Original Article

Correlation Between Serum Anti-Müllerian Hormone Levels and Antral Follicle Count in Prediction of Clinical Pregnancy in Women with Unexplained Infertility Undergoing ICSI Cycles

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ABSTRACT

Background: It is obvious that after years of publications worldwide. The question of whether the serum level of AMH predicts pregnancy after ICSI still unresolved. In the present study the correlation between serum AMH levels and pregnancy rate in a population of women undergoing their first ICSI cycle is investigated.

Aim: We assess accuracy of serum anti-müllerian hormone levels and anti follicle count in prediction of clinical pregnancy in women with unexplained infertility and undergoing ICSI cycles.

Materials and Methods: This is a retrospectively study in assisted reproductive technology unit at Maternity Hospital Ain Shams University from August 2018 to June 2019. This study included 71 women with unexplained primary infertility in ART unit undergoing ICSI cycles. Simple random sampling.

Results: We included (patients with unexplained primary infertility who underwent their first ICSI cycle aged 20-35 years with BMI ≤ 30 without associated medical problems as diabetes mellitus, hypertension and polycystic ovary. Serum AMH was measured within 3 months before the beginning of the ICSI cycle, ovarian stimulation was carried out, and all embryos transfer were carried out on a fresh cycle.

Conclusion: In this study, it was found that as the AMH level increases, the number of oocytes increases as well, but it is not a predictor of oocyte quality or pregnancy rate.

Key Words: Antral follicle count, anti-Müllerian hormone level

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INTRODUCTION

AMH, also known as mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor-β family. It is widely accepted that the reduction of AMH levels in serum is the first indication for decline in the follicular reserve of the ovaries and can be measured in the blood at any time in the menstrual cycle due to its stability [1].

Over the past decade there have been hundreds of publications regarding the ability of serum anti-Müllerian hormone level (AMH) to predict a positive pregnancy test in ICSI programe. Though the results have so far been controversial, it can be considered as established knowledge that serum AMH levels are positively correlated with the total number of retrieved oocytes[2, 3].

So that AMH would seem in fact to be highly predictive of poor ovarian response[4].

If the higher number of oocytes results in a higher number of available good quality embryos, it would be reasonable to expect a higher rate of positive pregnancy tests after in vitro fertilization treatment[5].

Indeed, a large number of studies have reported a positive correlation between serum AMH level and pregnancy rates[6].

However, there are also a number of studies showing limited predictive ability or no correlation between AMH levels and ICSI outcome[7].

The importance of such predictive ability is greater in women of advanced reproductive age due to the reduced success rates of this population[8].

Broer *et al*.[9] demonstrated that among various ovarian reserve tests (FSH, AMH, AFC, age), AMH and AFC had similar predictive ability for poor response, but age had a capacity of predicting ongoing pregnancy after ICSI.

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Iliodromiti *et al*.[10] showed that AMH, independent of age, is correlated with live birth after assisted conception, but with poor predictive accuracy.

Tal *et al*.[11] concluded that AMH is weakly correlated with clinical pregnancy rates.

Yao *et al*.[12] reported that AMH can predict pregnancy rates after ICSI as an independent parameter. It is obvious that after years of publications worldwide, the question of whether AMH predicts pregnancy after ICSI still unresolved.

AFC antral follicul count and AMH in predicting ICSI outcomes among patient in their first cycle[13]. Diminished ovarian reserve (DOR) is often associated with poor ovarian stimulation response[14].

AIM OF THE WORK

The aim of the work is to assess the accuracy of serum anti-müllerian hormone levels and anti follicle count in prediction of clinical pregnancy in women with unexplained infertility and undergoing ICSI cycles.

PATIENTS AND METHODS

This is a retrospectively study in assisted reproductive technology unit at Maternity Hospital Ain-Shams University from August 2018 to June 2019. This study included 71 women with unexplained primary infertility in ART unit undergoing ICSI cycles. Simple random sampling.

Sample size: Sample size was calculated using PASS 11.0 sample size calculation program and based on a study carried out by Spyridon et al.[15]. A sample size of all women undergoing their first ICSI (71 cases) attempt achieves 90% power to detect a difference of -0.48500 between the null hypothesis correlation of 0.00000 and the alternative hypothesis correlation of 0.48500 using a two-sided hypothesis test with a significance level of 0.01000.

Inclusion criteria: Patient with unexplained primary infertility. The patient underwent her first ICSI cycle. Age 20-35 years. BMI ≤ 30. Serum AMH measurement within 3 months before the beginning of the ICSI cycle. No associated medical problems as diabetes mellitus, hypertension and polycystic ovary. Ovarian stimulation was carried out and all embryos transfer were carried out on a fresh cycle.

Exclusion criteria: Abnormal baseline hormonal profile. Previous ovarian surgery. Abnormal gynaecological bleeding. Associated medical problems as DM, HTN, PCO. Female with any contraindication for pregnancy.

Study tools: Serum AMH levels were measured on any day of the woman's menstrual cycle, within 3 months of the beginning of the ICSI cycle. AMH concentration was measured using the AMH Gen II Elisa and antral folicualr count (AFC) by transvaginal U/S.

Research methodology: All participants were subjected from data saved in ART unit as: Medical history including: Personal history. Menstrual history. Past and Obstetric history.

Laboratory investigations: Routine investigations from data saved in ART unit: CBC, Bl group, Rh, Rubella and IgG. Hormonal profile in second day of menstrual cycle from data saved as: LH, FSH, TSH, Estrogen and Prolactin.

Induction of ovulation: On day 3 of spontaneous cycle, all the patients had basal hormonal profile (FSH, LH, E2, TSH and prolactin). Transvaginal (TV) ultrasound (U/S) on day 3 of non stimulated cycles was done by transvaginal probe of 5-9 MHZ. any patient found to have uterine abnormalities excluded. Ovarian hyper stimulation protocol was done according the unit protocol which is a long GnRH agonist protocol starting from midluteal phase by daily subcutaneous injection of triptoreline acetate (decapeptyl 0.05 mg, ferring pharmaceutical, Kid, Germany). Then on day 3 of next cycle after assessment of E2 level ovarian hyper stimulation was started by daily injection of gonapure75 IU/amp (Minapharm for pharmaceutical and chemical industries, Egypt). The starting dose of gonadotropines was prescribed according to the age, the BMI, hormonal profile, AFC and previous response of the patients. Then the dose was adjusted according to the ovarian response that was assessed by transvaginal folliculometry which was started on day six of the cycle. According to ovarian response, every other day TV U/S was performed and at the moment when the leading follicle reaches 16mm, daily TV U/S was performed daily till the largest follicle reaches a diameter of > 18mm. HCG (Choriomon 5,000 IU/amp. "IBSA, Switzerland") two apmules was administered for triggering ovulation.

Ovum pick up: 34-36 hours after HCG injection, ovum pick up was done under ultrasound guide. The direction of the guide beam was checked. The puncturing needle was connected to an aspiration apparatus attached by a fixation ring on the vaginal transducer. The aspiration was checked using test tubes.

The uterus, both ovaries and iliac vessels was identified by the visualization in both planes. Depth localization of the closest accessible follicle (distance from the upper vaginal pole to the center of the follicle) was done. Needle pushed forcefully to the center of the follicle (Aspiration pressure 90-100 mmHg).

ICSI: Intracytoplasmic sperm injection was performed on metaphase II occytes using the direct penetration technique, fertilization results was assessed 16 to 19 hours after insemination. Fertilization which considered normal by the presence of two pronuclei and or 2nd polar body.

Embryo transfer: Embryo transfer was done on 3rd day post insemination using labotect catheter under ultrasound guide at a distance about 1-1.5 cm from the fundus by the same gynaecologist. Number of maximum embryos transferred is 3 embryos on day 3.

Luteal phase support: This phase was supported by using per vaginal micronized progesterone. If positive progesterone was continued until 12 weeks of gestation.

Pregnancy test: Aserum βhCG was performed twelve days after day 3 embryos transfer followed by US six weeks after embryo transfer. To assess clinical pregnancy ECHO and pulsation.

Ethical consideration: The study was presented for the approval of the Ethical Committee of the Department of Obstetrics and Gynecology, and Assisted Reproduction Technology Unit at Faculty of Medicine, Ain Shams University.

STATISTICAL ANALYSIS

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 18.0, IBM Corp., Chicago, USA, 2009.

Descriptive statistics were done for quantitative data as minimum and maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data, median and 1st and 3rd inter-quartile range for quantitative non-normally distributed data, while it was done for qualitative data as number and percentage.

Inferential analyses were done for quantitative variables using Shapiro-Wilk test for normality testing, independent t-test in cases of two independent groups with normally distributed data and Mann whiteny U in cases of two independent groups with non-normally distributed data. In qualitative data, inferential analyses for independent variables were done using Fisher's Exact test for differences between proportions with small expected numbers. ROC curve was used to evaluate the performance of different tests differentiate between certain groups. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

Diagnostic characteristics were calculated as follows: Sensitivity = (True positive test / Total positive golden) x 100. Specificity = (True negative test / Total negative golden) x 100. Predictive positive value = (True positive test / Total positive test) x 100. Predictive negative value = (True negative test / Total negative test) x 100. LR+ = (sensitivity/ 1-specificity). LR- = (1- sensitivity / specificity). Diagnostic accuracy = ([True positive test + True negative test] / Total cases) x 100Youden's index = sensitivity + specificity − 1

RESULTS

Table 1 showed that demographic characteristics, AFC and laboratory findings among the studied cases.

Table 2 showed that stimulation, fertilization, cleavage and transfer among the studied cases.

Table 3 showed that pregnancy among the studied cases; chemical pregnancy was in less than half of the studied cases, clinical pregnancy was in more than third of cases, while twin was in more than tenth of cases.

Table 4 showed that no significant difference according to chemical pregnancy regarding AMH. Case with positive chemical pregnancy significantly had lower TSH.

Table 5 showed that no significant difference according to chemical pregnancy regarding stimulation, fertilization, cleavage and transfer.

Table 6 showed that no significant difference according to clinical pregnancy regarding AMH.

Table 7 showed that no significant difference according to clinical pregnancy regarding stimulation, fertilization, cleavage and transfer.

Table 8 showed that no significant difference according to twin pregnancy regarding AMH.

Table 9 showed that case with twin pregnancy significantly had higher stimulation, fertilization, cleavage and transfer day.

Table 10 showed that laboratory findings had non-significant low diagnostic performance in prediction of chemical pregnancy.

Table 11 showed that laboratory findings had non-significant low diagnostic performance in prediction of clinical pregnancy.

Table 12 showd that only AMH had signifciant moderate diagnostic performance in prediction of twin pregnancy.

Table 13 showed that AMH ≥1.5 ng/mL has high sensitivity and NPV in prediction of twin pregnancy (excluding test).

Table 14 showed that age, AMH and TSH had no significant effect on pregnancy among the studied cases.

Table 1: Demographic characteristics, AFC and laboratory findings among the studied cases

Variables	Mean±SD	Median (IQR)	Range
Age (years)	29.4±3.8	29.0 (27.0–31.0)	21.0–37.0
BMI (kg/m^2)	28.5±4.5	29.0 (26.0–31.1)	18.0–38.8
Duration (years)	4.7±3.4	4.0 (2.0–6.0)	1.0–16.0
AFC (total)	13.2±5.6	12.0 (9.0–17.0)	4.0–29.0
AMH (ng/mL)	3.1±2.7	2.4 (1.3–4.4)	0.1–14.3
FSH (IU/L)	7.1±4.7	6.4 (5.4–7.3)	3.6–43.6
LH (IU/L)	6.4±2.8	5.7 (5.0–7.5)	2.9–20.7
E2 (pg/mL)	43.6±23.5	40.0 (25.0–56.0)	3.0–120.0
Prolacting (ng/mL)	14.8±14.7	12.0 (10.0–15.0)	4.3–106.0
TSH (mIU/L)	2.5±1.6	2.0 (1.3–3.2)	0.3–7.0

Total=71

Table 2: Stimulation, fertilization, cleavage and transfer among the studied cases

Variables		N	%
Collected oocytes		67	94.4
Fertilized oocyte		66	93.0
Cleavage oocyte		66	93.0
Embryo formed		66	93.0
Embryo transfer		66	93.0
	Mean±SD	Median (IQR)	Range
Stimulation days	13.0±2.7	12.0 (11.0–15.0)	9.0–20.0
Stimulation ampoules	42.0±17.0	40.0 (30.0–52.0)	14.0–96.0
Collected oocytes	6.6±4.1	6.0 (3.0–9.0)	1.0–20.0
Fertilized oocyte	4.6±3.0	4.0 (2.0–7.0)	1.0–14.0
Cleavage oocyte	3.7±1.8	3.0 (3.0–5.0)	1.0–9.0
Embryo formed	3.3±1.9	3.0 (2.0–4.0)	1.0–9.0
Embryo transferred	2.1±0.8	2.0 (1.0–3.0)	1.0–5.0
Transfer day	3.5±1.0	3.0 (3.0–5.0)	2.0–5.0

Total=71

Table 3: Pregnancy among the studied cases

Variables	N	%
Chemical	34	47.9
Clinical	28	39.4
Twin	9	12.7

Total=71

Table 4: Comparison according to chemical pregnancy regarding demographic characteristics, AFC and laboratory findings

Variables	Positive (N=34)	Negative (N=37)	^p
Age (years)	30.1±3.6	28.7±3.9	0.111
BMI (kg/m^2)	29.0±4.8	28.2±4.2	0.459
Duration (years)	4.9±3.3	4.5±3.4	0.596
AFC (total)	13.9±6.3	12.6±4.8	0.327
AMH (ng/mL)	3.4±3.3	2.9±1.9	0.411
FSH (IU/L)	6.5±2.0	7.7±6.3	0.317
LH (IU/L)	7.0±3.5	5.9±1.8	0.101
E2 (pg/mL)	39.9±20.6	47.0±25.6	0.205
Prolacting (ng/mL)	12.5±4.8	16.8±19.8	0.224
TSH (mIU/L)	2.1±1.3	2.9±1.7	0.035*

^Independent t-test. *Significant

Table 5: Comparison according to chemical pregnancy regarding stimulation, fertilization, cleavage and transfer

Variables	Positive (N=34)	Negative (N=37)	*p*
Collected oocytes	34 (100.0%)	33 (89.2%)	§0.116
Fertilized oocyte	34 (100.0%)	32 (86.5%)	§0.055
Cleavage oocyte	34 (100.0%)	32 (86.5%)	§0.055
Embryo formed	34 (100.0%)	32 (86.5%)	§0.055
Embryo transfer	34 (100.0%)	32 (86.5%)	§0.055
Stimulation days	12.0 (11.0–14.0)	13.0 (11.0–15.0)	#0.148
Stimulation ampoules	37.5 (33.0–48.0)	42.0 (30.0–55.0)	#0.564
Collected oocytes	6.5 (3.0–10.0)	6.0 (3.0–8.0)	#0.450
Fertilized oocyte	4.0 (2.0–8.0)	3.5 (2.0–6.0)	#0.505
Cleavage oocyte	3.0 (3.0–5.0)	3.0 (2.5–5.0)	#0.824
Embryo formed	3.0 (2.0–4.0)	3.0 (2.0–4.5)	#0.744
Embryo transferred	2.0 (2.0–3.0)	2.0 (1.0–3.0)	#0.717
Transfer day	3.0 (3.0–5.0)	3.0 (3.0–4.0)	#0.338

#Mann Whitney test. §Fisher's Exact test

Table 6: Comparison according to clinical pregnancy regarding demographic characteristics, AFC and laboratory findings

Variables	Positive (N=28)	Negative (N=43)	^*p*
Age (years)	30.5±3.7	28.7±3.7	0.051
BMI (kg/m^2)	29.3±5.1	28.1±4.0	0.248
Duration (years)	5.0±3.6	4.5±3.2	0.525
AFC (total)	13.0±6.0	13.3±5.4	0.789
AMH (ng/mL)	3.1±3.5	3.1±2.0	0.955
FSH (IU/L)	6.7±2.1	7.4±5.9	0.515
LH (IU/L)	7.1±3.8	6.0±1.8	0.107
E2 (pg/mL)	41.3±21.1	45.1±25.0	0.509
Prolacting (ng/mL)	12.4±5.0	16.3±18.4	0.268
TSH (mIU/L)	2.1±1.3	2.8±1.7	0.077

^Independent t-test

Table 7: Comparison according to clinical pregnancy regarding stimulation, fertilization, cleavage and transfer

Variables	Positive (N=28)	Negative (N=43)	p
Collected oocytes	28 (100.0%)	39 (90.7%)	§0.148
Fertilized oocyte	28 (100.0%)	38 (88.4%)	§0.149
Cleavage oocyte	28 (100.0%)	38 (88.4%)	§0.149
Embryo formed	28 (100.0%)	38 (88.4%)	§0.149
Embryo transfer	28 (100.0%)	38 (88.4%)	§0.149
Stimulation days	12.0 (11.0–13.5)	13.0 (11.0–15.0)	#0.143
Stimulation ampoules	39.5 (30.0–50.0)	40.0 (30.0–55.0)	#0.850
Collected oocytes	6.0 (3.0–9.0)	6.0 (3.0–9.0)	#0.873
Fertilized oocyte	3.0 (2.0–7.0)	4.0 (2.0–7.0)	#0.917
Cleavage oocyte	3.0 (3.0–5.0)	3.0 (3.0–5.0)	#0.962
Embryo formed	3.0 (2.0–4.0)	3.0 (2.0–5.0)	#0.488
Embryo transferred	2.0 (1.5–3.0)	2.0 (1.0–3.0)	#0.879
Transfer day	3.0 (3.0–5.0)	3.0 (3.0–5.0)	#0.480

#Mann Whitney test. §Fisher's Exact test

Table 8: Comparison according to twin pregnancy regarding demographic characteristics, AFC and laboratory findings

Variables	Singlton (N=19)	Twin (N=9)	^*p*
Age (years)	30.6±3.9	30.2±3.3	0.817
BMI (kg/m^2)	28.6±4.2	30.8±6.6	0.288
Duration (years)	4.6±3.5	5.9±4.0	0.403
AFC (total)	13.2±6.6	12.6±4.6	0.807
AMH (ng/mL)	3.1±4.2	3.2±1.3	0.937
FSH (IU/L)	7.0±2.5	6.0±1.0	0.254
LH (IU/L)	7.8±4.2	5.6±1.9	0.150
E2 (pg/mL)	41.9±24.1	39.9±14.1	0.816
Prolacting (ng/mL)	11.7±5.3	13.7±4.4	0.347
TSH (mIU/L)	2.4±1.5	1.5±0.7	0.094

^Independent t-test

Table 9: Comparison according to twin pregnancy regarding stimulation, fertilization, cleavage and transfer

Variables	Singlton (N=19)	Twin (N=9)	*p*
Stimulation days	12.0 (11.0–14.0)	12.0 (11.0–12.0)	#1.000
Stimulation ampoules	40.0 (26.0–52.0)	36.0 (33.0–48.0)	#1.000
Collected oocytes	4.0 (2.0–8.0)	9.0 (7.0–12.0)	#0.012*
Fertilized oocyte	3.0 (2.0–6.0)	7.0 (4.0–9.0)	#0.019*
Cleavage oocyte	3.0 (3.0–3.0)	5.0 (5.0–5.0)	#<0.001*
Embryo formed	3.0 (2.0–3.0)	4.0 (2.0–5.0)	#0.332
Embryo transferred	2.0 (1.0–3.0)	2.0 (2.0–2.0)	#0.629
Transfer day	3.0 (3.0–3.0)	5.0 (5.0–5.0)	#0.003*

#Mann Whitney test. Significant

Table 10: Diagnostic performance of laboratory findings in prediction of chemical pregnancy

Factors	AUC	SE	*P*	95% CI
AMH	0.500	0.070	0.995	0.364–0.637
FSH	0.572	0.069	0.298	0.437–0.707
LH	0.596	0.068	0.164	0.463–0.729
E2	0.577	0.068	0.267	0.443–0.710
Prolacting	0.508	0.069	0.908	0.372–0.644
TSH	0.638	0.066	0.055	0.510–0.767

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table 11: Diagnostic performance of laboratory findings in prediction of clinical pregnancy

Factors	AUC	SE	*P*	95% CI
AMH	0.577	0.071	0.276	0.437–0.717
FSH	0.549	0.071	0.491	0.410–0.687
LH	0.568	0.071	0.335	0.429–0.708
E2	0.536	0.070	0.609	0.398–0.674
Prolacting	0.526	0.072	0.711	0.386–0.666
TSH	0.634	0.068	0.057	0.501–0.768

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table 12: Diagnostic performance of laboratory findings in prediction of twin pregnancy

Factors	AUC	SE	*P*	95% CI	Cut off
AMH	0.754	0.093	0.032*	0.572–0.937	≥1.5
FSH	0.596	0.109	0.417	0.383–0.810	--
LH	0.661	0.108	0.176	0.449–0.873	--
E2	0.512	0.108	0.922	0.300–0.724	--
Prolacting	0.576	0.111	0.523	0.358–0.794	--
TSH	0.678	0.102	0.134	0.478–0.879	--

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table 13: Diagnostic characteristics of AMH ≥1.5 ng/mL in prediction of twin pregnancy

Characters	Value	95% CI
Sensitivity	100.0%	66.4%–100.0%
Specificity	57.9%	33.5%–79.7%
Diagnostic accuracy (DA)	71.4%	51.3%–86.8%
Youden's index	57.9%	35.7%–80.1%
Positive Predictive value (PPV)	52.9%	27.8%–77.0%
Negative Predictive value (NPV)	100.0%	71.5%–100.0%
Positive likelihood ratio (LR+)	2.38	1.40–4.02
Negative likelihood ratio (LR-)	0.00	0.00–0.00
Diagnostic odd ratio (LR)	>100.0	>100.0–>100.0
Kappa	0.469	0.206–0.733

CI: Confidence interval

Table 14: Logistic regression models for factors affecting pregnancy among the studied cases

Scores	β	SE	*P*	OR (95% CI)
Chemical pregnancy				
Age ≤31.0 years	-0.19	0.40	0.636	0.83 (0.38–1.81)
AMH ≥2.5	0.30	0.47	0.520	1.35 (0.54–3.37)
TSH ≥2.3	-0.65	0.44	0.146	0.52 (0.22–1.25)
Clinical pregnancy				
Age ≤31.0 years	-0.33	0.41	0.419	0.72 (0.32–1.60)
AMH ≥2.5	-0.23	0.48	0.630	0.79 (0.31–2.04)
TSH ≥2.3	-0.61	0.46	0.187	0.54 (0.22–1.34)
Twin pregnancy				
Age ≤31.0 years	-2.11	0.68	0.002	0.12 (0.03–0.46)
AMH ≥2.5	1.00	0.74	0.179	2.72 (0.63–11.70)
TSH ≥2.3	-2.88	1.09	0.008	0.06 (0.01–0.47)

β: Regression coefficient, SE: Standard error, OR: Odds ratio, CI: Confidence interval, *significant

DISCUSSION

AMH, also known as mullerian-inhibiting substance, is a dimeric glycoprotein that belongs to the transforming growth factor-β family. It is widely accepted that the reduction of AMH levels in serum is the first indication for decline in the follicular reserve of the ovaries and can be measured in the blood at any time in the menstrual cycle due to its stability[1].

Over the past decade, there have been hundreds of publications regarding the ability of serum anti-Müllerian hormone level (AMH) to predict a positive pregnancy test in ICSI programe. Though the results have so far been controversial, it can be considered as established knowledge that serum AMH levels are positively correlated with the total number of retrieved oocytes[2,3].

Therefore, that AMH would seem in fact to be highly predictive of poor ovarian response[4].

If the higher number of oocytes results in a higher number of available good quality embryos, it would be reasonable to expect a higher rate of positive pregnancy tests after in vitro fertilization treatment[5].

Indeed, a large number of studies have reported a positive correlation between serum AMH level and pregnancy rates[6].

However, there are also a number of studies showing limited predictive ability or no correlation between AMH levels and ICSI outcome[7].

The importance of such predictive ability is greater in women of advanced reproductive age due to the reduced success rates of this population[8].

AFC antral follicular count and AMH in predicting ICSI outcomes among patient in their first cycle[13].

Diminished ovarian reserve (DOR) is often associated with poor ovarian stimulation response[14].

The current study was a retrospective study conducted at assisted reproductive technology unit at Maternity Hospital Ain-Shams University, to assess the accuracy of serum anti-müllerian hormone levels and anti-follicular count in prediction of clinical pregnancy in women with unexplained infertility and undergoing ICSI cycles.

In this study, 71 women were included to predict accuracy of AMH levels and AFC in clinical pregnancy also number of cycle, number of oocyte retrieved, quality of oocyte, number of embryo, grade of embryo and embryo transfer.

Serum AMH levels were measured on any day of the woman's menstrual cycle, within 3 months of the beginning of the ICSI cycle. AMH concentration was measured using the AMH Gen II Elisa and AFC by vaginal u/s.

In current study, we included (patients with unexplained primary infertility who underwent their first ICSI cycle aged 20-35 years with BMI ≤ 30 without associated medical problems as diabetes mellitus, hypertension and polycystic ovary. Serum AMH was measured within 3 months before the beginning of the ICSI cycle, ovarian stimulation was carried out, and all embryos transfer were carried out on a fresh cycle.

We excluded women with (abnormal baseline hormonal profile, previous ovarian surgery, and abnormal gynaecological bleeding associated medical problems as DM, HTN, PCO and female with any contraindication for pregnancy).

Our study found that there was no significant difference according to chemical and clinical pregnancy regarding stimulation, fertilization, cleavage and transfer and AMH.

No significant difference according to twin pregnancy regarding AMH. Case with twin pregnancy significantly had higher stimulation, fertilization, cleavage and transfer day. Laboratory findings had non-significant low diagnostic performance in prediction of chemical and clinical pregnancy.

Only AMH had significant moderate diagnostic performance in prediction of twin pregnancy. AMH ≥1.5 ng/mL has high sensitivity and NPV in prediction of twin pregnancy (excluding test).

Lia *et al.* in their study were in line with our results and found that there were no significant differences of serum AMH levels between success and failure of pregnancy. After adjusting age, the duration of the stimulation, total recombinant FSH dose used, serum estradiol levels, endometrial thickness, and number of intermediate sized (12-15 mm) and dominant follicles (≥16 mm) on the day of human choriogonadotropin injection, there was still no difference. Based on the above finding, the authors suggested that AMH was not a valuable biomarker in the prediction of clinical pregnancy. Furthermore, the authors hypothesized that serum AMH concentration was not associated with oocyte and/or embryo quality and AMH concentration did not reflect the oocyte genetic competence; both might be the determinant factor for successful embryo implantation (pregnancy)[16].

Against to our study, Lee *et al.* found on their study that patients aged over 40, AFC and AMH shown to be good biomarkers for the prediction of clinical pregnancy and live birth. Although AMH was positively correlated with clinical pregnancy and had no association with live birth, the predictive value of AFC and AMH were similar for both clinical pregnancy and live birth. To predict the live birth, age < 41, AFC > 3 and total retrieved oocytes > 6 appeared

to be meaningful. This study demonstrated the significance of AMH and AFC as predictors of clinical pregnancy and live birth for old aged women at their first IVF cycle with gonadotropin-releasing hormone antagonist protocol[13].

Borges *et al.* partially disagreed with us and found that serum levels of AMH are a useful predictor of ovarian response to controlled ovarian stimulation, oocyte quality, and fertilization. However, AMH levels may also compromise clinical outcomes; lower AMH levels did not impair embryo development[17].

Kaur and Mahajan in their study, which compare ovarian reserve and response to gonadotropin stimulation in fertile and infertile Indian women based on ovarian reserve markers, anti−Mullerian hormone and antral follicle count and evaluate whether infertile Indian women below the age of 35 years have an earlier depletion of their ovarian reserve and a lower ovarian response to gonadotropin in comparison to age−matched fertile controls agreed with our results and found that there were no difference in the AMH and AFC between the fertile and infertile women. In addition, there was no difference in the ovarian response; the mean number of oocytes. AMH had the strongest correlation with the number of oocytes retrieved in comparison to AFC and age. Finally, there is no difference in ovarian reserve and response in fertile and infertile Indian having similar demographics and basal characteristics[18].

Mantzavinos *et al.* in their study also disagreed with us and proved that Serum AMH levels are a strong predictive marker of clinical pregnancy in women undergoing a short agonist IVF protocol. There is also a strong association with cancellation rate, number of oocytes retrieved, poor response (≤3 oocytes), number of embryos, embryo transfer rate and live birth rates[8].

Ashrafi *et al.* study which compare the predictive values of serum anti-mullerian hormone (AMH), antral follicle count (AFC) and ovarian response prediction index (ORPI) ([AFC×AMH] / age) for in vitro ertilization/ intracytoplasmic sperm injection (IVF/ICSI) cycle outcomes partially disagreed with our results and showed that both AMH and AFC were good predictors of ovarian response; even it seems that AFC was being a better predictor. Combining these variables is necessary, as ovarian response prediction index will not improve the prediction value. All the variables had poor predictive ability for clinical pregnancy and live birth rates. Logistic regression analysis showed the AMH less than 0.4 ng/ml and quality of transferred embryos were significant predictors for clinical pregnancy rate[19].

Park *et al.* study which determined the predictive value of anti-Müllerian hormone (AMH) levels for pregnancy outcomes in patients over 40 years of age who underwent in vitro fertilization or intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) cycles was against with our results and showed that AMH levels were predictive of clinical pregnancy in infertility patients over 40 years of age[20].

Hassan *et al.* study which aimed to determine whether follicular output rate (FORT) could predict the clinical pregnancy rate in women with unexplained infertility undergoing IVF/ICSI disagreed with us and revealed that the correlation between FORT and pregnancy was independent of potential confounding factors. They concluded that FORT is an independent variable affecting the clinical pregnancy rate in IVF/ICSI cycles. Higher FORT values had better oocyte yield and clinical pregnancy rates in women with unexplained infertility undergoing IVF/ICSI with potentially normal ovarian response[21].

CONCOLUSION

In this study, it was found that as the AMH level increases, the number of oocytes increases as well, but it is not a predictor of oocyte quality or pregnancy rate. This might be because pregnancy is affected by many other factors such ass embryo quality, transfer technique, and endometrial receptivity. Furthermore, treatment success is also affected by sperm properties in patients who receive the treatment due to male factor.

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