Mohammed and Haddad



Iraqi Journal of Science, 2024, Vol. 65, No. 9, pp: 4901-4911 DOI: 10.24996/ijs.2024.65.9.7



ISSN: 0067-2904

# Relationship Between Anti-Mullerian Hormone (AMH) and Other Influencing Factors with *in Vitro* Fertilization (IVF) Outcome of Iraqi Infertile Women

### Lubna S. Mohammed\*, Namir I.A. Haddad

Department of Chemistry, College of Science, University of Baghdad, Al-Jadriya, Baghdad, Iraq

Received: 15/6/2023 Accepted: 12/8/2023 Published: 30/9/2024

#### Abstract

The clinical application of Anti-Mullerian Hormone (AMH) measurement has been proposed in the prediction of qualitative and quantitative aspects of assisted reproductive technologies (ART). The aim of this study is to investigate the role of AMH level and other influencing factors in the outcomes of in vitro fertilization (IVF) treatment. The study focused on AMH and its association with infertile women undergoing IVF. The population included in this study was 131 infertile women who sought therapy for infertility at Kamal Al-Samarra'ay Specialized Hospital. They were divided into three groups according to the level of AMH hormone; group A (0.5-1.5 ng/mL, n=52), group B (1.6-4 ng/mL, n=70), and group C (> 4 ng/mL, n=9). Data collection was performed by means of a specially designed questionnaire, independent of demographic data. It included questions related to infertility. Statistical analysis was performed with SPSS 26. Clinical parameters of infertile women, infertility hormones and their relationship with AMH, as well as FMS-like Tyrosine kinase 1 levels, were all investigated. The study findings revealed a significant difference in the concentration of AMH between infertile women (p < 0.01). Our analysis further demonstrated that the level of AMH reduces with advancing age in infertile women. Additionally, a positive correlation was observed between the number of oocytes obtained and AMH levels in infertile women. Furthermore, the study found that AMH levels had an effect on clinical pregnancy outcomes. Specifically, the highest success rates for IVF were observed in women with normal AMH levels falling within the ranges of group B (6-4 ng/mL). Additionally, the study demonstrated significant differences in AMH and TSH levels among infertile groups (p < 0.05). Our study concluded that there is a significant correlation between clinical parameters and IVF outcomes, which can provide information for future clinical interventions aimed at improving success rates. Our analysis highlighted the role of FMS-like Tyrosine kinase 1 in infertility, where higher FMS-like Tyrosine kinase 1 levels were associated with higher success rates in IVF. The results indicate that the FMS-like Tyrosine kinase 1 level is a valuable marker for confirming the success or failure of IVF and can help determine the appropriate treatment plan.

Keywords: AMH levels, FMS-like Tyrosine kinase 1, Infertile women, IVF.

<sup>\*</sup>Email: <u>lobna.saleem1205m@sc.uobaghdad.edu.iq</u>

# العلاقة بين هرمون المضاد لمولر (AMH) و العوامل المؤثرة الأخرى مع نتائج التلقيح الصناعي (IVF)في النساء العراقيات المصابات بالعقم

لبنى سليم محمد \* , نمير ابراهيم عباس حداد قسم الكيمياء، كلية العلوم، جامعة بغداد، الجادرية، بغداد، العراق

#### الخلاصة

تم اقتراح التطبيق السريري لقياس هرمون (AMH) في التنبؤ بالجوانب الكمية والنوعية في تقنيات المساعدة على الإنجاب (ART). الهدف من هذه الدراسة هو التحقق من دور مستوى هرمون AMH والعوامل المؤثرة الأخرى في نتائج علاج الإخصاب في المختبر (IVF). ركزت الدراسة على هرمون AMH وعلاقته بالنساء المصابات بالعقم اللواتي يخضعن لعمليات التلقيح الصناعي. شملت الدراسة مجتمع الدراسة 131 امرأة مصابة بالعقم اثناء تلقيهم للعلاج من العقم في مستشفى كمال السامرائي التخصصي. تم تقسيمهم إلى ثلاث مجموعات وفقًا لمستوى هرمون AMH ، المجموعة أ (0.5–1.5 نانوغرام / مل ، n= 52) ، المجموعة ب (1.6 - 4 نانوغرام / مل ، n = 70) ، والمجموعة ج (> 4 نانوغرام / مل ، n = 9). تم إجراء جمع البيانات عن طريق استبيان مصمم خصيصًا لذلك ، بصرف النظر عن البيانات الديموغرافية ؛ تضمنت أسئلة تتعلق بالعقم. تم إجراء التحليل الإحصائي باستخدام SPSS 26. تم فحص جميع المعايير السربرية للنساء المصابات بالعقم ، وهرمونات العقم وعلاقتها مع AMH ، وكذلك مستوى FMS-like Tyrosine kinase P ) لمنعت نتائج الدراسة عن اختلاف معنوى في تركيز هرمون AMH بين النساء المصابات بالعقم 10.01>). أظهر تحليلنا كذلك أن مستوى هرمون AMH انخفض مع تقدم العمر لدى النساء المصابات بالعقم. بالإضافة إلى ذلك ، لوحظ وجود علاقة إيجابية بين عدد البوبضات التي تم الحصول عليها ومستوبات هرمون AMH في النساء المصابات بالعقم. علاوة على ذلك ، كشفت الدراسة أن مستويات هرمون AMH لها تأثير على نتائج الحمل السريرية. كذالك ، لوحظت أعلى معدلات نجاح لعمليات التلقيح الصناعي في النساء ذوات مستويات هرمون AMH الطبيعية التي تقع ضمن نطاقات المجموعة ب بالإضافة إلى ذلك ، أظهرت نتائج الدراسة فروق ذات دلالة إحصائية في مستويات AMH و TSH بين مجموعات العقم (0.05> P). خلصت دراستنا إلى أن هناك علاقة كبيرة بين الدوال السريرية ونتائج التلقيح الاصطناعي ، والتي يمكن أن توفر معلومات للتدخلات السريرية المستقبلية التي تهدف إلى تحسين معدلات النجاح. سلط تحليلنا الضوء على دور FMS-like Tyrosine kinase 1 في العقم ، حيث ارتبطت مستويات مرتفعة من FMS-like Tyrosine kinase 1 بمعدلات نجاح أعلى في التلقيح الاصطناعي. تشير النتائج إلى أن مستوى FMS-like Tyrosine kinase 1 هو دالة سريرية قيمة لتأكيد نجاح أو فشل التلقيح الاصطناعي ويمكن أن يساعد في تحديد خطة العلاج المناسبة.

#### **1. Introduction**

Infertility is a condition of the human reproductive system that is one of the most frequent health concerns all over the world. It is the inability of a man or woman to produce children, whether due to psychological or biological factors. According to the data, between 10 and 15% of couples between the ages of 18 and 45 are infertile, and as a result of certain lifestyle and environmental changes, the prevalence of infertility has risen rapidly, becoming the third most hazardous disease after cancer and heart disease [1]. There are two types of infertility: primary and secondary. Primary infertility is the inability to conceive after 12 Months, whereas secondary infertility is infertility after having conceived at least once previously [2]. About 12.7% of women of childbearing age seek infertility treatment each year. Approximately 85% of infertile couples have identifiable causes. However, because female fertility declines with age, immediate assisted ART techniques, including IVF, may be considered a first-line treatment for women older than 35 years. Although the success rate

varies by diagnosis and age, accurate diagnosis, efficient treatment, and shared decisionmaking can help many infertile couples accomplish their goals for a successful pregnancy [3]. The granulosa cells of the ovarian follicle produce anti-Mullerian hormone, a glycol-protein hormone that affects female fertility. It has the same structure as inhibin and activin, which are part of the transforming growth factor beta superfamily. The main jobs of inhibin and activin are to stop growth and make folliculogenesis happen [4]. Anti-Mullerian hormone is a glycolprotein with a molecular weight of 140 kDa that belongs to the growth factor superfamily. The human AMH gene, which is situated on the short arm of chromosome 19, is made up of 275 base pairs that are separated into five exons [5]. Also, AMH, known as the inhibitory Mueller factor or substance, was identified in 1947 as a measure of ovarian reserve and is an important part of infertility testing [6]. Additionally, since the ovaries secrete AMH into the circulation in women, AMH is detectable in serum. Since serum AMH levels primarily reflect the ovarian follicular pool, a decrease in the number of small, growing follicles may be followed by a decrease in circulating AMH [7]. Higher levels indicate a high number of eggs in the body [8]. And its highest levels are usually recorded when a woman reaches about 25 years old, and then its levels begin to decline after reaching the age of thirty. Studies have indicated that the AMH hormone can be analyzed on any day of the menstrual cycle, implying that its levels can be analyzed on any day of the menstrual cycle. It does not change [9]. Anti-Mulleria hormone is often used to examine ovarian function and reproductive concerns in women, particularly during pregnancy-assisted procedures like in vitro fertilization, or what is known as in vitro fertilization. Many more reasons include forecasting the beginning of menopause, or what is known as menopause, determining the cause of early menopause, and determining the cause of the lack of menstruation or the absence of a period [5]. Female infertility may be due to hormonal imbalances such as polycystic ovary syndrome (PCO), which is an endocrine condition clinically defined as chronic anovulation associated with hyperandrogenism [10,11]. FMS-like Tyrosine kinase 1 is the soluble variant of the Vascular Endothelial Growth Factor (VEGF) receptor-1, a protein FMS-like Tyrosine kinase with anti-angiogenic characteristics. It was first discovered by Kendall and Thomas in 1993. Also, FMS-like Tyrosine kinase 1 is a physiological regulator of angiogenesis in the skeleton and growth of the embryo and is also involved in vasculogenesis, angiogenesis, and lymphangiogenesis during embryonic and postnatal development in reproduction [12]. The main source of FMS-like Tyrosine kinase 1 in pregnancy is the placenta, which plays a major role in the establishment of a balance between angiogenesis and vascular [13]. FMS-like Tyrosine kinase 1 has the potential to predict serious adverse pregnancy events, such as severe pneumonia, which may cause preeclampsia symptoms, intensive care unit admission, viral sepsis, and maternal death [14,15]. The aim of this study is to investigate the role of AMH levels in the outcomes of in vitro fertilization (IVF).

# 2. Experimental part

# 2.1. Study subjects

The current prospective study includes 131 infertile patients who were attending the Kamal Al-Samarra'ay Specialized Hospital for IVF treatment between October 2021 and October 2022. This study was approved by the ethics committee of the hospital, and all patients had given their signed informed consent to participate.

# 2.2. Questionnaire and clinical data

The study included 131 infertile women receiving IVF (+/-ICSI) therapy and was conducted using a convenient sampling approach. Demographic and pregnancy outcomes data were analyzed, including Body Mass Index (BMI), name, age, husband's age, infertility duration, causes of infertility, follicle number, mature oocytes number, basal concentrations of

AMH, endometrial thickness, number of embryos transferred, fertilization rate, and FMS-like Tyrosine kinase 1 level.

# 2.3 Collecting patients

Samples were selected for women ages ranging from 20 to 45 who suffered from primary or secondary infertility and patients who underwent the IVF procedure more than once. On the second or third day of the menstrual cycle, a fasting blood sample was taken to evaluate the hormones LH, FSH, estrogen, testosterone, progesterone, PRL, TSH, and AMH. All 131 infertile patients who completed the inclusion requirements were recruited and were then separated into three groups according to the AMH hormone level: group A (0.5-1.5 ng/mL, n=52), group B (1.6-4 ng/mL, n=70), and group C (> 4 ng/mL, n=9). Frozen embryos and those with a BMI < 18.5 kg/m<sup>2</sup> and an AMH of less than 0.5 were excluded from the study. Biochemical pregnancy was defined as a blood human chorionic gonadotropin (HCG) level of more than 25 units/L 12-14 days after the transfer of the embryo.

# 2.4 Hormonal assay

In the hospital's endocrinology laboratory, all hormone levels were assessed on the same day. Estrogen and progesterone were measured on the morning of HCG administration. On the day of oocyte retrieval, a specialist used transvaginal ultrasonography to determine endometrial thickness.

# 2.5 Protein FMS-like Tyrosine kinase1 analysis

The blood sample was taken on the day of the oocyte retrieval. The blood specimens were collected and placed in a glass tube containing a gel separator. Following a 10-minute incubation period at 37 °C the samples were subjected to centrifugation at  $3000 \times g$  for 3 minutes to isolate the serum. The resulting serum was then transferred to an Eppendorf tube and stored at a temperature of -20 °C until required for parameter estimation. The ELISA was measured and the O.D. absorbance read at 450 nm in a microplate reader, and then the concentration of FMS-like Tyrosine kinase 1 can be calculated. 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. The mean and standard deviation (SD) were employed to represent data with a normal distribution. The sample characteristics were established using means and frequencies. When comparing categorical data, chi-square tests were performed, and when comparing the means of several groups for quantitative variables, ANOVA was utilized. A p-value less than 0.05 was considered statistically significant, and a p-value of less than 0.01 was considered statistically highly significant [16].

# 3. Results

One hundred and thirty-one infertile women who were referred to Kamal Al-Samarra'ay Specialized Hospital were recorded in our study. Table 1 shows the demographic characteristics of infertile women depending on the range of the AMH hormone. Our study revealed the most successful IVF group in terms of the effect of AMH on infertility. In this study, the most common age-related group for AMH levels among 70 women having IVF was the range of 1.6 to 4 ng/mL. Thirty-two of them (24.4%) had successful pregnancies, while 38 (29.0%) experienced failure. The results showed that the second most successful group was AMH group A (0.5 to 1.5 ng/mL) in 52 women who underwent IVF, 20 of them (15.2%) had

successful pregnancies, while 32 (24.4%) did not. Finally, the lowest pregnancy success rate was in the AMH group C range (>4 ng/mL); in 9 of the women who underwent IVF, only 2 (1.5%) had gotten pregnant and 7 (5.3%) failed. As for a comparison between polycystic ovarian syndrome (PCO) and the range of the AMH, our study revealed that when comparing the AMH groups to other causes of infertility, the B group with an AMH range of 1.6 to 4 ng/mL had the lowest percentage (8 out of 70 women) that were affected by PCO. This group also had the highest success rate in achieving pregnancy. Additionally, our study revealed that group B, which consisted of 33 women out of 70 women, had the male factor as the cause of infertility, representing the highest percentage (25.1%). The results presented in Table 1 indicated that there were highly significant differences (p-value < 0.01) in AMH levels across the three groups studied. Our results also revealed statistically significant differences when comparing the AMH range to TSH. However, when comparing the AMH range to other infertility hormones or parameters such as Endometrial Thickness (E.T.), Oocyte number, mature Oocyte, number of embryos, age, BMI, duration of infertility, and repeated IVF cycles, the results showed no significant differences. However, there is no statistically significant FMS-like Tyrosine kinase 1 that exhibits clear variations across groups of infertile women.

Parameter	Groups				<i>p</i> -value		
	Group A	Group B	Group C	<i>p1</i>	<i>p2</i>	р3	
	(n=52)	(n=70)	(n=9)				
AMH range	0.5-1.5 ng/mL	1.6-4 ng/mL	> 4 ng/mL				
IVF Success				-	-	-	
Yes,n,(%)	20(15.2%)	32(24.4%)	2(1.5%)				
No,n,(%)	32(24.4%)	38(29.0%)	7(5.3%)				
РСО		8(6.1%)	1(0.7%)	-	-	-	
Yes, n, (%)	6(4.5%)	62(47.3%)	8(6.1%)				
No, n (%)	46(35.1%)						
Causes	18(13.7%)	33(25.1%)	4(3.05%)	-	-	-	
Male factor, n, (%)	4(3.05%)	5(3.8%)	2(1.5%)				
Tubal, n, (%)	16(12.2%)	13(9.9%)	1(0.71%)				
Unexplained, n, (%)	8(6.1%)	11(8.3%)	1(0.7%)				
Mixed, n, (%)							
Oocyte number	9.03±2.59		8.66±4.69	0.89	0.94	0.83	
(mean±SD)		9.30±3.26					
Mature Oocyte	6.59±2.10	6.88±2.24	6.33±3.20	0.76	0.94	0.77	
No of Embryo	5.78±1.77	5.78±1.69	5.33±1.93	1.00	0.75	0.74	
Age (mean±SD) year	32.01±6.15	30.7±4.97	30.44±4.36	0.38	0.70	0.99	
BMI (mean±SD) kg/m <sup>2</sup>	27.62±4.32	26.20±4.33	29.06±5.58	0.18	0.64	0.16	
Duration of Infertility	6.19±3.97	5.13±2.91	$4.00 \pm 2.29$	0.20	0.16	0.60	
(mean ±SD)							
Endometrial Thickness (mean ±SD)	7.90±1.44	$7.93 \pm 1.26$	7.82±0.94	0.99	0.98	0.96	
Embryo Transfers	3.42±1.10	3.04±1.14	3.22±0.97	0.15	0.87	0.89	
Fertilization Rate	52.14±18.81	53.25±20.81	53.09 ±15.81	0.95	0.99	1.00	
LH (µIU/mL)	3.16±1.63	3.06±1.37	3.31±2.23	0.92	0.96	0.89	
FSH (µIU/mL)	5.01±1.87	4.90±1.28	4.81±1.52	0.91	0.93	0.98	
FSH/LH Ratio	0.64±0.30	0.63±0.28	0.76±0.61	0.96	0.59	0.50	
E2 (pg/mL)	1275.68±532.12	1343.003±556.8	1238.25±436.02	0.77	0.98	0.84	
		6					
Testosterone (ng/mL)	0.44±0.16	0.43±0.16	0.49±0.19	0.99	0.67	0.62	

**Table 1:** The demographic information and clinical features of the studied groups were

 distributed based on their AMH levels

Progesterone (ng/mL)	0.37±0.11	0.38±0.08	0.37±0.08	0.95	0.98	0.94
PRL (ng/mL)	18.03±6.51	16.90±6.08	19.06±5.59	0.58	0.89	0.59
TSH(µIU/mL)	$2.09 \pm 1.35$	2.01±0.97	$0.95 \pm 0.34$	0.93	0.01	0.022
					5	
AMH (ng/mL)	1.02±0.30	2.29±0.75	4.84±1.30	< 0.0	< 0.0	< 0.01
				1	1	
Repeated IVF Cycles	1.44±0.72	1.24±0.46	$1.11 \pm 0.33$	0.14	0.25	0.79
Tyrosine kinase1 (pg/mL)	1302.70	1575.60±780.41	1243.36 ±360.92	0.08	0.96	0.36
	±601.14					

The results were expressed as mean  $\pm$  SD, range (minimum-maximum), or number (percentage). *P1* = the group A compared with group B; *P2* = the group A compared with group C; *P3* = the group B compared with group C, (*p*>0.05) = non-significant differences; (*p*<0.05) = significant differences; (*p*≤0.01) = high significant differences.

#### 4. Discussion

The purpose of our study was to determine the relationship between infertility and AMH in women undergoing IVF treatment. Patients were divided into three categories based on AMH range, group A (low AMH 0.5-1.5 ng/mL), group B (normal AMH 1.6-4 ng/mL), and group C (High AMH >4 ng/mL). This study is a retrospective of 131 infertile patients with the purpose of knowing how important this diagnostic marker is to IVF success.

A high AMH level is associated with PCO and is therefore suggested as a diagnostic criterion. However, no universally acknowledged AMH value may be utilized to diagnose PCO [17]. Also, AMH levels and pregnancy outcomes are still debated [18]. Furthermore, AMH is supposed to be one of the most significant predictors of response to ovarian stimulation that can be measured before initiating IVF or ICSI therapy [19]. A lower AMH level was indicative of a weakened ovarian response and fewer retrievable Oocytes. Nelson *et al.* conducted a separately controlled prospective study [20].

Similarly, it shows that AMH is an accurate predictor of ovarian responsiveness. They found that low levels of AMH were associated with reduced rates of fertilization and oocyte production. Serum AMH calculates ovarian reserve, helps in dosage selection for ovarian stimulation, and forecasts response to stimulation. AMH is a decent indicator of the number of Oocytes, but it does not indicate the health of the oocytes or the likelihood of pregnancy [21]. The higher the number of oocytes, the higher the success rate in IVF, especially in women who had infertility due to normal AMH, although the results do not show any significant differences. This study is consistent with a previous study by Magnusson *et al.* [22] and disagrees with Abdulwahid *et al.* [23]. The number of Oocytes in women gradually decreases with advanced age. Decreased oocyte quality is also a major factor that worsens pregnancy outcomes with age [24].

According to the current study, the greater the number of embryos and the number of mature embryos, the higher the success rate in IVF. Infertile women who had normal AMH (group B) had a higher number of mature embryos ( $6.88 \pm 2.24$ ) and the highest success rates, which may be related to a higher number of embryos and Oocytes compared to other AMH groups of infertility. This result is in agreement with a previous study by Cadenas *et al.* and Ma *et al.* [25,26]. Furthermore, the study found that group C with an AMH level >4 ng/mL had a lower number of embryos ( $6.33 \pm 3.20$  which could be attributable to a reduction in the number of embryos and oocytes, although this result disagreed with a previous study by Abdulwahid *et al.* 

Maternal age has a well-established and consistent effect on AMH levels and Oocyte retrieval. According to the current study, all women who participated in assisted reproduction programs were between the ages of 20 and 45, and this is consistent with a recent study done by Al-Abbasi *et al.* in 2023 that found that women under the age of 35 years have more chances of pregnancy compared to women over the age of 35 years related to levels of AMH [27]. Low levels of AMH before 39 years may be associated with a higher risk of menopause between 40 and 45 years [28]. Age is one of the important factors that affect hormone levels, and generally, older women are more susceptible to AMH deficiency due to their insufficient ovaries production and decreased fertility due to age, especially when they are over 35. However, our study contradicts a previous study conducted by Gomez *et al.* [29]. Table 1 revealed a significant decrease in the level of AMH in infertile women with increasing age, which confirms that the decrease in AMH concentration is a sign that increases the risk of infertility.

Through our study, we have noticed that infertile women have a normal AMH level in group B. It achieved the highest pregnancy success rate, and they were younger, with an average age  $(30.7 \pm 4.97)$ , and this is consistent with previous studies [30]. Statistics in Iraq show that older women are more involved in assisted reproduction. It was found that the success rate of IVF decreases with age [31]. Our research's findings agree with those of a prior study by Roupa *et al.* [32].

The BMI of the women in our study was between 27 and 29 kg/m<sup>2</sup>, indicating that they were overweight and may be connected with infertility since it affects ovulation and hormones. Particularly group C with (AMH > 4 ng/mL) has the highest BMI (29.06± 5.58 kg/m<sup>2</sup>). A higher BMI had a clear negative effect on fertility. Our study agrees with a previous studies by Sneed *et al.* [33], Ahmed and Al-Lami [34]. Another study showed that elderly and obese women are more likely to give birth to poor-quality fetuses [35].

Based on the impact of infertility duration on the results of IVF, those who have been infertile for a long period are not good candidates for IVF. According to the study, patients in group A had the longest period of infertility ( $6.19\pm3.97$  years). Furthermore, the study found that individuals with normal and high AMH had the shortest period of infertility. Moreover, the association between infertility duration and IVF success was not significant in our study, which was consistent with previous studies by Ludwig *et al.* [36] and Wolff *et al.* [37].

The current study showed that the thickness of the endometrium ranged between 7-8 mm, and it was discovered that it play 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.* [24]. Previous studies found that endometrial thickness has a limited ability to identify women who have a low chance of getting pregnant after IVF. Endometrial thickness and its response during early IVF stimulation appear to be a better predictor of success than endometrial thickness at the beginning or end of an IVF cycle [38].

According to our findings, women in group A with low levels of AMH (0.5-1.5 ng/mL) had the highest value of embryo transfer  $(3.42 \pm 1.10)$ . The association between embryo transfer and IVF success was not significant (*P*>0.01), which could be attributed to the fact that patients in group A had the longest period of infertility (6.19 ± 3.97 years) and had undergone more repeated cycles of IVF.

The total quantity of embryos that will be transferred at one time is finally based on the woman's age. Ovarian stimulation is crucial to the success of *in vitro* embryo transfer because it allows for the recruitment of multiple healthy, fertile oocytes and, consequently, multiple embryos. Our result is consistent with the previous research by Hashem *et al.* [39]. Lower AMH levels were associated with a poorer ovarian response and fewer retrievable oocytes, and AMH was also found to be a good predictor of ovarian reactivity. In another prospective, controlled investigation, they observed that reduced AMH correlates with poorer Oocyte

yields and fertilization rates. They also discovered that patients with low AMH levels had a greater probability of miscarriage [20].

According to the current study, women in group C have the highest levels of LH, AMH, the LH/FSH ratio, and testosterone, while FSH and TSH were high in the A group. Our findings contradict prior work by Abdulwahid *et al.* [23]. Patients in the B group (normal AMH 1.6-4) had higher levels of estrogen, progesterone, PRL, and FMS like-tyrosine kinase 1. Although there were no significant differences in our data, most hormones play a vital role in the success of fertility.

However, AMH and TSH levels were low in our sample and associated with significant differences between infertile groups, which could be linked to infertility and IVF failure. In addition, most of the patients had AMH hormone levels that were relatively low, with the normal range being 1.6 to 4 ng/mL, and there were significant differences. Regardless of whether AMH levels are low, women still have an acceptable chance of becoming pregnant. Our study agrees with a previous study conducted by Gomez *et al.* [29], Marca *et al.* [40], and Brodin *et al.* [41], who showed a significant relationship between FSH and AMH levels and that patients with low AMH levels had a higher risk of cycle cancellation, mostly due to decreased ovarian response [42-44]. Another study by Marca *et al.* Has shown that AMH can predict the chance of success in both younger and older women [45].

We observed through our study that group B with a normal AMH level had the highest *in vitro* fertilization success rate due to the fact that it had the highest number of mature oocytes, E2, Progesterone, PRL, FMS-like Tyrosine kinase 1, Lowest BMI, Embryo Transfer, LH Hormone, and Testosterone hormone.

Through our study and in terms of causes of infertility, women whose husbands cause infertility have the highest incidence of infertility. According to the current study, women in group C have the highest levels of LH, AMH, the LH/FSH ratio, and testosterone.

The study found that the majority of women who had more Repeat cycles of IVF had a poor success rate, with no significant difference. According to our study, women who experienced infertility due to group C (AMH >4 ng/mL) had the lowest IVF success rate despite trying more than once  $(1.11 \pm 0.33)$ . The results of our study are not consistent with a previous study by Ni *et al.* [46]. The success rate of IVF remains limited in some women despite good-quality embryo transfers on repeated attempts [47].

FMS-like Tyrosine kinase 1 levels were observed to be directly correlated to gestational age and increased with gestational age in early pregnancy [48]. Furthermore, a large variation in FMS-Like Tyrosine kinase 1 level was observed between study groups; patients in group B with normal AMH levels (1.6-4 ng/mL) showed higher levels of FMS-like Tyrosine kinase 1 (1575.6 pg/mL) compared to other groups. Studies have reported changes in FMS-like Tyrosine Kinase 1 concentration. Although there is no evidence for the optimum value of FMS-like Tyrosine Kinase 1 levels during pregnancy, it is difficult to assess how these differences are intended or to determine normal versus excessive concentrations. Moreover, there is little information about the effect of maternal factors such as BMI and smoking on FMS, like Tyrosine kinase 1 levels [49]. The difference in FMS-like Tyrosine kinase 1 concentrations also emphasizes the need for understanding and ensuring that results are comparable between different types of biological samples.

Results from measurements of serum and plasma may not be comparable [50]. Our data suggest that FMS-like Tyrosine kinase 1 can serve as a biomarker of infertility associated

with negative outcomes in women with low AMH. Despite our study, no significant differences were shown for tyrosine kinase 1, but it has an important role, as we noticed that its concentration increases in pregnant women, and the highest success rates of IVF were for women with a normal level of AMH.

# **5.** Conclusion

Our study concluded that AMH, which is considered to be of high predictive value in evaluating ovarian reserve, was closely associated with IVF outcomes, which could provide information for future clinical interventions to increase success rates. As AMH may permit the identification of both extremes of ovarian stimulation, a possible role for its measurement may be in the individualization of treatment strategies in order to reduce the clinical risk of ART and optimize treatment burden.

# **Ethics clearance**

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

### References

- [1] S. Ahmed, K. F. Al-Rawi, and R. M. Murshid, "The relationship of anti-mullerian hormone (AMH) with calcyphosina in polycystic ovary syndrome women", *Journal of Optoelectronics Laser*, vol. 41, no. 10, pp. 307-311, 2022.
- [2] J. T. Choy and M. L. Eisenberg, "Male infertility as a window to health", *Fertility and Sterility*, vol. 110, no. 5, pp. 810-814, 2018.
- [3] S. A. Carson and A. N. Kallen, "Diagnosis and management of infertility: a review", *Jama*, vol. 326, no. 1, pp. 65-76, 2021.
- [4] R. Kucera, V. Babuska, Z. Ulcova-Gallova, V. Kulda, and O. Topolcan, "Follicular fluid levels of anti-mullerian hormone, insulin-like growth factor 1 and leptin in women with fertility disorders", *Systems Biology in Reproductive Medicine*, vol. 64, no. 3, pp. 220-223, 2018.
- [5] M. Rzeszowska, A. Leszcz, L. Putowski, M. Hałabiś, J. Tkaczuk-Włach, J. Kotarski, and G. Polak, "Anti-mullerian hormone: structure, properties and appliance", *Ginekologia Polska*, vol. 87, no. 9, pp. 669-674, 2016.
- [6] A. M. Kotlyar and D. B. Seifer, "Ethnicity/race and age-specific variations of serum AMH in women a review", *Frontiers in Endocrinology*, vol. 11, Article no. 593216, 2021.
- [7] A. L. Marca, G. Sighinolfi, D. Radi, C. Argento, E. Baraldi, A. C. Artenisio, G. Stabile, and A. Volpe, "Anti-mullerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART)", *Human Reproduction Update*, vol. 16, no. 2, pp. 113-130, 2010.
- [8] B. S. Kadhum and S. A. W. Al-Shammaree, "Association of iron status in follicular fluid with pregnancy outcomes in infertile women undergoing IVF/ICSI", *Iraqi Journal of Science*, vol. 62, no. 6, pp. 1779-1786, 2021.
- [9] N. Josso, "Women in reproductive science: anti-müllerian hormone: a look back and ahead", *Reproduction*, vol. 158, no. 6, pp. F81-F89, 2019.
- [10] T. R. Segal and L. C. Giudice, "Before the beginning: environmental exposures and reproductive and obstetrical outcomes", *Fertility and Sterility*, vol. 112, no. 4, pp. 613-621, 2019.
- [11] Z. M. Almusawi, N. I. A. Haddad, and Ekhlas A. Husein, "Dectin-1 levels in obese and overweight women with polycyctic ovary syndrome (PCOS)", *International Journal of Pharmaceutical Research*, vol. 12, no. 2, pp. 1095-1102, 2020.
- [12] S. Selvarajan, J. Ramalingam, and V. Venugopal, "Soluble FMS-like tyrosine kinase-1: an overview", *International Journal of Medical Biochemistry*, vol. 6, no. 2, pp. 117-123, 2023.
- [13] V. Phupong, W. Areeruk, P. Tantbirojn, and R. Lertkhachonsuk, "Soluble fms-like tyrosine kinase 1 and placental growth factor ratio for predicting preeclampsia in elderly gravida", *Hypertension in Pregnancy*, vol. 39, no. 2, pp. 139-144, 2020.
- [14] J. T. Torres, S. E. Sosa, L. C. Poon, J. M. S. Paredes, G. E. Gutierrez, A. E. Nuñez, and A. J. Reyes, "Increased levels of soluble fms-like tyrosine kinase-1 are associated with adverse

outcome in pregnant women with COVID-19", *Ultrasound in Obstetrics and Gynecology*, vol. 59, no. 2, pp. 202-208, 2022.

- [15] M. M. Abdulla, N. I. A. Haddad, and E. A. Hussein, "Correlations of serum vitamin D and thyroid hormones with other biochemical parameters in iraqi pregnant women with preeclampsia disease". *Journal of Global Pharma Technology*, vol. 11, no. 2, pp. 441-450, 2019.
- [16] V. E. Johnson, "Revised standards for statistical evidence", *Proceedings of the National Academy* of Sciences, vol. 110, no. 48, pp. 19313-19317, 2013.
- [17] Z. M. Almusawi, N. I. A. Haddad, and Ekhlas A. Husein, "Dectin-1 levels in obese and overweight women with polycyctic ovary syndrome (PCOS) ", *International Journal of Pharmaceutical Research*, vol. 12, no. 2, pp. 1095-1102, 2020.
- [18] B. Dilbaz and Ş. A. Mert, "Evaluation and interpretation of AMH in female infertility", *Duzce Medical Journal*, vol. 24, no. Special issue, pp. 82-85, 2022.
- [19] K. Jayaprakasan, Y. Y. Chan, R. Islam, Z. Haoula, J. Hopkisson, A. Coomarasamy, and N. R. Fenning, "Prediction of in vitro fertilization outcome at different antral follicle count thresholds in a prospective cohort of 1,012 women", *Fertility and Sterility*, vol. 98, no. 3, pp. 657-663, 2012.
- [20] S. M. Nelson, R. W. Yates, and R. Fleming, "Serum anti-mullerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles—implications for individualization of therapy", *Human Reproduction*, vol. 22, no. 9, pp. 2414-2421, 2007.
- [21] M. I. Cedars, "Evaluation of female fertility-AMH and ovarian reserve testing", *The Journal of Clinical Endocrinology & Metabolism*, vol. 107, no. 6, pp. 1510-1519, 2022.
- [22] A. Magnusson, K. Källen, A. T. Kjellberg, and C. Bergh, "The number of oocytes retrieved during IVF: a balance between efficacy and safety", *Human Reproduction*, vol. 33, no.1, pp. 58-64, 2018.
- [23] H. H. Abdulwahid, Z. S. Omran, S. A. Alhasanawy, and R. M. Hussein, "Evaluation of the oocyte quality versus ICSI outcomes in sub fertile Iraqi women with polycystic ovary syndrome", *Journal of Health Sciences*, vol. 6, no. S2, pp. 1108-1114, 2022.
- [24] Y. Sun, J. Zhang, Y. Xu, Z. Luo, Y. Sun, G. Hao, and B. Gao, "Effects of age on pregnancy outcomes in patients with simple tubal factor infertility receiving frozen-thawed embryo transfer", *Scientific Reports*, vol. 10, Article no. 18121, 2020.
- [25] J. Cadenas, L. C. Poulsen, D. Nikiforov, M. L. Grøndahl, A. Kumar, K. Bahnu, and A. L. M. Englund, "Regulation of human oocyte maturation in vivo during the final maturation of follicles", *Human Reproduction*, vol. 38, no. 4, pp. 686-700, 2023.
- [26] L. Ma, L. Cai, M. Hu, J. Wang, J. Xie, Y. Xing, J. Shen, Y. Cui, X. J. Liu, and J. Liu, "Coenzyme Q10 supplementation of human oocyte in vitro maturation reduces postmeiotic aneuploidies", *Fertility and Sterility*, vol. 114, no. 2, pp. 331-337, 2020.
- [27] I. A. Alabbasi and Z. A. M. Al-Jawadi, "The relationhip of anti-mullerian hormone (AMH) with infertile women," *College Of Basic Education Research Journal*, vol. 19, no.1, pp. 753-762, 2023.
- [28] S. Desongnis, G. Robin, D. Dewailly, P. Pigny, and S. Catteau-Jonard, "AMH assessment five or more years after an initially low AMH level", *European Journal of Obstetrics and Gynecology and Reproductive Biology*, vol. 256, pp. 70-74, 2021.
- [29] R. Gomez, M. Schorsch, T. Hahn, A. Henke, I. Hoffmann, R. Seufert, and C. Skala, "The influence of AMH on IVF success", *Archives of Gynecology and Obstetrics*, vol. 293, no. 3, pp. 667-673, 2016.
- [30] L. Ying, L. H. Wu, and A. Y. Loke, "The effects of psychosocial interventions on the mental health, pregnancy rates, and marital function of infertile couples undergoing in vitro fertilization: a systematic review", *Journal of Assisted Reproduction and Genetics*, vol. 33, no. 6, pp. 689-701, 2016.
- [31] N. Nkangana, Outcomes of assisted reproductive technologies in women 40 years and older at Tygerberg Hospital. Diss. Stellenbosch: Stellenbosch University, 2021.
- [32] Z. Roupa, M. Polikandrioti, P. Sotiropoulou, E. Faros, A. Koulouri, G. Wozniak, and M. Gourni, "Causes of Infertility in Women at Reproductive Age", *Health Science Journal*, vol. 3, no. 2, pp. 80-87, 2009.
- [33] M. L. Sneed, M. L. Uhler, H. E. Grotjan, J. J. Rapisarda, K. J. Lederer, and A. N. Beltsos, "Body mass index: impact on IVF success appears age-related", *Human Reproduction*, vol. 23, no. 8, pp. 1835-1839, 2008.

- [34] R. E. Ahmed, and M. Q. Al-lami, "Anti-mullerian hormone and follicle stimulating hormone as markers of ovarian agingin a sample of iraqi women." *Iraqi Journal of Science*, vol. 57, no. 3A, pp. 1671-1679, 2016.
- [35] I. T. Ali, N. I. A. Haddad, and E. A. Hussein. "Assessment of monocyte chemo-attractant protein-1 (MCP-1) and other biochemical parameters in iraqi pregnant women." *Iraqi Journal of Science*, vol. 6, no. 10, pp. 4152-4162, 2022.
- [36] M. Ludwig, D. F. Finas, S. Al-Hasani, K. Diedrich, and O. Ortmann, "Oocyte quality and treatment outcome in intracytoplasmic sperm injection cycles of polycystic ovarian syndrome patients", *Human Reproduction*, vol. 14, no. 2, pp. 354-358, 1999.
- [37] M. V. Wolff, A. K. Schwartz, N. Bitterlich, P. Stute, and M. Fäh, "Only women's age and the duration of infertility are the prognostic factors for the success rate of natural cycle IVF", *Archives of Gynecology and Obstetrics*, vol. 299, no. 3, pp. 883-889, 2019.
- [38] M. Williams, G. De, and J. L. Frattarelli, "Changes in measured endometrial thickness predict in vitro fertilization success", *Fertility and Sterility*, vol. 88, no. 1, pp. 74-81, 2007.
- [**39**] R. Hashem, S. A. Wadood, and Q. A. Mahdi, "The impact of follicular fluid growth differentiation factor 8 levels on IVF/ICSI outcomes", *Biochemistry Cell Biology Archives.*, vol. 19, no. 1, pp. 215-221, 2019.
- [40] A. L. Marca, S. Malmusi, S. Giulini, L. F. Tamaro, R. Orvieto, P. Levratti, and A. Volpe, "Antimullerian hormone plasma levels in spontaneous menstrual cycle and during treatment with FSH to induce ovulation," *Human Reproduction*, vol. 19, no. 12, pp. 2738-2741, 2004.
- [41] T. Brodin, N. Hadziosmanovic, L. Berglund, M. Olovsson, and J. Holte, "Antimüllerian hormone levels are strongly associated with live-birth rates after assisted reproduction", *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 3, pp. 1107-1114, 2013.
- [42] J. Penarrubia, F. Fábregues, D. Manau, M. Creus, G. Casals, R. Casamitjana, F. Carmona, J. A. Vanrell, and J. Balasch, "Basal and stimulation day 5 anti-mullerian hormone serum concentrations as predictors of ovarian response and pregnancy in assisted reproductive technology cycles stimulated with gonadotropin-releasing hormone agonist-gonadotropin treatment", *Human Reproduction*, vol. 20, no. 4, pp. 915-922, 2005.
- [43] T. Ebner, M. Sommergruber, M. Moser, O. Shebl, E. Schreier-Lechner, and G. Tews, "Basal level of anti-mullerian hormone is associated with oocyte quality in stimulated cycles", *Human Reproduction*, vol. 21, no. 8, pp. 2022-2026, 2006.
- [44] F. Lamazou, F. Fuchs, M. Grynberg, V. Gallot, E. Herzog, R. Fanchin, N. Frydman, and R. Frydman, "Cancellation of IVF-ET cycles: poor prognosis, poor responder, or variability of the response to controlled ovarian hyperstimulation? an analysis of 142 cancellations", *Journal De Gynecologie, Obstetrique Et Biologie De La Reproduction*, vol. 41, no. 1, pp. 41-47, 2011.
- [45] A. L. Marca, S. M. Nelson, G. Sighinolfi, M. Manno, E. Baraldi, L. Roli, S. Xella, T. Marsella, D. Tagliasacchi, R. D'Amico, and A. Volpe, "Anti-mullerian hormone-based prediction model for a live birth in assisted reproduction", *Reproductive Biomedicine Online*, vol. 22, no. 4, pp. 341-349, 2011.
- [46] Y. Ni, L. Huang, C. Tong, W. Qian, and Q. Fang, "Analysis of the levels of hope and influencing factors in infertile women with first-time and repeated IVF-ET cycles", *Reproductive Health*, vol. 18, no. 1, pp. 1-9, 2021.
- [47] Q. Qiu, Y. Li, S. W. Fong, K. C. Lee, A. C. H. Chen, H. Ruan, and K. F. Lee, "Endometrial stromal cells from women with repeated implantation failure display impaired invasion towards trophoblastic spheroids", *Reproduction*, vol. 165, no. 3, pp. 335-346, 2023.
- [48] S. Selvarajan, J. Ramalingam, J. Vijayaraghavan, and Z. Bobby, "Evaluation of soluble Fms-like tyrosine kinase-1 in first trimester of pregnancy: A cross-sectional study", *Journal of Clinical and Diagnostic Research*, vol. 13, no. 12, BC01-BC04, 2019.
- [49] O. Erez, R. Romero, J. Espinoza, W. Fu, D. Todem, J. P. Kusanovic, F. Gotsch, "The change in concentrations of angiogenic and anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and smallfor-gestational age", *The Journal of Maternal-Fetal and Neonatal Medicine*, vol. 21, no. 5, pp. 279-287, 2008.
- [50] M. Jacobs, N. Nassar, C. L. Roberts, R. Hadfield, J. M. Morris, and A. W. Ashton, "Levels of soluble fms-like tyrosine kinase one in first trimester and outcomes of pregnancy: a systematic review", *Reproductive Biology and Endocrinology*, vol. 9, no. 1, pp. 1-8, 2011.