ng/ml), respectively, as predictors of positive pregnancy in women undergoing IVF/ICSI cycles.

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KEYWORD Obesity, BMI, SFRP5, wnt5a, ICSI Outcome

1. Introduction

There has been a growing epidemic of obesity worldwide, which has given rise to other serious public health concerns. In the preceding decades, the prevalence of overweight and obesity has reached alarming levels. Nearly 40% of people worldwide can be labeled obese or overweight (Wang H et al.,2023[1]). We can use body mass index (BMI) to determine a person's optimal weight and obesity level. Adipose tissue, the primary energy storage and transport organ, controls energy balance; when the body stores too much energy as fat, this is called obesity. Obesity is directly hazardous to health and is closely linked to various chronic diseases like cardiovascular disease, type 2 diabetes, respiratory disease, and reproductive abnormalities. Obesity decreases fertility; women with a BMI of more than 30 kg/m2 had a 2.7-fold increased risk of infertility. (Wang H et al.,2023[1]& Al-yasiry RZ et al., 2022[2]).

Indeed, obesity is a tissue-specific and systemic disorder that can cause oxidative stress and chronic inflammation that produces an unregulated and persistent synthesis and secretion of cytokines and chemokines by innate immune cells and adipokines from adipocytes (García-Ferreyra J et al.,2021[3]). Secreted frizzle-related protein-5 (SFRP5) is a novel antagonist adipokine of the wingless-type mouse mammary tumour virus integration site family (Wnt) signalling pathway and one of the five members of the SFRP family. It regulated lipid metabolism, negatively regulated adipogenesis, reduced insulin resistance (IR) and decreased metabolic dysfunction. It was secreted by adipocytes and acted as an anti-inflammatory adipokine that modulated metabolic functions (Inal ZO et al.,2018[4]& Mohsin HAH et al.,2023[5]). Human and animal

studies on the association between obesity and SFRP5 are limited in number and conflicting results (Rydzewska M. et al.,2021[6]).

Increasing evidence from rodent and cellular models shows that wnt-signaling regulates adipose tissue growth and inflammation, thereby representing a possible prevention target for obesity-related comorbidities. In this regard, it has been demonstrated that adipose tissue macrophages release the glycoprotein wnt5a, which modulates the innate immune response and promotes low-grade inflammation. Human data on wnt5a serum concentrations about obesity have been explored in smaller cohorts in numerous studies, with contradictory results. While some research revealed that wnt5a levels were higher in obese people, others could not confirm these findings (Relling I. et al.,2018[7]).

According to the best knowledge, no studies have studied the association between serum and follicular fluid SFRP5 and serum and follicular fluid wnt5a and IVF/ICSI outcomes in patients with different BMI. Therefore, the aims of this study were (i)to assess the effect of serum and follicular levels of SFRP5 as well as serum and follicular levels of wnt5a on IVF/ ICSI outcomes with different BMI, (ii) to determine the potential roles of SFRP5 as a predictor of positive pregnancy in Iraqi infertile females undergo IVF/ ICSI with different BMI.

2. Patients and Methods

The Ethics Committee on Human Research of the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, and written informed consent was obtained from each participant. The study group consisted of 90 women who underwent IVF/ICSI cycles at the "High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University" between

September 2021 and July 2023. Patients with poor ovarian reserve, hyperprolactinemia, chronic diseases like diabetes, hypertension, chronic renal disease, male partners with azoospermia, female patients with pelvic inflammatory disease, and all cases of endometrioses, except mild ones were excluded. Full history (medical, surgical, and obstetrical) was obtained from infertile couples, and infertile women were examined entirely, including general and gynecological examinations. The height and weight to obtain BMI (BMI = weight (kg) / [height (m)]2) (Al Hourani HM et al.,[8]) were determined for each participant woman.

Intracytoplasmic Sperm Injection Program :

The baseline hormonal analysis (FSH, LH, E2, Prolactin, TSH) was assessed for female participants on day 2 of the menstrual cycle by enzyme-linked fluorescent assay technique (ELFA), using Mini vids analysis equipment (BIOMRIEUX/ Italy). Controlled Ovarian Hyperstimulation with flexible Gonadotropin Releasing Hormone (GnRH) antagonist protocol was used for all patients according to the guidelines of the institute. This protocol included ovarian stimulation with recombinant follicular stimulating hormone (Gonaf -F®, Merk-Serono/ Switzerland) started on the second day of the menstrual cycle with or without human menopausal gonadotropin (Menogone®, Ferring, GmbH/ Germany). The gonadotropin starting dose depended on the BMI, age of the women, previous response to ovarian stimulation, and antral follicular count (AFC).

A transvaginal ultrasound was performed on the fifth day of stimulation, and the consequent scan was done every two to three days as required. A daily subcutaneous injection of 0.25 mg of cetrorelix acetate (Cetrotide®, Merck Serono,

Geneva, Switzerland) was initiated as soon as the leading follicles reached a diameter of 13-14 mm with multiple dose regimen, in which it was given daily until the trigger day. Final oocyte maturation established by the administration of subcutaneous recombinant human chorionic gonadotropin (r-hCG)500 µg (Ovitrelle® Merk-Serono/ Switzerland) when two to three leading follicles observed by ultrasound at an average diameter of 17-18 mm or r-hCG 250 µg and Triptorelin 0.1 mg (Decapeptyl® Ferring / Germany) (dual triggering). On the day of triggering, the serum E2 levels using an ELFA technique were measured, and the total gonadotrophin dose used was calculated for each participant.

Ovum pickup (OPU) was performed under general anesthesia with transvaginal ultrasound guidance after 34-36 hours of trigger administration. The follicular fluid was immediately brought to an embryology specialist to gather the retrieved "cumulus-oocytes complexes (COC)". Assessment of each oocyte (after denudation) was carefully done, observing the existence or lacking the first polar body (PB) or germinal vesicle. Ova that were morphologically intact and extruded from the first polar body were selected for microinjection. Intracytoplasmic sperm injection (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. 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)" (Magaton IM et al.,2023[9]) were calculated. Embryo morphology was established on the number of blastomeres, the percentage of fragmentation "(≤10% = score 0; 11–20% = 1; 21–30% = 2; >30% = 3)" and blastomere symmetry "(equal = score 1, different = 2)" (Al Hourani HM et

al.,[8]) and embryo transfer was done. Luteal support was started on the day of oocyte retrieval by progesterone injection depot 250mg 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. (Zhang Y et al.,2022 [10])

Collection of blood serum and follicular fluid samples and biochemical assay:

Venous blood samples from the antecubital veins were collected on the day of OPU for SFRP5 and wnt5a levels measurement of all female patients. These blood samples (3ml) were taken into a serum-separating tube (gel and clot activator) through a disposable syringe and allowed to coagulate for 10-20 minutes at room temperature. After being centrifuged for 20 minutes at a rate of 3000 rpm, the serum was obtained and transferred to a labeled Eppendorf tube. The obtained follicular fluid on the day of oocyte retrieval following the isolation of COC was collected in a plain tube and centrifuged for 20 minutes at 3000 rpm for the separation of debris and cellular contents. The resulting supernatant of follicular fluid is transferred into a labeled collecting tube. Both serum and follicular fluid samples were stored at -20°C for SFRP5 and wnt5a levels assay time. The enzyme-linked immunosorbent assay (ELISA) technique used in the current study for measurement of SFRP5 and wnt5a levels utilizing a detection kit (YL Biotech Co., Ltd., Shanghai, China) and microplate reader capable of measuring absorbance at 450 nm (Huma reader HS, Human, USA) .

3.Statistical Analysis

Microsoft Office 2010 and version 23.0 of the Statistical Package for Social Sciences (SPSS) were used to analyze the data. Descriptive statistics were calculated to describe the data,

including frequency, range, mean, and standard deviation. The groups were contrasted using the independent sample t-test (Comparison between two groups), chi-square (Comparison of non-continuous variables and percentages), and analysis of variance (ANOVA) (Comparison between more than two groups). The receiver operating characteristic (ROC) curve was used to determine the cut-off value, sensitivity, and specificity. The results were deemed significantly different when the p-value was less than 0.05. Pearson's correlation coefficient (r) determined the degree of relationship between continuous variables.

4. Results

Thirty of 120 female patients who were not eligible for the study were excluded (17 froze all, five poor responses, three empty follicles, two abnormal embryos, and three atretic embryos). The remaining 90 female patients were categorized into three groups according to BMI ranking as follows: group 1: normal-weighted females (23%), group 2: overweighted females (47%) and Group 3: obese females.(%30)

The comparison of demographic data among the three studied groups (average weight, overweight and obese females) is shown in Table 1. There were no significant differences regarding the mean female's age (31.48 ± 4.56 versus 32.93 ± 5.02 and 34.22 ± 6.87 with (p-value=0.240) and duration of infertility (5.97 ± 4.49 versus 6.61 ± 2.63 and 6.32 ± 4.84) with (p-value=0.768). Females with primary infertility were 19 (90.5%), 36 (85.7%) and 20 (74.1%), respectively. On the other hand, females with secondary infertility were 2(9.5%), 6(14.3%), and 7(25.9%), respectively, with no significant differences among the three studied groups (p-value=0.271). Furthermore, there was no significant difference among the three studied groups regarding the causes of infertility (p-value=0.279.(

Regarding the stimulation characteristics, there was a significant

difference in mean total gonadotrophin dose used among average weight, overweight and obese females, 1805.95± 345.86 versus 1908.33± 460.27 and 2133.52± 415.91 I.U., respectively (p = 0.023). It was higher in overweight and obese females than in average-weight females. However, there was no significant difference in triggering among normal-weight, overweight, and obese females. Females with hcg triggering were 15 (16.6%), 30 (33.3%), and 18 (20%), respectively. On the other hand, females with dual triggering were 6(6.67%), 12(13.3%) and 9(10%) respectively as shown in table 2. Moreover, there was a significant positive correlation between serum or follicular fluid wnt5 and gonadotropin dose (table 3) .

The comparison of secreted frizzle-related protein 5 (SFRP5) levels among the studied groups is demonstrated in Table 4. Serum and follicular fluid SFRP5 levels were significantly higher (p < 0.001) in normal-weight females. Serum SFRP5 levels in normal weight, overweight and obese females were 22.22 ± 5.90 versus 16.92 ± 5.43 and14.44 ± 4.36, respectively, follicular fluid SFRP5 levels were18.95 ± 5.26 versus13.47 ± 4.93 and11.44 ± 3.99 .There was a significant difference in mean fertilization rate among normal weight, overweight and obese female groups, 79.99 ± 14.05 versus 69.26 ± 21.84 and 67.17 ± 17.97, respectively (p = 0.05), being higher in normal weight than overweight and obese females. However, there was no significant difference in mean maturation rate (MR) among the three studied groups (p = 0.77). The MR was 67.68 ± 17.74 in normal weight and 68.00 ± 20.63 in overweight, while the obese female group was 64.79 ± 18.92, insignificantly higher in normal-weight females. as shown in Table 5 .

There was a significant positive correlation between serum SFRP5 and MR and FR (table 6) (r= 0.289 and p = 0.006, r=0.261 and p = 0.013, respectively). In addition, there were

significantly higher serum (p-value=0.002) and follicular fluids (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) respectively as shown in Table 8 However, there were insignificantly ($p > 0.05$) 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 7 .

The receiver Operative Characteristic (ROC) curve has been used to calculate serum and follicular fluid SFRP5 cut-off values to predict a positive pregnancy with acceptable sensitivity, specificity, and accuracy. The results are demonstrated in Table 8 and Figure 1,2. Accordingly, the cut-off value of serum SFRP5 was ≥ 15.86 (ng/ml) with a sensitivity of 88.9 %, specificity of 60.3 %, an accuracy of 70.3 %, and an area under the curve (AUC) equal to 0.730 with a p-value equal to 0.001. On the other hand, the follicular fluid SFRP5 cut-off value was ≥ 11.3(ng/ml) with sensitivity=92.6 %, specificity=57.1 %, the area under the curve =0.724, and p-value. 001. Finally, there was a positive significant correlation between serum and follicular fluid SFRP5 with MR and FR ($p < 0.05$). Also, there was a positive significant correlation between serum and follicular fluid wnt5a and total gonadotropin dose ($p < 0.05$).

Table (1): Comparison of demographic features among the studied groups

Parameter Mean ± SD Range	Normal weight group n=21	Overweight group n=42	Obese group n=27	p-value
Age (years)	31.48 ± 4.56	32.93 ± 5.02	34.22 ± 6.87	0.240 ∀ NS
	24 - 40	23 - 45	18 - 40	
BMI (kg/m²)	23.13 ± 1.34	27.52 ± 1.47	32.96 ± 2.98	< 0.001 ∀ HS
	21.05 – 24.91	25.00 – 29.97	30.00 – 40.68	
Duration of infertility (years)	5.97 ± 4.49	6.61 ± 3.63	6.32 ± 4.84	0.768 ∀ NS
	1-19	1.5- 16	1.5-19	
Type of infertility n (%)				
Primary	19 (90.5 %)	36 (85.7 %)	20 (74.1 %)	0.271 € NS
Secondary	2 (9.5 %)	6 (14.3 %)	7 (25.9 %)	
Causes of infertility n (%)				
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 %)	

SD: standard deviation; **n:** number of patients; **BMI:** body mass index; **PCOS:** poly cystic ovarian syndrome; **HS:** highly significance at $p \leq 0.01$ **NS:** non-significant at $p > 0.05$; **∀:** ANOVA test; **€:** chi square test

Table (2): Comparison of stimulation characteristics among the studied groups

Mean ± SD Range	Normal weight group n=21	Overweight group n=42	Obese group n=27	p-value
Gn dose (IU)	1805.95±345.86	1908.33±460.27	2133.52±415.91	**0.023** ∀ S
	1275-2300	3000 1350-	3300 1575-	
E2 on the day of the trigger (pg/ mL)	1496.86 ± 768.51	1415.77 ± 612.72	1092.36 ± 394.09	0.039 ∀ **S**
	400 – 2967	412 - 2836	600 - 2146	
Triggering n (%)				
HCG	15(16.6%)	30 (33.3%)	18 (20 %)	0.9 € NS
Dual	6 (6.67 %)	12 (13.3) %	9 (10 %)	

SD: standard deviation; **n:** number; **Gn:** gonadotropin; **IU:** international unit; **S:** significant at p ≤ 0.05; **∀:** ANOVA test; **€:** chi square test

Table (4): Comparison of SFRP5 levels among 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	16.92 ± 5.43	14.44 ± 4.36	**< 0.001** **∀** **S**
	8.00 – 30.10	6.40 - 29.00	5.90 – 22.40	
Follicular fluid SFRP5	18.95 ± 5.26	13.47 ± 4.93	11.44 ± 3.99	**< 0.001** **∀** **S**
	7.00 - 27.00	6.00 – 27.00	5.40 – 20.80	

SFRP5: Secreted frizzle related protein 5; **SD:** Standard deviation; **n:** numbers: Significant at p≤ 0.05; **∀:** ANOVA test

Table (5): Comparison of maturation rate and fertilization rate among the studied groups

Parameter (Mean ± SD) Range	Normal weight group n=21	Overweight group n=42	Obese group n=27	p-value
Maturation rate	67.68 ± 17.74	68.00 ± 20.63	64.79 ± 18.92	0.77 V NS
	33-100	33.7 - 100	30.7 - 100	
Fertilization rate	79.99 ± 14.05	69.26 ± 21.84	67.17 ± 17.97	0.05 V S
	40 - 100	25 - 100	40 - 100	

SD: Standard deviation; **NS:** Non-significant at **p** > 0.05; **V**: ANOVA test

Table (6): Correlations between serum SFRP5 levels and maturation rate as well as fertilization rate

Characteristic	Serum SFRP5	
	r	***p***
Maturation Rate	**0.289**	**0.006****
Fertilization Rate	**0.261**	**0.013***

SFRP5: Secreted frizzle related protein 5; ***:** Significant correlation; **r:** Pearson's correlation coefficient

Table (7): 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 Ŧ S
Follicular fluid SFRP5	14.86 ± 8.98	10.60 ± 4.59	0.005 Ŧ S
Serum Wnt5a	2.05 ± 1.53	2.55 ± 1.54	0.167 Ŧ NS
Follicular fluid Wnt5a	1.94 ± 1.58	2.36 ± 1.33	0.200 Ŧ NS

SFRP5: secreted frizzle related protein 5; **Wnt5a**: wingless type mouse mammary tumor virus integration site5a; **SD**: standard deviation; **NS**: son-significant at $p > 0.05$; **S**: significant at $p \leq 0.05$; **Ŧ**: independent sample t test

Table (8): Receiver Operative Characteristic curve of serum and follicular fluid SFRP5 as a predictor of a positive pregnancy

ROC curve characteristics	Serum SFRP5	Follicular fluid SFRP5
Cut-off value (ng/ml)	≥ 15.86	≥ 11.3
The area under the curve (AUC)	0.730	0.724
95 % CI	0.603 to 0.799	0.620 to 0.813
Sensitivity	88.9 %	92.6 %
Specificity	60.3 %	57.1 %
Accuracy	70.3 %	70.2 %
p-value	0.001 S	0.001 S

SFRP5: secreted frizzle related protein 5; **ROC**: Receiver Operative Characteristic; **S**: Significant at $p \leq 0.05$

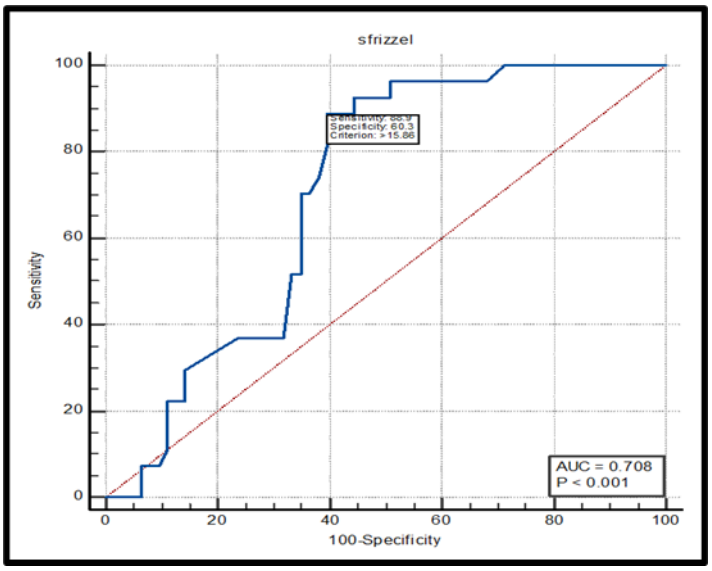

Figure (1): Serum SFRP5 ROC curve as a predictor of the positive pregnancy

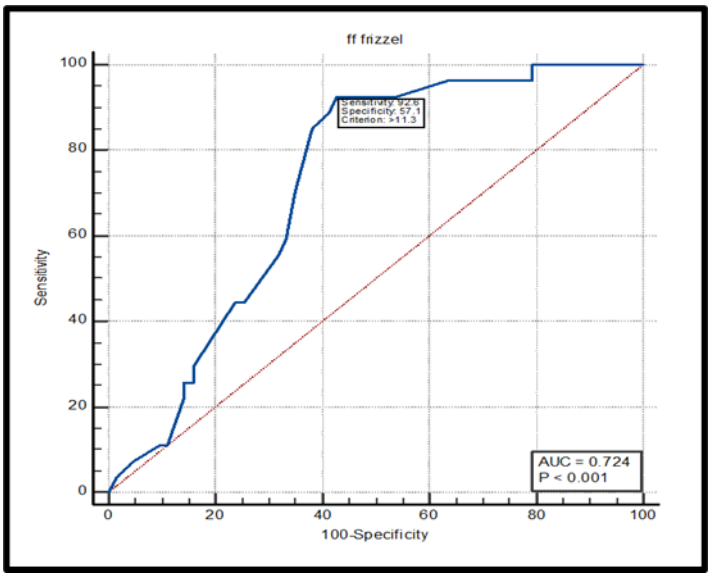

Figure (2): Follicular fluid SFRP5 ROC curve as a predictor of a positive pregnancy

4. Discussion

In this study, there were 23 % normal-weighted females (Group 1), 47 % overweighted females (Group 2), and 30 % obese females (Group 3). Thus, it appears that a significant proportion of enrolled infertile women are either overweight or obese since they are in combination, accounting for 77 %. Two suggestions can explain this finding. The first one is that in our community, being overweight and obese are common, based on data from various Iraqi reports (Pengpid S& Peltzer K,2021[11]; Shabu, S.A, 2019[12]; Jasim HM et al.,2018[13]). The second possibility is the association between high BMI (overweight and obesity) and infertility in women (Gautam, D et al.,2023[14]; Marinelli S et al.,2024[15]). Obese women's fertility appears to be reduced in assisted reproductive programs. Indeed, due to low oocyte quality, a reduced preimplantation rate, and decreased uterine receptivity, being overweight or obese is also linked to unfavorable results for patients having IVF/ICSI) (Al-yasiry RZ et al., 2022[2]).

The results showed no significant differences in demographic characteristics, age, duration of infertility, type of infertility, and cause among the three studied groups. The lack of significant differences in demographic parameters ensures statistical matching among the three groups. Any difference in IVF/ICSI outcomes can be attributed to factors other than these demographic characteristics .

Regarding the stimulation characteristics, there was a significant difference in mean total gonadotrophin dose, higher in overweight and obese females than normal-weight females. These results agree with Dornelles et al. (Dornelles VC et al.,2022 [16]). Also, a meta-analysis done by Christensen et al. (Christensen MW et al.,2016[17]), which included 5,342 treatment cycles during the period 1999–2009, both

obese and overweight women did, on usual, get a higher dose than normal-weight women. The total FSH dose increased with increasing BMI, which aligns with our observation.

The need for higher doses of gonadotrophins in obese and overweight women may be attributed to altered pharmacodynamics, larger distribution volume in overweight and obese patients, altered estradiol metabolism, and decreased sex hormone binding globulin (SHBG) concentrations. The absorption, metabolism, and clearance of gonadotrophins injected subcutaneously have been observed to differ in obese women (Marinelli S et al.,2024[15]). Also, a decreased ovarian sensitivity to gonadotropin in overweight and obese women was likely due to hyperinsulinemia, which can influence follicular growth and contribute to prolonged ovarian stimulation.(Li Y et al.,2023[18])

Leptin levels are also higher in obese women, and it has been hypothesized that high intrafollicular leptin levels throughout ovarian stimulation in IVF/ICSI cycles are linked to relative gonadotrophin resistance due to a blocking impact on granulosa cells' FSH-stimulated steroid synthesis (Dawood SA et al.,2023[19]). Moreover, our study found a significant positive correlation between serum or follicular fluid wnt5a and the gonadotropin dose. Experimental studies have shown that higher levels of wnt5a result in impaired steroidogenesis by granulosa cells with ultimately reduced estradiol production in addition to reduced FSH receptor expression by these cells (Abedini A et al.,2015 [20]), and eventually, there will be retarded follicular development and to overcome such consequences, higher doses of gonadotropin will be required .

On the other hand, there was no significant difference in the triggering for the final maturation of oocytes. Several authors have shown the variation in the type of cause for final

oocyte maturation associated with variation in reproductive outcomes following IVF/ICSI cycles (Hu KL et al.,2021[21]). So, this absence of considerable difference
may lead to avoidance of any bias in fertility outcome related to dual or hcg type of triggering for final oocyte maturation. This will restrict the justification of any viable difference in fertility outcome or other fertility parameters to variation in BMI among the three studied groups .

In the present study, it has been found that serum and follicular fluid levels of SFRP5 were higher in women with normal BMI when contrasted to overweight or obese women. This finding is consistent with cross-sectional studies that found SFRP5 negatively correlated with obesity indices such as BMI, waist-to-hip ratio, body fat percent, glycolipid metabolism, and inflammation. (He X& Ma H 2020[22]; Akoumianakis I et al. , 2019[23]). In addition, SFRP5 was negatively correlated with adiposity in two extensive studies of primarily overweight and obese subjects (Carstensen-Kirberg M et al., 2017[24]; Black MH, et al.,2018 [25])

The possible explanation for this finding could be related to the involvement of SFRP5 in the maturation of fat cells, as the SFRP5 gene is a significant target of transcription factor peroxisome proliferator-activated receptor- γ (PPARγ) (Koutaki D et al.,2021 [26]). The PPARγ is a transcription factor that plays an important role in the differentiation and function of mature adipocytes and adipogenesis. Obesity has been associated with a decrease in activity and quantity of PPARγ. This association appears to be substantially linked to the etiology of obesity (Motawi TK et al.,2017[27]). According to studies, SFRP5 increases PPARγ and appears to be a marker for mature adipocytes (Koutaki D et al.,2021 [26]).

The results of this study revealed that the mean fertilization rate (FR) was

higher in normal weight than in overweight and obese females. However, there was no significant difference in the mean maturation rate (MR). The insignificantly higher MR agrees with Dornelles et al. (Dornelles VC et al.,2022 [16]). Regarding the FR, the higher FR in normal-weight patients agrees with Liu et al. (Liu X et al.2023[28]) and the systematic review from Amiri & Ramezani Tehrani (Amiri M & Tehrani FR, 2020[29]), as these studies showed a poorer fertilization rate with increasing BMI. Nevertheless, it is inconsistent with the results of García-Ferreyra et al. in 2021who found no significant difference in mean FR among normal, overweight, and obese groups.

An obese female patient's lower oocyte quality than women of average weight may account for this finding. There is evidence of altered gene expression in the oocytes of obese and overweight women compared to normal-weight women. Expression of oocyte genes linked to inflammation, oxidative stress, and lipid metabolism rises when obesity levels out (Ruebel ML et al., 2019[30]). According to clinical trials, obese women receiving fertility treatments have higher levels of the hormones leptin, C-reactive protein, and follicular fluid triglycerides, all of which were found to be positively correlated with body mass index (BMI), as well as lower oocyte quality, fewer fertilized oocytes, and less successful pregnancy outcomes (Song J. et al.,2020 [31]) This study found a significant positive correlation between serum SFRP5 and FR and MR. The plausible explanation for this result might be related to inflammation and oxidative stress and the association between wnt5a and oxidative stress. It has been found that wnt5a might directly increase reactive oxygen species (ROS) production by increased nicotinamide adenine dinucleotide phosphate oxidase activity and reduced nitric oxide bioavailability (Koutaki D et al.,2021 [26]). The sequestration of wnt5a by SFRP5

(Akoumianakis I et al.,2022[32]; Huang X , et al., 2021[33]) may reduce the oxidative stress, and thereby the net result will be increasing total oocyte count, MII oocyte, MR, and FR. This interaction between wnt5a and SFRP5 in determining oocyte quality suggests that these two markers affect FR by affection oocyte quality.

In the present study, there were significantly higher serum and follicular fluid SFRP5 levels in pregnant females; however, the serum and follicular fluid wnt5a levels were lower in pregnant women than non-pregnant women, but the difference was not significant from a statistical perspective. Molecules present in the follicular fluid, including adipokines such as wnt5a and SFRP5, may play an essential role in communication between somatic and germ cells, which is necessary for the oocyte to acquire competence. Thus, assessing these markers in the follicular fluid may provide a non-invasive valuable tool in predicting the outcome of IVF/ICSI cycles. The wnt5a glycoprotein is an important mediator of the wnt signaling pathway that may regulate the formation of the primordial germ cells (Chen F et al., 2022[34]). Akino et al. (Akino R et al., 2020[35]) showed that the transcription levels of canonical pathway signaling molecules were lower in human dysmature cumulus cells than in control cells .

Habara et al. in 2021 discovered that canonical wnt signaling is essential for primordial follicle activation via regulation of pre-granulosa cell differentiation and subsequent maturation of the oocyte, which is another indicator of how canonical wnt signalling influences oocyte quality and subfertility. The wnt5a, the non-conical pathway, has been shown to antagonize the canonical pathway (Habara O et al., 2021[36]); therefore, its higher level in non-pregnant women may lead to retarded maturation of cumulus cells, thereby affecting the growth of the follicles, leading to the production of poor-quality oocytes, less

fertilization, poor quality embryo, less chance for implantation and less chance for pregnancy.

Moreover, as mentioned above, wnt5a has been associated with oxidative stress and increased ROS production (Akoumianakis I et al.,2022[32]; Huang X , et al., 2021[33]). Thus, it may enhance endoplasmic reticulum stress, leading to poor oocyte quality, poor embryo quality, impaired endometrial receptivity, less chance for implantation, and a lower pregnancy rate. In the current study, we were able to identify two cutoff values for serum and follicular fluid SFRP5 as ≥15.86 (ng/ml) ≥ 11.3(ng/ml), respectively, with acceptable levels of sensitivities, specificities, and accuracies. Indeed, this finding is one of the original points of the current study that added to the predictors of positive pregnancy in women undergoing IVF/ICSI cycles.

Inal et al. 2018 performed a study investigating serum and follicular fluid SFRP5 levels in non-obese, non-hyperandrogenic patients with polycystic ovarian syndrome undergoing IVF/ICSI, and they found no significant difference in mean serum and follicular fluid SFRP5 levels between pregnant and non-pregnant women .

After a thorough search in the available published articles, we did not find a study that is similar to our study in estimating serum and follicular fluid levels of SFRP5 and wnt5a among various groups of women undergoing IVF/ICSI cycles based on their corresponding BMI or to compare these levels between pregnant and non-pregnant women. Thus, this is the first study to investigate serum and follicular fluid levels of SFRP5 and wnt5a among various groups of women undergoing IVF/ICSI cycles based on their corresponding BMI and IVF/ICSI outcomes of these patients. The points of originality in our study are the synchronous measurements of serum and follicular fluid (reflecting the oocyte microenvironment) SFRP5 and

wnt5a levels. Analyses of SFRP5 and wnt5a association with IVF/ ICSI outcomes among various groups of women undergoing IVF/ ICSI cycles.

5- Conclusion

Both secreted frizzled-related protein 5(SFRP5) and wingless-type mouse mammary tumor virus integration site family 5a(wnt5a) glycoproteins were detected in serum and follicular fluid of infertile women. Serum and follicular fluid SFRP 5 levels positively associated with IVF/ICSI outcome. The cutoff values for serum and follicular fluid SFRP5 were ≥15.86 (ng/ml) and ≥ 11.3(ng/ml), respectively, as predictors of positive pregnancy in women undergoing IVF/ICSI cycles.

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Author Contribution

Hameedah A. Mohsin, performed the study Hayder A. L. Mossa, and Mufeda Ali Jwad supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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