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Predictive factors of ovarian response to GnRH antagonist stimulation protocol: AMH and age are potential candidates

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Abstract

Background: Prediction of ovarian response prior to the ovarian stimulation cycle is useful in determining the optimal starting dose of recombinant follicle-stimulating hormone (r-FSH). This study was designed to (I) evaluate which of the following parameters (age, AMH, and FSH) can be used as a predictor of ovarian response to GnRH antagonist stimulation protocol, (II) determine the cutoff value of AMH and age for predicting poor and high ovarian response, and (III) investigate the relationship between age, AMH level, and other clinical parameters. It is a retrospective study. A total of 318 women with a mean age of 28.2 ± 5.9 years old were included in this study. Hormone levels (FSH, LH, PRL, E2, and AMH) and the number of collected oocytes were determined. Based on the number of retrieved oocytes, the participants were divided into three groups: poor response (oocytes < 4, *n*= 51), normal response (oocytes 4–14, *n*= 192), and high response (oocytes > 14, *n*= 75).

Results: A significant increase has been found in AMH level and number of retrieved oocytes and mature oocytes from low to normal and high ovarian response group ($P < 0.001$). Also, the age in the poor ovarian response group was significantly greater than normal and high ovarian response groups ($P < 0.001$). A significant positive correlation has been found between the number of retrieved oocytes and mature oocytes and level of AMH ($P < 0.001$). The receiver operating characteristic (ROC) curves showed that both AMH and age had the highest accuracy in the prediction of poor ovarian response with a cutoff value < 1.45 and > 31.5 years, respectively. Additionally, the ROC analysis has shown that the AMH had the highest accuracy, followed by age in the prediction of high ovarian response with a cutoff value > 3.55 and < 27.5 years, respectively.

Conclusions: This study demonstrates that AMH level and women's age may be used as potential predictors of ovarian response to GnRH antagonist stimulation protocol.

Keywords: Age, AMH, GnRH antagonist, Infertility, Oocyte, Ovarian response

Background

Around 10 to 18% of couples have a problem with having children [1]. Female infertility is considered a primary cause of about 37% of infertile couples [2]. In females, the fecundity will begin to decline significantly when women age arrives at 30–35 years, and it declines sharply after age 37 years [3]. The decline in female fecundity occurs normally as a result of the continuous process of oocyte atresia [4]. The reduction in fertility associated with the female age is characterized by decreases in the quality and quantity of oocytes, gradual elevation in the level of FSH, and reductions in the level of anti-Müllerian hormone (AMH) and inhibin B levels [3]. Nowadays, intracytoplasmic sperm injection (ICSI) is considered one of the most important ways to overcome infertility problems and one of the effective treatment

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approaches that are used by infertility clinics [5]. In assisted reproduction technology, the females undergo ovarian stimulation in order to permit retrieval of multiple oocytes during one cycle, and it is occurring through the administration of exogenous gonadotropins [6]. During this period, maintaining the LH and FSH levels above a critical threshold is considered a very necessary step [7]. The GnRH agonist or antagonist protocol can be used to ensure the prevention of a premature spike of LH that would induce ovulation [8]. During ovarian stimulation protocol, the embryologists use several ovarian reserve tests which include the anti-Müllerian hormone, basal FSH, and antral follicle count as a predictor of ovarian response, ICSI outcomes, and occurrence of pregnancy [9]. However, the use of these tests is still limited because they have a low predictive value, show cycle-dependent fluctuations, and lack clear cutoff values [10]. A previous study showed a negative correlation between the number of antral follicles and ovarian aging [11].

Anti-Müllerian hormone is a glycoprotein produced exclusively in the gonads [12]. In female, the AMH arrived at a high peak of around 25 years and then gradually decline until becoming undetectable before menopause [13]. A previous study has noted that the AMH is better in the prediction of ovarian response than age and basal FSH [14]. Other studies showed that the level of AMH cannot be used as an indicator of embryo quality and pregnancy chances [15]. Other studies found a relationship between the high level of AMH hormone before the start of the stimulation protocol and the increase in the risk of ovarian hyperstimulation syndrome (OHSS) [16]. Several studies showed an association between the level of AMH and the follicular pool, and it can be used as an ovarian reserve marker [17]. AMH also can be used to predict poor as well as excessive response in IVF [18]. This study was designed to (I) assess which of the following parameters (age, AMH, and FSH) can be used as a predictor of ovarian response to GnRH antagonist stimulation protocol, (II) determine the cutoff value of AMH and age for predicting poor and high ovarian response, and (III) investigate the relationship between age, AMH level, and other clinical parameters.

Methods

Study population

This retrospective study included three hundred and eighteen women with a mean age of 28.2 ± 5.9 years old; all cases attended to the Al Bassma Fertility Center in the Palestinian Territories between May 2010 and December 2011. All the participants were selected according to the following inclusion criteria: women age between 18 and 48 years old, women undergoing GnRH antagonist protocols, first ICSI cycle, a normal body mass index, women have a regular menstrual cycle, and the male partner has normal semen parameters. In contrast, the women were excluded from the present study depending on the following criteria: cigarette smokers, diabetes mellitus, women using an oral contraceptive, endocrine abnormality, endocrine disorders (polycystic ovarian syndrome or polycystic ovaries), women with a history of ovarian surgery, and women suffering from a recurrent abortion. The calculations of sample size were based on the formula for a cross-sectional study where the EPI-INFO statistical package version 7.2 was used with a 99.9% confidence interval (CI), 80% power, 0.4 ratios, 3.75 risk ratio, 4.9 odds ratio, and 30% outcome in the exposed group. Consequently, the total sample size was 314 persons. The medical records were used by the researcher to gather the general and medical information that included females' age; body mass index; menstrual history; hormone profile; number of collected oocytes, mature oocytes, immature oocytes, and fertilized oocytes; number of embryos transferred; and the pregnancy results.

Hormone profile and ovarian stimulation

All women included in this study have been undergoing ovarian stimulation by using GnRH antagonist protocols with a recombinant FSH. The ultrasonographic and blood samples were collected from all women on the third day of the menstrual cycle. Briefly, the serum was separated by centrifugation at 3500 rpm for 15 min, and then, all of the following hormones (basal level of E2, FSH, LH, PRL, TSH, and AMH) were measured using the Tosoh Instrument (AIA-360, Tokyo, Japan). The oocyte pickup was scheduled 33–36 h after the administration of 5000 to 10,000 IU of hCG (Pregnyl) depending upon the age of females and the degree of ovarian response. According to the ovarian response, the samples were divided into three groups: a poor response (< 4 oocytes retrieved, n = 51), a normal response (4 to 14 oocytes retrieved, n = 192), and a high response (> 14 oocytes retrieved, n = 75) [19]. Embryo cleavage was evaluated after 16–18 h from ICSI, where the high-quality embryos (grade I or II) were transferred into the uterine cavity after 3 days from ICSI. The embryos were transferred, and all patients received luteal support with vaginal progesterone until a pregnancy test was performed after 2 weeks from embryo transferred. The cases were classified as pregnant women when the level of the β-hCG hormone arrived at more than 5 mIU/mL. For this study, multiple pregnancies were regarded as one pregnancy.

Statistics analysis

All the data were analyzed using IBM SPSS for Windows software package version 24.0 (SPSS, Inc., Chicago, IL, USA). Samples of this study were non-normally

distributed (non-parametric) according to the value of the skewness test, kurtosis test, and Z-value. Kruskal–Wallis (H-test) and Mann-Whitney (U-test) were applied to compare the means of quantitative variables among the study groups. Spearman's test was used to evaluate the correlation coefficient between the clinical parameters. Receiver operating characteristic (ROC) curves were generated for ovarian reserve markers (female age, AMH level, and basal level of FSH) to compare their ability to predict low or high ovarian response. The results in the abovementioned procedures were accepted as statistically significant when $P < 0.05$.

Results

Clinical parameters of the study population and their ovarian response

Table 1 shows that the age in the poor ovarian response group was significantly greater than normal and high ovarian response groups ($P < 0.001$). A significant variation has been found in the basal level of E2 and the oocyte fertilization rate among the study groups ($P = 0.041$ and $P < 0.001$, respectively). In contrast, the levels of AMH and E2 on hCG day; the number of retrieved oocytes, mature oocytes, immature oocytes, and fertilized oocytes; and number of embryos transferred, as well as the value of ß-hCG, were increased significantly from low ovarian response to normal and high ovarian response ($P < 0.001$).

Correlation between clinical parameters of the study population

As indicated in Table 2, a positive significant correlation was found between AMH level ($r = 0.707$, $P < 0.001$); the number of mature oocytes ($r = 0.867$, $P < 0.001$), immature oocytes ($r = 0.779$, $P < 0.001$), and fertilized oocytes ($r = 0.843$, $P < 0.001$); number of embryos transferred ($r = 0.496$, $P < 0.001$) and ß-hCG level ($r = 0.333$, $P < 0.001$); and the number of retrieved oocyte. Conversely, a significant negative association was found between the basal level of FSH and the number of retrieved oocyte ($r = -0.112$, $P = 0.046$). There was also a significant positive association between the number of mature oocytes ($r = 0.558$, $P < 0.001$), immature oocytes ($r = 0.599$, $P < 0.001$), and fertilized oocytes ($r = 0.573$, $P < 0.001$); number of embryos transferred ($r = 0.268$, $P < 0.001$); and ß-hCG level ($r = 0.212$, $P < 0.001$) and AMH level. A significant negative correlation has been shown between AMH level and FSH level ($r = -0.221$, $P < 0.001$). A significant positive correlation was reported between the number of immature oocytes and fertilized oocytes, number of embryos transferred, and ß-hCG level ($r = 0.445$, $P < 0.001$; $r = 0.936$, $P < 0.001$; $r = 0.583$, $P < 0.001$; and $r = 0.374$, $P < 0.001$, respectively) and the number of mature oocytes. In contrast, a significant negative correlation was noted between the number of mature oocytes and the basal level of E2 hormone ($r = -0.125$, $P = 0.025$). A significant positive

Table 1 Clinical parameters of the study population and their ovarian response

Clinical parameters	Study population ($n = 318$)	Poor response ($n = 51$)	Normal response ($n = 192$)	High response ($n = 75$)	*P*-value
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
Female age (year)	28.2±5.9	32.33±7.09	27.98±5.58	25.91±4.31	< 0.001
Basal E2 level (pg/mL)	35.87±19.18	41.56±21.43	33.66±18.33	37.67±18.97	0.041
Basal FSH level (mIU/mL)	7.34±2.65	8.37±4.32	7.31±2.21	6.73±1.93	0.120
Basal LH level (ng/mL)	5.83±3.44	5.79±4.95	5.71±3.11	6.18±3.01	0.055
Basal PRL level (ng/mL)	15.73±13.16	15.22±10.39	15.94±15.60	15.53±6.37	0.410
Basal TSH level (uIU/mL)	2.01±1.35	2.49±1.82	1.95±1.30	1.86±0.98	0.071
AMH level (ng/mL)	3.57±3.08	1.14±2.05	2.88±1.77	6.99±3.53	< 0.001
E2 level on hCG day (pg/ml)	1986.60±893.81	1253.37±680.14	2095.55±887.36	2206.29±791.9	< 0.001
Number of retrieved oocytes	9.90±5.31	2.86±2.01	8.97±2.95	17.04±2.77	< 0.001
Number of mature oocytes	6.96±3.59	2.08±0.89	6.96±2.47	10.28±3.37	< 0.001
Number of immature oocytes	2.89±2.88	0.78±1.45	2.02±1.63	6.55±2.96	< 0.001
Number of fertilized oocytes	5.55±2.62	1.82±0.74	5.63±1.92	7.87±2.11	< 0.001
Oocyte fertilization rate	83.09±14.92	90.36±17.51	82.72±12.87	79.07±16.31	< 0.001
Number of embryos transferred	3.74±1.64	2.10±1.58	3.92±1.52	4.40±1.24	< 0.001
Value of ß-HCG (mIU/mL)	6.75±5.56	3.88±4.00	7.00±5.87	8.08±5.04	< 0.001

The values are expressed as mean ± SD

SD standard deviation, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone, *ß-HCG* beta human chorionic gonadotropin

$P < 0.05$, significant; $P \geq 0.05$, not significant

Table 2 Correlation between clinical parameters of the study population (*n*=318)

Clinical Parameters		Basal FSH Level (mIU/mL)	AMH Level (ng/mL)	E2 Level on hCG day (pg/ml)	Number of Retrieved Oocyte	Number of Mature Oocytes
Basal E2 Level (pg/mL)	r	-0.071	0.036	-0.027	-0.045	-0.125
	P-Value	0.209	0.522	0.637	0.429	0.025
Basal FSH Level (mIU/mL)	r	1.000	-0.221	-0.043	-0.112	-0.073
	P-Value		< 0.001	0.447	0.046	0.193
AMH Level (ng/mL)	r	-0.221	1.000	0.236	0.707	0.558
	P-Value	< 0.001		< 0.001	< 0.001	< 0.001
Number of Retrieved Oocytes	r	-0.112	0.707	0.355	1.000	0.867
	P-Value	0.046	< 0.001	< 0.001		< 0.001
Number of Mature Oocytes	r	-0.073	0.558	0.360	0.867	1.000
	P-Value	0.193	< 0.001	< 0.001	< 0.001	
Number of Immature Oocytes	r	-0.119	0.599	0.249	0.779	0.445
	P-Value	0.034	< 0.001	< 0.001	< 0.001	< 0.001
Number of Fertilized Oocytes	r	-0.035	0.573	0.330	0.843	0.936
	P-Value	0.532	< 0.001	< 0.001	< 0.001	< 0.001
Number of Embryos Transferred	r	0.046	0.268	0.139	0.496	0.583
	P-Value	0.415	< 0.001	0.013	< 0.001	< 0.001
Value of ß-HCG	r	0.037	0.212	0.040	0.333	0.374
	P-Value	0.506	< 0.001	0.476	< 0.001	< 0.001

Spearman's test, *r* correlation coefficient, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone, *ß-HCG* beta human chorionic gonadotropin
$P < 0.05$, significant; $P \geq 0.05$, not significant

association was found between AMH level ($r = 0.236$, $P < 0.001$); the number of retrieved oocyte ($r = 0.355$, $P < 0.001$), mature oocytes ($r = 0.360$, $P < 0.001$), immature oocytes ($r = 0.249$, $P < 0.001$), and fertilized oocytes ($r = 0.330$, $P < 0.001$); and number of embryos transferred ($r = 0.139$, $P = 0.013$) and E2 level on hCG day.

Predictive value of AMH and age during the different ovarian response

As illustrated in Table 3, the ROC curve analysis in poor response showed that both AMH and age had the highest accuracy (AUC = 0.894, 95% CI = 0.832–0.955, $P < 0.001$; AUC = 0.675, 95% CI = 0.588–0.761, $P < 0.001$, respectively) in predicting poor response when compared with the basal FSH level (AUC = 0.529, 95% CI = 0.433–0.626, $P = 0.519$). The selected cutoff value of AMH and age for prediction of poor response was < 1.45 ng/mL (80.7% sensitivity, 78.4% specificity) and > 31.5 years (52.9% sensitivity, 77.6% specificity), respectively (Figs. 1, 2, and 3). In high response, the ROC curve analysis showed that AMH had the highest accuracy (AUC = 0.888, 95% CI = 0.849–0.928, $P < 0.001$), followed by age (AUC = 0.613, 95% CI = 0.542–0.684, $P = 0.004$) and the basal level of FSH (AUC = 0.570, 95% CI = 0.496–0.644, $P = 0.075$) (Table 3). In the prediction of high ovarian response, the optimum cutoff value of AMH was > 3.55 ng/mL (sensitivity 81.3%, specificity 74.5%), that of age was < 27.5 years (sensitivity 52.1%, specificity 76.0%) (Figs. 4, 5, and 6).

Clinical parameters among the different age groups

As pointed in Table 4, a significant decrease has been found among the different age groups in the basal levels of LH ($P = 0.026$), AMH ($P < 0.001$), and E2 on hCG

Table 3 Test characteristics for AMH, age, and FSH as a predictor of the poor and high ovarian response

Parameters	Area under the curve	95% CI		Cut-off value	% Sensitivity	% Specificity	*P*-value
		Lower bound	Upper bound				
Poor ovarian response							
AMH level (ng/mL)	0.894	0.832	0.955	< 1.45	80.7	78.4	< 0.001
Female age (year)	0.675	0.588	0.761	> 31.5	52.9	77.6	< 0.001
Basal FSH level (mIU/mL)	0.529	0.433	0.626	> 8.85	33.3	75.0	0.519
High ovarian response							
AMH level (ng/mL)	0.888	0.849	0.928	> 3.55	81.3	74.5	< 0.001
Female age (year)	0.613	0.542	0.684	< 27.5	52.1	76.0	0.004
Basal FSH level (mIU/mL)	0.570	0.496	0.644	< 6.35	64.6	46.7	0.075

AMH anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone
$P < 0.05$, significant; $P \geq 0.05$, not significant

day ($P = 0.002$); the number of retrieved oocytes ($P < 0.001$), mature oocytes ($P < 0.001$), immature oocytes ($P < 0.001$), and fertilized oocytes ($P < 0.001$); number of embryos transferred ($P < 0.001$); and the value of ß-hCG ($P < 0.001$) where the highest value in the mentioned parameters was observed in the younger women.

Correlation between clinical parameters and women's age

As shown in Table 5, significant negative correlation has been found between levels of LH ($P = 0.005$), AMH ($P < 0.001$), and E2 on hCG day ($P = 0.002$); numbers of retrieved oocytes ($P < 0.001$), mature oocytes ($P < 0.001$), immature oocytes ($P < 0.001$), and fertilized oocytes ($P < 0.001$); and number of embryos transferred ($P < 0.001$), the value of ß-hCG ($P < 0.001$), and women's age. Conversely, a significant positive correlation was found between the FSH level ($P < 0.011$), the oocyte fertilization rate ($P < 0.001$), and women's age.

Study population in relation to clinical pregnancy outcome

Table 6 reveals a significant reduction in the age and basal level of E2 of pregnant women compared to non-pregnant women ($P < 0.001$). A significant increase has been found in AMH level ($P = 0.002$); the number of retrieved oocytes ($P < 0.001$), mature oocytes ($P < 0.001$), immature oocytes ($P = 0.013$), and fertilized oocytes ($P < 0.001$); and number of embryos transferred ($P < 0.001$) of pregnant women compared to non-pregnant women.

Correlations between clinical parameters in pregnant women

As illustrated in Table 7, there is a negative correlation between the AMH level ($r = -0.185$, $P = 0.020$); the number of retrieved oocytes ($r = -0.232$, $P = 0.003$),

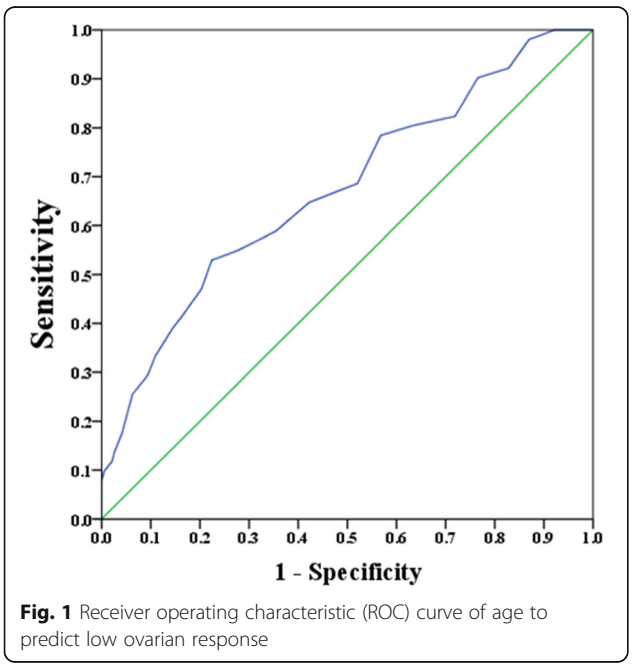

Fig. 1 Receiver operating characteristic (ROC) curve of age to predict low ovarian response

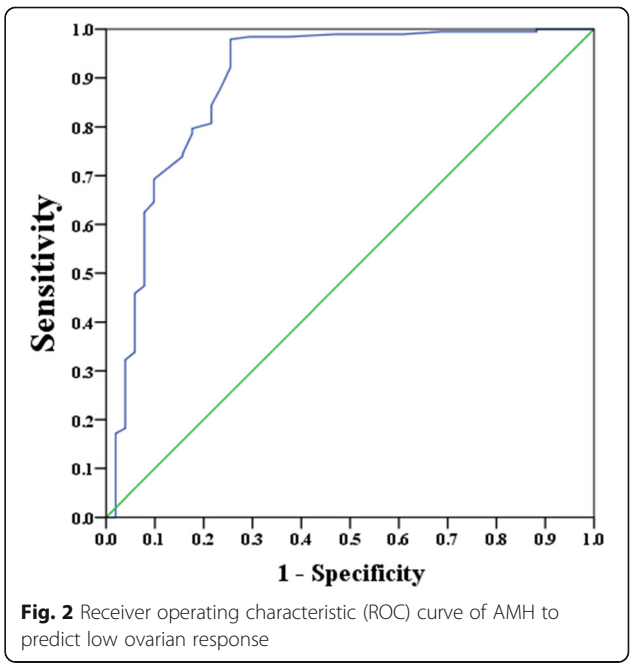

Fig. 2 Receiver operating characteristic (ROC) curve of AMH to predict low ovarian response

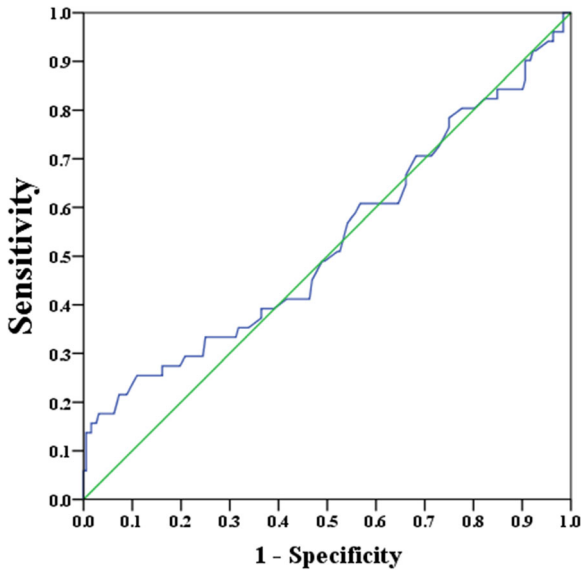

Fig. 3 Receiver operating characteristic (ROC) curve of basal level of FSH to predict low ovarian response

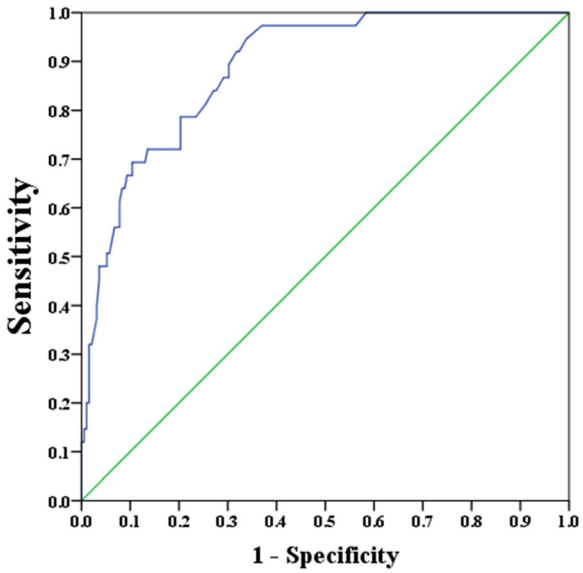

Fig. 5 Receiver operating characteristic (ROC) curve of AMH to predict high ovarian response

mature oocytes (r = −.208, P = 0.009), immature oocytes (r = −0.178, P = 0.025), and fertilized oocytes (r = −0.222, P = 0.005); number of embryos transferred (r = −0.162, P = 0.043); and the age of pregnant women. On the other hand, a positive correlation has been found between the basal FSH level and the age of pregnant women (r = 0.161, P = 0.043). In addition, a significant positive correlation was noted between the basal E2 level; the number of retrieved oocyte, mature oocytes, immature oocytes, and fertilized oocytes (P = 0.035, P < 0.001, P < 0.001, P < 0.001, and P < 0.001, respectively), and the level of AMH. In contrast, a negative significant correlation was found between AMH level and the basal level of FSH (r = −0.196, P = 0.014). A positive significant correlation was noted between the AMH level; the number of retrieved oocytes, mature oocytes, immature oocytes, and fertilized oocytes (P < 0.001, P < 0.001, P < 0.001, P < 0.001, and P < 0.001, respectively); and E2 level on hCG day. A negative significant correlation has been observed between the E2 level on hCG day (P = 0.003) and the age of pregnant women.

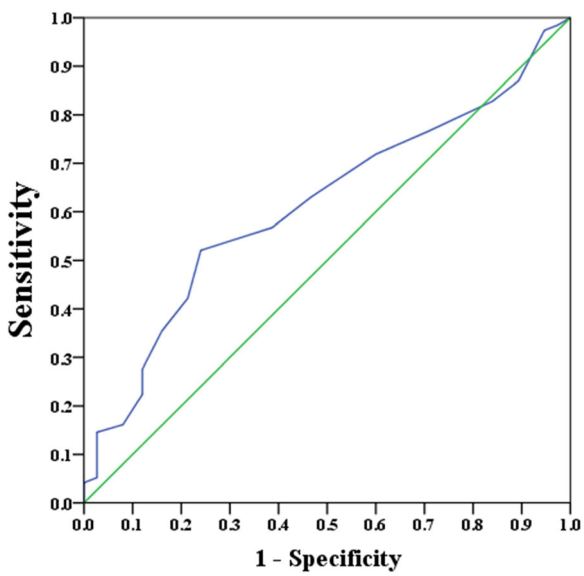

Fig. 4 Receiver operating characteristic (ROC) curve of age to predict high ovarian response

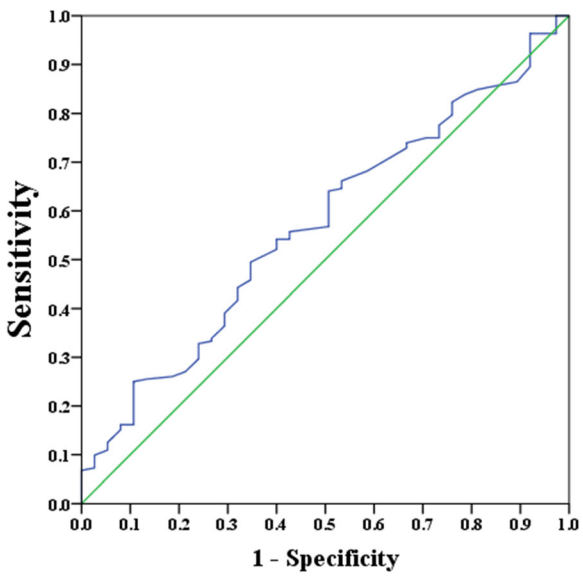

Fig. 6 Receiver operating characteristic (ROC) curve of basal level of FSH to predict high ovarian response

Table 4 The comparison of clinical parameters among the different age groups (*n*=318)

Clinical parameters	(Age ≤ 25) (*n* = 121)	(Age= 26–35) (*n* = 154)	(Age > 35) (*n* = 43)	*P*-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Basal E2 level (pg/mL)	32.78±16.64	38.26±20.81	36.01±18.94	0.155
Basal FSH level (mIU/mL)	7.07±2.77	7.48±2.43	7.63±3.05	0.155
Basal LH level (ng/mL)	6.12±3.87	5.90±3.26	4.78±2.53	0.026
Basal PRL level (ng/mL)	15.41±7.57	15.26±8.79	18.28±29.23	0.831
Basal TSH level (uIU/mL)	1.91±1.35	2.10±1.42	2.00±1.07	0.432
AMH level (ng/mL)	4.16±2.86	3.54±3.32	2.04±2.15	< 0.001
E2 level on hCG day (pg/ml)	2111.31±791.31	1989.87±959.94	1624.00±839.29	0.002
Number of retrieved oocyte	11.42±4.73	9.83±5.35	5.84±4.61	< 0.001
Number of mature oocytes	7.93±3.10	6.99±3.50	4.12±3.77	< 0.001
Number of immature oocytes	3.36±3.02	2.84±2.88	1.72±2.10	< 0.001
Number of fertilized oocytes	6.24±2.18	5.63±2.63	3.30±2.53	< 0.001
Number of embryos transferred	4.10±1.49	3.72±1.69	2.79±1.54	< 0.001
Value of ß-HCG (mIU/mL)	7.75±5.94	6.56±5.26	4.60±4.90	< 0.001

The values are expressed as mean ± SD
SD standard deviation, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone, *ß-HCG* beta human chorionic gonadotropin
$P < 0.05$, significant; $P \geq 0.05$, not significant

Discussion

Despite the success of assisted reproductive technology in overcoming female reproductive problems in several cases, there are still some cases that respond poorly to the stimulation protocol which in turn leads to the ICSI failure. The prediction of ovarian response plays a critical role in the determination of the optimal starting dose from recombinant FSH which leads to increases in the ability of ovarian response and reduces the number of doses needed during stimulation [20]. A previous study indicated that there are several factors, including age, length of the menstrual cycle, basal level of FSH, AMH level, and antral follicle count, that can be used as clinical predictors of oocyte yield and ovarian response during ovarian stimulation [21]. However, there are few studies investigating the ability to predicting ovarian response in GnRH antagonist protocols [22, 23]. To improve the ovarian response and ICSI outcomes, it is necessary to study the association between the AMH level, age, and ovarian response and to estimate the cut-off value of the AMH level and women's age at which the ovarian response may be poor and high [24]. The present study has found a significant increase among the responder groups in AMH level; E2 level on hCG day; number of retrieved oocytes, mature oocytes, immature oocytes, and fertilized oocytes; number of embryos transferred; and the value of ß-hCG. Besides, a significant reduction was found in the age, basal E2 level, and oocyte fertilization rate among the responder groups. All of the mentioned results are in agreement with a previous study prepared by Seifer and his colleagues who found a relationship between circulating AMH levels and ovarian response to gonadotropin treatment. They showed that women with ≥ 11 oocytes retrieved had serum AMH concentrations 2.5 times higher than those of women with ≤ 6 oocytes retrieved [25]. Other studies supported such findings [19, 26].

Table 5 Correlation between clinical parameters and women's age (*n*=318)

Women's age (year)		
Clinical parameters	***r***	***P*-value**
Basal E2 level	0.072	0.199
Basal FSH level	0.143	0.011
Basal LH level	−0.157	0.005
Basal PRL level	−0.021	0.713
Basal TSH level	0.061	0.280
AMH level	−0.301	0.001
E2 level on hCG day	−0.170	0.002
Number of retrieved oocyte	−0.332	< 0.001
Number of mature oocytes	−0.321	< 0.001
Number of immature oocytes	−0.219	< 0.001
Number of fertilized oocytes	−0.302	< 0.001
Oocyte fertilization rate	0.199	< 0.001
Number of embryos transferred	−0.231	< 0.001
ß-HCG level	−0.202	< 0.001

Spearman's test, *r* correlation coefficient, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone, *ß-HCG* beta human chorionic gonadotropin
$P < 0.05$, significant; $P \geq 0.05$, not significant

Table 6 Study population in relation to clinical pregnant outcome (n = 318)

Clinical parameters	Pregnant (n = 158)	Non-pregnant (n = 160)	*P*-value
	Mean ± SD	Mean ± SD	
Female age (year)	26.79±5.37	29.57±6.12	< 0.001
Basal E2 level (pg/mL)	32.36±19.21	39.34±18.56	< 0.001
Basal FSH level (mIU/mL)	7.37±2.18	7.31±3.05	0.233
Basal LH level (ng/mL)	5.87±3.24	5.80±3.64	0.514
Basal PRL level (ng/mL)	15.25±6.49	16.20±17.41	0.306
Basal TSH level (ulU/mL)	1.93±1.17	2.10±1.50	0.555
AMH level (ng/mL)	3.90±2.93	3.25±3.19	0.002
E2 level on hCG day (pg/ml)	2016.37±832.07	1957.21±952.56	0.404
Number of retrieved oocytes	11.54±5.45	8.28±4.64	< 0.001
Number of mature oocytes	8.25±3.72	5.69±2.97	< 0.001
Number of immature oocytes	3.19±2.93	2.59±2.81	0.013
Number of fertilized oocytes	6.42±2.58	4.68±2.36	< 0.001
Number of embryos transferred	4.30±1.40	3.19±1.68	< 0.001

The values are expressed as mean ± SD
SD standard deviation, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone
$P < 0.05$, significant; $P \geq 0.05$, not significant

The present study showed a significant negative association between the number of retrieved oocytes, AMH level, and the basal level of FSH. Conversely, a significant positive correlation was found between the number of retrieved oocytes, mature oocytes, immature oocytes, and fertilized oocytes; number of embryos transferred; and AMH level. Similar results demonstrated that antral follicle count (AFC) was closely related to serum AMH level on the third day of the cycle in women suffering from infertility problems [27, 28]. A review manuscript reported that five of the studies showed that AFC and AMH had a correlation similar to the number of oocytes retrieved, whereas four other studies indicated that AMH was either less good or better [29].

In the present study, the cutoff values of the age and AMH as a predictor of poor response were > 31.5 years (AUC= 0.675) and < 1.45 ng/ml (AUC= 0.894), respectively. These findings are in line with previous studies that found that the cutoff value of AMH for predicting poor ovarian response is between 0.30 and 1.40 ng/ml [30–32]. Another meta-analysis study that included 28 studies of women undergoing ART exhibited that AMH (area under the curve, AUC= 0.78) is a better predictor of poor response to ovarian stimulation than age (AUC= 0.61) [33]. In addition, the La Marca study observed that low AMH cutoff values (0.1 to 1.66 ng/mL) have 76% sensitivities and 79% specificities for the prediction of poor response to gonadotropin stimulation [12]. Nevertheless, these findings disagree with another study that found that the cutoff value for AMH of a poor response from a normal response was 0.1 and 2.97 ng/ml [34]. This variation was supported by Kelsey et al.'s study which reported that the best cutoff value for AMH was 0.7 ng/mL in predicting poor response [13].

The optimum cutoff value of AMH and age of the high response in this study were > 3.55 ng/mL (AUC= 0.888) and < 27.5 years (AUC= 0.613), respectively. The value of AMH cutoff is in agreement with a systematic review of two studies that used AMH to predict high response to gonadotropin stimulation and found that high AMH cutoff values (3.36 to 5.0 ng/mL) have sensitivities and specificities ranging between 53 and 90.5% and 70 and 94.9%, respectively [12, 33]. In contrast, these findings do not match with a previous article found that the cutoff of AMH level in predicting high stimulation was > 4.89 ng/mL (AUC= 0.82, sensitivity= 55%; a specificity = 85%) [32].

Analysis ROC revealed that AMH and women's age are the most accurate of other tests in predicting ovarian response compared to the basal level of FSH. The AUC for AMH was higher than age and basal FSH, and these findings match with previous studies [35, 36]. In contrast, a previous study found the antral follicle count (AFC) to be better than the AMH level [37]. The variation in the cutoff values of AMH and age for poor and high response prediction is probably resulting from the use of different assay methods, differences in the definition of poor and high response, different study populations, and different stimulation protocol. However, one can say that AMH is overall able to identify a large percentage of expected poor and high responders [21].

Table 7 Correlations between clinical parameters in pregnant women (*n*=158)

Clinical Parameters		Women Age (Year)	Basal E2 Level (pg/mL)	Basal FSH Level (mIU/mL)	AMH Level (ng/mL)	E2 Level on hCG day (pg/ml)
Women Age (Year)	r	1.000	0.036	0.161	-0.185	-0.236
	P-Value		0.657	0.043	0.020	0.003
Basal E2 Level (pg/mL)	r	0.036	1.000	-0.083	0.168	-0.033
	P-Value	0.657		0.299	0.035	0.682
Basal FSH Level (mIU/mL)	r	0.161	-0.083	1.000	-0.196	0.014
	P-Value	0.043	0.299		0.014	0.860
AMH Level (ng/mL)	r	-0.185	0.168	-0.196	1.000	0.331
	P-Value	0.020	0.035	0.014		< 0.001
Number of Retrieved Oocyte	r	-0.232	0.143	-0.082	0.677	0.388
	P-Value	0.003	0.073	0.304	< 0.001	< 0.001
Number of Mature Oocytes	r	-0.208	0.070	-0.063	0.495	0.324
	P-Value	0.009	0.381	0.434	< 0.001	< 0.001
Number of Immature Oocytes	r	-0.178	0.157	-0.158	0.639	0.346
	P-Value	0.025	0.049	0.047	< 0.001	< 0.001
Number of Fertilized Oocytes	r	-0.222	0.053	0.009	0.513	0.311
	P-Value	0.005	0.510	0.915	< 0.001	< 0.001
Number of Embryos Transferred	r	-0.162	-0.007	0.133	0.137	0.062
	P-Value	0.043	0.935	0.096	0.085	0.436

Spearman's test, *r* correlation coefficient, *AMH* anti-Müllerian hormone, *E2* estradiol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *PRL* prolactin hormone, *TSH* thyroid-stimulating hormone, *ß-HCG* beta human chorionic gonadotropin
$P < 0.05$, significant; $P \geq 0.05$, not significant

The results showed a significant decrease among the age groups in AMH level; the number of retrieved oocytes, mature oocytes, and fertilized oocytes; and number of embryos transferred. Such results are in the line with a previous study that used Chinese women across different age groups and found a significant reduction in AMH level with an increase in the women's age [38]. A significant negative correlation was found between AMH level; E2 level on hCG day; the number of retrieved oocytes, mature oocytes, and fertilized oocytes; number of embryos transferred; and the women's age. A previous study recommended that the level of AMH may be considered as the best marker of ovarian aging and the time to menopause [39]. While another study mentioned that the reduction in the fecundity ability was shown in women with very low AMH levels [40].

Finally, the present study observed a significant reduction in the women's age and basal level of E2 in pregnant women compared to non-pregnant women; a significant increase was found in the AMH level, the

number of retrieved oocytes and mature oocytes, number of embryos transferred in pregnant women compared to non-pregnant women. A negative correlation was observed between AMH level, the number of retrieved oocytes and mature oocytes, number of embryos transferred, and the age of pregnant women. These findings match with a previous study that noted that AMH level is associated with the oocyte yield and could be used to predict pregnancy outcomes [41]. In addition, other authors observed a correlation between higher baseline serum AMH levels and higher clinical pregnancy rates [42]. Furthermore, several studies have found that low AMH levels correlate with lower rates of clinical pregnancies and higher cancelation rates, but it has a weak ability to predict the clinical pregnancy [14, 43]. Conversely, another study was unable to observe an association between baseline AMH levels and pregnancy rates [44].

Conclusions

This study demonstrates that AMH level and women's age were good biomarkers for the prediction of ovarian response to GnRH antagonist stimulation protocol. The AMH value (< 1.45 ng/mL) and women's age (> 31.5 years) can be considered as potential indicators of poor ovarian response. In contrast, the value of AMH (> 3.55 ng/mL) and the women's age (< 27.5 years) are considered potential indicators of high ovarian response.

Limitations

Future studies with a large sample size are needed in order to confirm these results.

Abbreviations

ICSI: Intracytoplasmic sperm injection; AMH: Anti-Müllerian hormone; FSH: Follicle-stimulating hormone; E2: Estradiol; LH: Luteinizing hormone; TSH: Thyroid-stimulating hormone; PRL: Prolactin; ß-HCG: Beta human chorionic gonadotropin

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Authors' contributions

M. M. L collected and processed the samples. Additionally, he contributed to data analysis, and he was a major contributor in writing the manuscript. M. M. Y performed a review for data analysis, discussion preparation, and the writing of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The current study was approved by the Institutional Ethics Committee (Reference. No. PHRC/HC/03/10), and consent was provided according to the Declaration of Helsinki. Besides, all participants signed an informed approval form to participate in the present study. All samples were analyzed according to the guidelines and standard procedures of Al Basma Fertility Center, Palestinian Territories.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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