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FMS-like Tyrosine Kinase 1 and Other Associated Factors to Predict in Vitro Fertilization (IVF) Outcomes in Different Causes of Infertility

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Abstract

As infertility became a significant public health problem, assisted reproductive technologies (ART) were introduced. The study aims to investigate infertility in women undergoing their IVF cycle, the role of FMS like Tyrosine kinase 1, and other associated factors causing infertility, as well as the relationship between FMS like Tyrosine kinase 1 and the IVF outcome. The study population consisted of 136 infertile women who sought medical help at Kamal Al-Samarra'ay Specialized Hospital for infertility treatment. The collection of data was performed by means of a specifically designed questionnaire, which, apart from the demographic data, included questions concerning the causes of infertility. According to the reasons for infertility, the patients who participated in our research were classified into five groups. The study showed that, by comparing the groups, the success rate of IVF was the highest when the causes of infertility were male factor, unexplained, mixed, PCO, and tubal factor. Additionally, the study showed a significant difference ($p<0.05$) in the level of FMS-like Tyrosine kinase 1, number of embryos, repeated IVF cycles, and embryo transfer (E.T.) between infertility groups. Our study concluded that the clinical parameters were closely associated with IVF outcomes, which could provide information for future clinical interventions to increase successful rates. Our research revealed that FMS-like Tyrosine Kinase 1 has a role in infertility; the greater the value, the higher the success rate. Furthermore, the findings show that there is a close association between FMS-like Tyrosine kinase 1 level and IVF success, and it can be used as a valuable marker to confirm the extent of IVF failure or success.

Keywords: Causes of infertility, infertile women, FMS-like Tyrosine kinase 1

FMS–like Tyrosine Kinase 1 و العوامل الأخرى المرتبطة بها للتنبؤ بنتائج الإخصاب في المختبر (IVF) في أسباب مختلفة من العقم

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الخلاصة

عندما أصبح العقم مشكلة صحية عامة كبيرة ، تم إدخال تقنيات المساعدة على الإنجاب. تهدف الدراسة الى التحقيق في العقم عند النساء اللواتي يخضعن لعمليات أطفال الأنابيب وكذلك التحقيق في العلاقة بين FMS–like Tyrosine kinase1 ونتائج عمليات اطفال الانابيب. تكون مجتمع الدراسة من 136 امرأة مصابة بالعقم طلبن المساعدة الطبية في مستشفى كمال السامرائي التخصصي لعلاج العقم. تم جمع البيانات

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عن طريق استبيان مصمم خصيصًا ، بصرف النظر عن البيانات الديموغرافية ؛ تضمنت أسئلة تتعلق بأسباب العقم. تم تقسيم المرضى الذين شملتهم دراستنا إلى خمس مجموعات وفقًا لأسباب العقم. أظهرت الدراسة ، من خلال مقارنة المجموعات ، أن معدل نجاح اطفال الانابيب هو الأعلى عندما تكون أسباب العقم من الرجال ، غير المبررة، مختلطة ، تكيسات المبايض والعامل البوقي ، بالإضافة إلى ذلك، اظهرت الدراسة تغييرات كبيرة ($p<0.05$) في مستوى FMS–like Tyrosine kinase 1 ، و عدد الأجنة ، ودورات التلقيح الاصطناعي المتكررة ونقل الأجنة بين مجموعات العقم. كشف بحثنا أن المعلمات السريرية كانت مرتبطة ارتباطًا وثيقًا بنتائج التلقيح الاصطناعي، والتي يمكن أن توفر معلومات للتدخلات السريرية المستقبلية لزيادة معدلات النجاح. لاحظنا أن FMS–like Tyrosine kinase 1 مرتبط في العقم. فكلما زادت القيمة، ارتفع معدل النجاح. علاوة على ذلك، تظهر النتائج أن هناك علاقة وثيقة بين مستوى FMS–like Tyrosine kinase 1 ونجاح التلقيح الاصطناعي ، ويمكن استخدامه كعلامة قيمة لتأكيد مدى فشل أو نجاح التلقيح الاصطناعي.

1. Introduction

Infertility is a disease of the male or female reproductive system that is defined by the failure to achieve pregnancy after one year or more of regular unprotected intercourse [1,2]. The World Health Organization (WHO) recognizes infertility as a public health issue worldwide, making it the fifth most severe disability among couples [3]. Infertility affects the lives of millions of people of childbearing age in all countries of the world and has an impact on their lifestyles, their families, and their societies [4]. It is estimated that between 186 million individuals and 48 million couples suffer from infertility worldwide [1]. Males are only responsible for 20 to 30% of cases but contribute up to 50% of all infertility cases [5]. Latest estimates suggest that women's infertility is the cause in about 37% of all cases of infertile couples [6], and 25-40% are due to combined problems in both parents [7]. There are several causes of infertility, including some that are unexplained or affect both men and women [8]. The causes of infertility vary based on the age of the partner and the duration of the marriage [3]. Infertility can be classified as either primary or secondary. Primary infertility is the inability to conceive after one year, whereas secondary infertility is infertility after having conceived at least once previously [9]. Approximately 12.7% of reproductive-age women seek treatment for infertility each year. In addition, approximately 85% of infertile couples have an identifiable cause. However, because female fecundity declines with age, immediate assisted reproductive technology (ART) techniques, including *in vitro* fertilization (IVF), can be considered a treatment for women, especially women older than 35 years of age. Although the success rate varies according to the diagnosis of the cause of infertility as well as age, accurate diagnosis and effective treatment can facilitate the achievement of pregnancy goals for many couples being treated for infertility [10,11]. Infertility is caused by many different factors in the female or male reproductive system. Sometimes the causes of infertility (unexplained infertility) cannot be explained [1]. Other causes, including lifestyle and environmental factors such as obesity, excessive alcohol intake, and smoking, can affect fertility. In addition, exposure to environmental toxins and pollutants can be directly toxic to gametes (sperm and eggs), resulting in their poor quality and decreased numbers and thus leading to infertility [12]. Male infertility can be due to a variety of causes, the most important of which are hormonal deficiency, sexually transmitted problems, physical causes, genetic factors, the environment, and lifestyle [13]. In the male reproductive system, infertility is often caused by other causes, including problems with semen output, low or absent sperm, movement (motility), and abnormal shape (morphology) of the sperm [14]. While the most common causes of female infertility are ovarian dysfunction with anovulation (25 to 35%), pathology of oviducts (20 to 25%), endometriosis (10 to 20%), uterine pathology (adhesions, fibroids) (5 to 10%), and unexplained infertility (20 to 30%), [15,16]. Female infertility may be caused by hormonal imbalances. These hormonal conditions include polycystic ovary syndrome (PCO), premature ovarian failure (POF), hypothalamic dysfunction, and hyperprolactinemia [15]. Female causes are responsible for 40-45% of the etiology of infertility [17]. Tubal factor infertility is one of the most frequent causes of female infertility and is responsible for 25–40% of female infertility [18,19]. PCO is a defined endocrine condition, clinically defined as chronic anovulation associated with hyperandrogenism [20]. PCO is one of the causes of irregular menstruation and thus impaired fertility, with an incidence of PCO in adult women ranging from 16 to 33% [21]. Unexplained infertility is a type of exclusive diagnosis that can only be made if no abnormality is identified on semen analysis, gynecological examination, ovulation monitoring, or fallopian tube examination. If necessary, laparoscopy may be used for diagnosis [22]. In other cases, the cause is suspected to be genetic or immunological; both partners may be fertile independently, but the couple cannot conceive together without assistance [23]. Serum FMS-like Tyrosine kinase 1 is a circulating antiangiogenic protein. It can also affect the growth of follicles, which in turn affects the normal function of the ovary [24]. On the one hand, FMS-like Tyrosine kinase 1 can negatively regulate vascular endothelial growth factor (VEGF) and block oocyte maturation; as well, it acts on steroid hormones to affect androgens in the granular cell layer, indirectly interfering with estrogen expression and disrupting ovarian function, thus promoting the occurrence of PCO [25]. A higher level of plasma FMS-like Tyrosine kinase 1 was associated with pregnancy [26, 27]. The main purpose of this study was to investigate the causes of infertility in women of childbearing age and to compare five causes of infertility in couples, as well as the relationship between FMS-like Tyrosine kinase 1 and the IVF outcome.

2. Materials and Methods

2.1. Study subjects

The current study includes 136 infertile patients who are attending the Kamal Al-Samarra'ay Specialized Hospital for IVF treatment between October 2021 and October 2022. All patients had given their signed informed consent to participate.

2.2. Questionnaire and clinical data

The convenient sampling method was used to include 136 infertile women not undergoing IVF (+/-ICSI) treatment. The following data on demography and pregnancy outcomes were analyzed: female name, female age, husband’s name, and age infertility duration, causes of infertility, number of Oocytes, number of mature Oocytes, BMI, basal gonadal hormone concentrations, endometrial thickness, number of embryos transferred and fertilization rate.

2.3. Collecting patients

Samples included women whose ages ranged from 20 to 45 years, who suffered from primary or secondary infertility, and who underwent the IVF procedure once, twice, and three times. On the second day of the menstrual cycle, blood was collected from all patients to evaluate hormones associated with infertility. All 136 patients who met the inclusion criteria were enrolled and divided into groups according to the cause of infertility. Group 1 (male factor, n = 57), group 2 (tubal, n = 9), group 3 (unexplained, n = 33), group 4 (PCO, n = 16), and group 5 (mixed, n = 21). The exclusion criteria included IVF that uses frozen embryos and those women with a BMI < 18.5 kg/m^2 and an AMH <0.5 ng/mL. Biochemical pregnancy was defined as serum HCG higher than 25 U/L in 12 to 14 days after transfer.

2.4. Hormonal assay

All hormones were measured on day two of the menstrual cycle in the endocrinology laboratory of the hospital to analyze hormones related to infertility. Estradiol and progesterone were measured on the morning of HCG administration. Endometrial thickness was measured on the day of oocyte retrieval by transvaginal ultrasound.

2.5. Protein FMS-like Tyrosine kinase1 analysis

The blood sample was taken on the day of the oocyte retrieval. Blood was transferred into a glass tube with a gel separator, and the blood samples were allowed to clot for 10 minutes at 37 ^{o}C in an incubator before being centrifuged at 3000 × g for 3 minutes to obtain the serum, put in an Eppendorf tube, and stored frozen at -20 ^{o}C until being used to estimate the parameters. The ELISA was measured and read for the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of Vascular Endothelial Growth Factor Receptor 1 (VEGFR1) could be calculated, which was Included in the study. Hemolyzed sera were excluded. The study protocol conforms to the ethical guidelines endorsed by the College of Science, University of Baghdad ethics committee.

2.6. Statistical analysis

All data were prospectively collected in SPSS version 26.0, a central database for electronic medical records. For each IVF cycle initiated, we collected data with the normal distribution, expressed as the mean ± standard deviation (SD) for data with the normal distribution. Sample characteristics were determined by means and frequencies. ANOVA for quantitative variables was used to compare means among multiple groups, and Chi-square tests were used for the comparison of categorical variables. A P value less than 0.05 was considered statistically significant, and a P value less than 0.01 was considered statistically highly significant.

3. Results

One hundred and thirty-six women who were enrolled in Kamal Al-Samarra'ay Specialized Hospital were enrolled in this study; they underwent IVF, and they were divided into five groups according to the causes of infertility. Table 1 shows the demographic characteristics of infertile women, male and female factors (tubal and PCO), unexplained factors, and mixed causes. The research provided explanations of the findings including IVF success and failure percentages. Our study revealed the most successful groups and explains the causes of infertility. The most frequent cause of infertility was the male factor in 57 of the women who underwent IVF; 19(13.9%) of them were successfully conceived, and 38 (27.9%) failed. The results showed that the second most successful group was the unexplained group in 33 of the women undergoing IVF, 15(11.02%) of whom succeeded while 18(13.2%) failed. This is followed by mixed causes, with 21 patients 11(8.08%), conceiving and 10(7.35%) failing. While the PCO group came next with 16 patients, 7(5.14%) women succeeded in getting pregnant and 9(6.6%) failed. Finally, the lowest pregnancy success rate was in the tubal group, which includes 9 women, with only 3(2.2%) of them successfully conceiving. According to the results presented in Table 1, we compared the causes of infertility with the number of embryos transferred and the number of e repeated IVF cycles, and there were statistically significant differences between them, $p<0.01$ and $p<0.05$ respectively. While the results showed there are no significant differences between the causes of infertility and infertility hormones, as for FMS-like Tyrosine kinase 1, it had an effect on infertility and had clear, significant differences $p<0.01$.

Table 1: The demographic data and clinical characteristics of the studied groups are distributed according to causes of infertility

Parameter	Groups					
	G1 (mean±SD) (n=57)	**G2 (mean±SD)** (n=9)	**G3 (mean±SD)** (n=33)	**G4 (mean±SD)** (n=16)	**G5 (mean±SD)** (n=21)	***p*-value**
Cause of infertility	**Male factor**	**tubal**	**unexplained**	**PCO**	**Mixed**	-
IVF success Yes, n (%) No, n (%)	19 (13.9%) 38 (27.9%)	3(2.2%) 6(4.4%)	15 (11.02%) 18 (13.2%)	7 (5.14%) 9 (6.6%)	11(8.08%) 10 (7.35%)	
Age (Year)	30.98 ±5.43	31.55±6.83	30.90±5.67	33.06±5.73	32.28±5.17	0.64
BMI (Kg/m^2)	27.25 ±4.97	26.44±3.08	26.46±4.46	28.15 ±3.56	26.88±4.40	0.77
Duration of infertility (Year)	5.35±3.68	6.88±4.59	5.03±3.35	5.43±2.87	6.88±4.11	0.31
Oocyte number	9.68±3.44	9.00±2.29	8.81±3.23	8.18±1.79	9.14±2.76	0.45
Number of embryos	6.00±1.91 *d* **	5.66±1.50 *g* **	5.72±1.68 *I* **	4.93±1.34 *j* *	3.33±1.23	<0.01
Repeated IVF cycles	1.24± 0.50 *a* **	1.88±1.16 *f* *, *g* **	1.39±0.55	1.18±0.40	1.23 ± 0.43	<0.05
Endometrial Thickness	7.14±2.36	7.98±1.48	8.04±1.38	7.74±1.19	7.86±1.39	0.18
Mature oocyte	7.14±2.36	6.55±2.18	6.63±2.31	5.62±1.50	6.61±1.96	0.19
E.T	3.15±1.08 *a* **	7.98±1.48 *e* **,*f* **, *g***	3.18±1.13	3.43±1.15	3.33±1.23	<0.01
F.R	47.10±18.68	49.62±17.43	58.42±19.60	54.49±17.59	57.12±20.96	0.059
LH (mIU/mL)	2.89±1.17	3.68±1.70	3.11±1.54	3.44 ±1.77	3.31±1.60	0.424
FSH (mIU/mL)	4.87±1.45	5.67±1.61	4.61±1.51	5.03±1.04	5.27±1.97	0.31
LH/FSH Ratio	0.61±0.27	0.75±0.36	0.69±0.24	0.68±0.31	0.59±0.20	0.36
E2 (pg/mL)	1436.27±602.50	1072.15±425.96	1291.16± 445.63	1093.48± 308.13	1238.41±522.65	0.07
Testosterone (ng/mL)	0.43±0.15	0.33±0.08	0.45±0.18	0.49±0.17	0.45±0.16	0.18
Progesterone (ng/mL)	0.39±0.09	0.38±0.09	0.38±0.12	0.35±0.10	0.36±0.07	0.69
PRL (ng/mL)	18.33±7.33	16.07±4.24	17.68±5.75	19.50±5.60	15.64±4.86	0.31
TSH (mIU/mL)	1.97±0.94	2.22±1.07	2.21±0.95	2.06±0.87	1.96±0.85	0.76
AMH (ng/ mL)	1.91±0.95	2.03±0.62	1.69±0.80	1.81±0.59	1.65±0.86	0.59
FMS-Like Tyrosinekinase1 (pg/mL)	1987.95±980.85 *b* ** , *d* **	1377.57±513.12	1187.3± 631.70	1528.98± 529.56	1208.64±480.89	<0.01

The results were expressed as mean ± SD (mean±standard deviation), range (minimum-maximum), or number (percentage), (A = Groups 1 vs 2), (B = Groups 1 vs 3), (C = Groups 1 vs 4), (D = Groups 1vs 5), (E = Groups 2 vs 3), (F= Groups 2 vs 4), (G = Groups 2 vs 5), (H = Groups 3 vs 4), (I = Groups 3 vs 5), (J = Groups 4vs 5).
**The difference is highly significant at $p \leq 0.01$. *The difference is significant at $p < 0.05$.

4. Discussion

The purpose of our study was to determine the causes of infertility in women undergoing IVF treatment. Patients were divided into five categories based on the reasons for the male component (sperm abnormalities) and female component (fallopian tubal obstruction, polycystic ovarian syndrome), unexplained, and mixed problems. According to the current study, all women who participated in assisted reproduction programs were between the ages of 20 and 45, with a mean of age 30.98±5.43 years. The male factor was the most common cause of infertility. Women who had infertility due to their husbands were younger than the rest of the women and had the highest success rate. Women who had infertility owing to PCO were on average 33.06 5.73 years old. Our results proved that the age group that reviewed the section was between 30 and 33 years old, and this is consistent with previous studies [28]. Statistics in Iraq show that older women participate more in assisted reproduction, and it was found that the success rate of IVF decreases with age [29]. The results from our study are consistent with a previous study conducted by Roupa *et al*. [30]. The body mass index of the women who participated in our study ranged from 26 to 28 kg/m^2, which indicates overweight, and this may be associated with infertility because it affects ovulation and hormones. In particular, the PCO group had the greatest ratio of 28.15 3.56 kg/m^2, while the tubal factor group had the lowest score (26.44 3.08 kg/m^2). The high BMI had a pronounced negative influence on fertility [31,32]. Our study does agree with a previous study by Sneed *et al*. [33]. Another study showed that older and obese women are more likely to create poor-quality embryos [31]. Based on the effect of infertility duration on IVF outcomes, the study discovered that the longer the duration of infertility, the lower the success rate. As a result, those who have been infertile for a long period of time are not good candidates for IVF. According to the study, patients with tubal factor had the longest period of infertility (6.88 ± 4.59 years) and the lowest success rate. Furthermore, the study found that individuals with unexplained infertility and a male factor had the shortest period of infertility and the highest IVF success rate. Moreover, the association between the length of infertility and IVF success was not significant in our study, which was consistent with previous studies by Ludwig *et al*. and Wolff *et al*. [34,35]. As for the number of Oocytes, the higher the count of Oocytes, the higher the success rate in IVF, especially in women who had infertility due to the male factor, although the results do not show any significant differences. This study is consistent with the previous study by Magnusson *et al*. [36] and disagrees with Abdulwahid *et al*. [21]. The number of Oocytes in women is gradually reduced with age. A decrease in Oocyte quality is also one of the main factors that worsen pregnancy outcomes with increasing age [37]. The study showed that the higher the number of embryos, the higher the success rate in IVF. It was found that the highest percentage of embryo numbers among women who were infertile was because the male factor had the highest number of Oocytes, with significant differences ($P<0.01$). Ovaries demonstrate that older and obese women are more likely to produce poor-quality embryos, suggesting that age and BMI were contributing factors in this case [33]. The repeated cycles of IVF varied between 1 and 2, and the study found that the majority of women who underwent IVF more than once had a poor success rate, with a significant difference ($P<0.05$). According to our study, women who suffered infertility due to the tubal factor had the lowest success rate for IVF despite having attempted it more than once (1.88 ± 1.16). The results from our study are consistent with a previous study conducted by Ni *et al*. [38]. The success rate of IVF remains limited in some women despite transfers of good-quality embryos in repeated attempts [39]. The current study showed that the thickness of the endometrium ranged between 7-8 mm, and it was discovered that it plays a role in the success of the fertilization process, although there was no significant difference. Our study does agree with a previous study by Sun *et al*. [37]. Previous studies found that endometrial thickness has a limited ability to identify women who have a low chance of pregnancy after IVF. Endometrial thickness and responsiveness during early IVF stimulation seem to be better prognostic predictors of success than endometrial thickness at the start or end of the IVF cycle [40]. According to the study, the greater the number of mature embryos, the higher the success rate in IVF. Infertile women due to the male factor have a higher number of mature embryos (7.14 ± 2.36) and the highest success rates, which may be related to a higher number of embryos and oocytes compared to other reasons for infertility. This result is in agreement with a previous study by Cadenas *et al*. [41] and Ma *et al*. [42]. The study found that the PCO group had a lower number of mature embryos (5.62 ± 1.50), which could be attributable to a decrease in the number of embryos and the number of oocytes, although this result disagreed with a previous study by Abdulwahid *et al*. [21]. According to our findings, women with tubal factor had the highest value of embryo transfer (7.98 ± 1.48), and the association between embryo transfer and IVF success was significant (P<0.01), which could be attributed to the fact that patients with tubal factor had the longest period of infertility (6.88 ± 4.59 years), and the majority of women who underwent more repeated cycles of IVF. The overall number of embryos to be transferred at one time is finally based on the woman's age and ovarian stimulation (OS), which is one of the key factors in the success of in vitro fertilization embryo transfer (IVF-ET) by enabling the recruitment of numerous healthy fertilizable oocytes and thereby multiple embryos. Our results disagree with the previous study by Hashem *et al*. [43]. According to the current study, women with PCO have the highest level of LH, PRL, and testosterone. In contrast, the tubal factor group had elevated levels of FSH, TSH, and AMH. Our findings contradict to a prior work by Abdulwahid *et al.* [21]. Patients with male factor had higher levels of estrogen, progesterone, and tyrosine kinase 1. Although there were no significant changes in our data, most hormones have a vital role in the success of infertility. However, AMH levels were low in our sample, which could be linked to infertility and IVF failure. Furthermore, the majority of the patients had relatively low levels of the AMH hormone (normal range 1.6 to 4 ng/mL), and no statistically significant differences in AMH were found. Regardless of whether AMH levels are low, women still have an acceptable chance of becoming pregnant, according to a different study [44]. FMS-like Tyrosine kinase 1 levels were observed to be directly correlated to gestational age and increased with gestational age in early pregnancy [45]. Furthermore, a large variation in FMS-like Tyrosine kinase 1 levels was observed between study groups in our study. Patients with male factors showed higher levels of FMS-like Tyrosine kinase 1 compared to patients with unexplained female causes and mixed causes. The median FMS-like Tyrosine kinase 1 level in male factor infertility was 1987.95 pg/mL, which was significantly higher than that in other infertility factors. Studies have reported changes in FMS-like Tyrosine kinase 1 concentrations [46]. Given that there are no established normative levels of FMS-like Tyrosine kinase 1 in pregnancy, it is difficult to assess how significant these differences are or to define normal versus abnormal concentrations. Moreover, there is little information about the effect of maternal factors such as body mass index and smoking on FMS-like Tyrosine kinase 1 levels [47]. The variation in FMS-like Tyrosine kinase 1 concentrations also underscores the need to understand and ensure results are comparable between different types of biological samples, as measurements of plasma and serum may not yield similar results [46]. FMS-like Tyrosine kinase 1 was able to distinguish infertility from intrauterine pregnancy, with a highly significant difference at (P<0.01) as stated in a previous study by Wataganara *et al*. [48]. Measurement of FMS-like Tyrosine kinase 1 therefore aids in identifying the implantation of the embryo at an ectopic site [49].

5. Conclusion

Our study concluded that the clinical parameters were closely associated with IVF outcomes, which could provide information for future clinical interventions to increase success rates. The findings show that there is a close association between FMS-like Tyrosine kinase 1 level and IVF success, and it can be used as a valuable marker to confirm the extent of IVF failure or success.

Ethics clearance

The research ethical committee at scientific research has the ethical approval of environmental, health, higher education, and scientific research ministries in Iraq

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