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Sensitivity and Specificity of Measuring Anti-Müllerian Hormone and Follicle Stimulating Hormone levels in Predicting Response to Ovarian Stimulation in Infertile Women

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ABSTRACT

Background and Objective: Assisted reproductive technologies (ART), including in vitro fertilization (IVF) and embryo transfer, have achieved considerable success, largely due to controlled ovarian hyperstimulation (COH). However, there remains a shortage of studies on the estimation of various ovarian response markers linked to the GnRH agonist-controlled ovarian stimulation (COS) regimen. The objective of the current study is to assess the productiveness of Follicle Stimulating Hormone (FSH) and Anti-Mullerian Hormone (AMH) in determining ovarian responses in infertile patients undergoing controlled ovarian stimulation.

Methods: This cohort study involved 90 females, aged 20 to 43 years, with primary and secondary infertility lasting between 3 and 13 years, attending the Al-Najef fertility center for ICSI cycles between June 2020 and January 2021. Patients with a history of ovarian surgery, polycystic ovary syndrome (PCOS), and premature ovarian failure (POF) were excluded. FSH and AMH levels were calculated on second 2 of the menstrual cycle, and the association between these indicators and outcome factors was evaluated.

Findings: AMH demonstrated a negative link with age and a positive link with the antral follicle count, total number of follicles after induction, and retrieved oocyte count ($p<0.05$). FSH exhibited a significant negative link by the total number of follicles after induction ($p<0.05$). AMH demonstrated the best sensitivity and specificity, positive predictive value (PPV) and negative predictive value (NPV) for predicting ovarian response. Specificity, sensitivity, PPV and NPV for AMH with cut-off values >2 ng/mL test providing 84%, 80%, 86%, 57% in comparison to FSH with cut-off values >4 IU/mL yielded 67%, 68%, 50% and 54%, respectively.

Conclusion: In conclusion, our findings suggest that AMH is a superior predictor of ovarian response compared to FSH.

Keywords: *AMH, Ovarian Response, FSH, Assisted Reproductive Technologies*.

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Introduction

Controlled Ovarian Hyperstimulation (COH) has significantly contributed to the success of ART, containing in vitro fertilization and embryo transfer (ET). However, for patients to fully benefit from these therapies, individualized COH protocols are necessary. Unfortunately, the majority of women do not exhibit a predictable or consistent response to ovarian stimulation procedures. Moreover, there is limited documentation on the assessment of various ovarian response indicators in relation to the GnRH agonist controlled ovarian stimulation (COS) protocol (1).

AMH is a glycoprotein chemical predominantly identified with inhibin and activin from the transforming development parameter beta superfamily. Its primary functions include development, separation and folliculogenesis (2).

AMH is currently the normally used serum marker for assessing ovarian capacity, including the evaluation of AMH levels. AMH is transmitted by granulosa cells of growing follicles, starting from the crucial stage to the small antral step (3). Although the day 3 FSH test is the most common method for assessing ovarian reserve, AMH remains the most crucial biochemical test (4-6). Polycystic Ovarian Syndrome is characterized by high quantities of progesterone, which affects female ripening, and this combination is sometimes referred to as the Biological Body Clock Test.

AMH is transmitted via antral follicles, and it inhibits early-stage follicle recruitment while decreasing the pre-antral and small antral follicles responsiveness to FSH (7, 8). Unlike other hormones, the levels of AMH remain relatively fixed over the menstrual cycle, allowing for determination on any time of cycle (9). The clinical success of treatment largely depends on the ovary's reaction to gonadotrophin, making it essential to detect and predict the ovarian reaction before undertaking assisted reproductive innovation therapy, based on factors associated with the projected ovarian response. This analysis can lead to optimal treatment outcomes, including a decreased risk of ovarian hyperstimulation disorder (OHSD) and an improved ovarian response.

Several factors, such as age, antral follicle count (AFC), estradiol concentration, baseline FSH, inhibin B, and AMH, can be utilized to determine ovarian responsiveness (10). The objective of the present investigation is to evaluate the predictive abilities of FSH and AMH in determining ovarian responses in IVF patients undergoing controlled ovarian stimulation.

Methods

After being confirmed by the ethics committee of University of Babylon with the code ANH2021-3-22, a prospective cohort research was done in June 2020 and January 2021 at the Al-Najef fertility clinic. The study population consisted of ninety sub-fertile women undergoing infertility therapy.

On days 1-2 of their spontaneous menstrual periods, lateral scans were performed by the same researcher on all individuals to determine the amounts of antral follicles in the range of 1-5 mm in radius per ovary. The AFC was measured as the sum of the counts from both ovaries. Additionally, FSH and AMH levels were measured at the same time. The serum AMH concentration was evaluated employing the AMH Enzyme-Linked Immunosorbent Assay (ELISA) from Beckman Coulter. Levels of FSH are measured using the Enzyme-linked Fluorescence Assay (ELFA) on a VIDAS analyzer from BioMerieux SA.

The data were evaluated based on the ROC curve. For the p-values less than 5%, significance of data was confirmed for each of the mentioned methods.

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Results

The demographic data of the investigated group are summarized in Table 1. The majority of cases had female factor causes, and a significant proportion of them experienced primary infertility. The AMH and FSH hormone levels in day 2 of MC in the studied group with ultrasound findings are shown in table 2. All patients underwent the ovarian stimulation protocol, and the results of the stimulation were presented in Table 3. The duration of induction averaged 13.40±2.40 days, with a range of 10 to 14 days. The median total number of follicles after induction was 15, and the median amount of obtained oocytes was 11. The majority of patients exhibited a good ovarian response, accounting for 87.78% of the total cases.

Table 1. Demographics and clinical data of the studied group

Characteristics	Patients (n=90) Mean±SD or Number(%)
Age (years)	31.46±5.41 (20-43)
BMI (kg/m^2)	27.77±6.16 (22-35)
Duration of infertility (years)	8.55±4.11 (3-13)
Cause of infertility	
Male	32(53.55)
Female	38(42.22)
Unexplained	4(4.44)
Both	16(17.78)
Type of infertility	
Primary	78(86.67)
Secondary	12(13.33)
Occupation	
Employer	28(31.11)
House wife	62(68.89)
Address	
Rural	31(34.44)
Urban	59 (65.56)

Table 2. The AMH and FSH hormone with ultrasound findings in day 2 of MC in the studied group

Day2 MC and Measured parameters	Mean±SD
US findings	
Antral count, median (range)	13 (8-18)
Endometrium thickness mm	5.91±1.41
Hormonal analysis	
FSH (IU/L)	0.447±1.42
AMH (ng/mL)	2.90±1.83

Table 3. Outcome of ovarian stimulation in the studied group

Variable	
Duration of induction, days (Mean±SD)	13.40±2.40 (10-14)
No. of follicles after induction, median (range)	
<14 mm	3 (1-4)
14-18 mm	5 (1-7)
>18 mm	8 (5-10)
Total	15 (6-20)
Oocytes retrieved, median (range)	11 (5-14)
Response, number(%)	
Poor	11(12.22)
Good	79(87.78)

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Table 4 presents the relationship between the levels of FSH and AMH and the studied characteristics. A significant negative link was seen in age and AMH level ($p<0.05$), showing that as age increased, AMH levels tended to decrease.

In other words, there are significant positive links between the antral follicle count, total number of oocytes, and total amounts of obtained oocytes with level of AMH ($p<0.05$). This proposes that higher levels of AMH were linked with a greater number of antral follicles and a higher yield of oocytes during the ovarian stimulation process.

Furthermore, a significant negative link was found between the total amounts of oocytes and the duration of induction with FSH level ($p<0.05$). This implies that a longer duration of induction was associated with lower FSH levels, and a higher number of retrieved oocytes correlated with lower FSH levels.

In summary, Table 4 provides practical understandings in the relationship between age, ovarian response markers (AMH and FSH), and the outcomes of ovarian stimulation in the studied group.

Table 4. FSH and AMH levels with studied characteristics

Factors	FSH	AMH
Age		
r	0.128	-0.413
P	0.316	0.007
BMI		
r	0.309	-0.203
P	0.023	0.229
Duration		
r	0.006	-0.059
P	0.960	0.709
Antral count		
r	-0.212	0.389
P	0.095	0.01
Follicle size<14		
r	-0.064	0.294
P	0.629	0.07
Follicle size 14-18		
r	-0.272	-0.033
P	0.037	0.84
Follicle size>18		
r	-0.187	0.287
P	0.157	0.076
Total		
r	-0.314	0.33
P	0.015	0.04
Oocyte retrieved		
r	-0.196	0.603
P	0.163	0.001
Induction duration		
r	-0.447	0.23
P	0.001	0.13

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The findings of the binomial regression procedure in Table 5 revealed significant associations between AMH levels and response types. An increase in AMH was discovered to be linked with a reduced risk of poor ovarian response, with an odds rate of 4.652 and a 95% confidence rate between 0.970 to 9.298. Low response rates were used as the benchmark for comparison.

Table 5. Logistic regression analysis for AMH and FSH in good vs poor ovarian response

Good ovarian response	p-value	Odds ratio	95% Confidence Interval	
			Lower range	Upper range
FSH	0.015	1.421	0.875	2.307
AMH	0.045[a]	4.652	0.970	9.298

[a]**Poor ovarian response**

The ROC curves for AMH and FSH as predictors of a poor or good ovarian response were constructed (Figure 1). AMH exhibited the best sensitivity for predicting a favorable ovarian response (AUC 0.803, 95% CI: 0.655-0.950) and was better regarding FSH (AUC 0.658, 95% CI: 0.396-0.92, p=0.03). The AMH assay was 80% sensitive and 84% specific, whereas the FSH assay was 68% sensitive and 67% specific. As a result, the most desirable specificity and sensitivity for foreseeing ovarian response was demonstrated at an AMH level of >2 ng/mL.

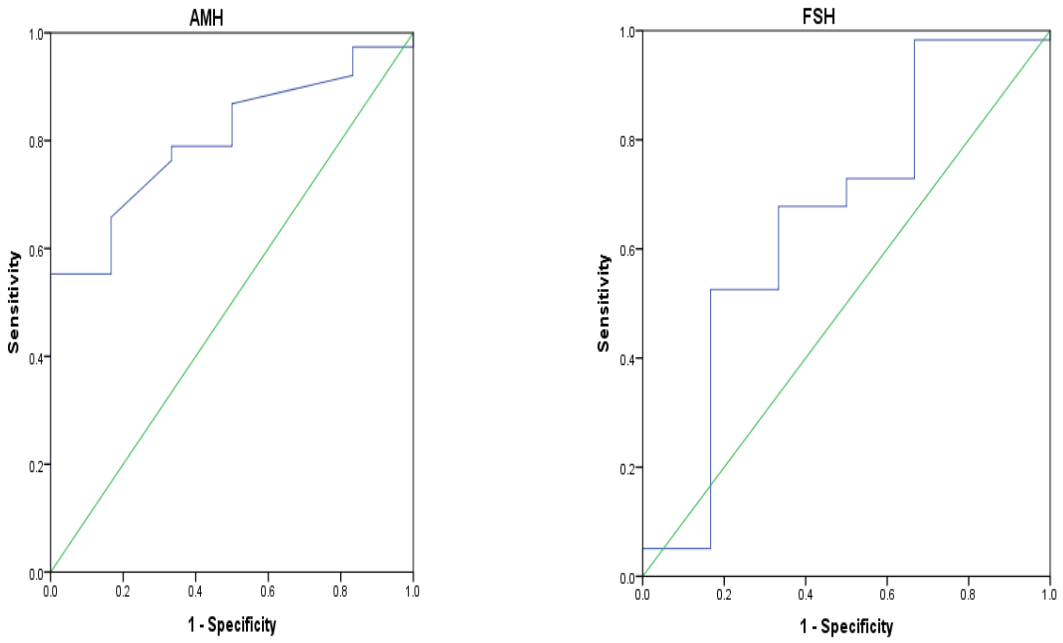

Figure 1. ROC curves for AMH and FSH predicting good against poor ovarian response

As presented in table 6, sensitivity, specificity, PPV and NPV for AMH test are 84%, 80%, 86%, 57% and for FSH are 67%, 68%, 50% and 54% respectively.

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Table 6. Diagnostic value of AMH and FSH

Hormone	Area	Cut of Value	Sensitivity	Specificity	PPV	VPV	p-value	95% CI	
								Lower	Upper
AMH	0.803	2 ng/ml	80%	84%	86%	57%	0.015	0.655	0.950
FSH	0.658	4 IU/ml	68%	67%	50%	54%	0.045	0.542	0.754

Discussion

The outcomes of the present research show a significant link between blood AMH and the number of antral follicles and retrieved oocytes. Numerous previous studies have also highlighted AMH as the most accurate predictor of ovarian reserve and responding the stimulation (11), and this investigation further reinforces its predictive value. Normal ovarian reactivity (2 ng/ml) is aligned with findings from previous studies (12), despite varying tests and contrasting results. An AMH level below or equivalent to 1 ng/ml was suggested as a possible predictor of a poor ovarian response. However, the majority of participants in this test were younger than 35 years of age, which may limit the extending of the results to women above this age.

Regarding the relatively small number of low response cases, the findings about poor ovarian response may lack robustness and require confirmation from further studies focusing on this specific population. Additionally, the results of these tests might be influenced by specific AMH interventions.

Our results align with prior studies showing that AMH is a superior predictor compared to FSH and serves as a reliable indicator of ovarian reserve (13). Notably, AMH consistently outperformed FSH across models, independent of the cycle or treatment group, in predicting ovarian response (14).

Other studies have attempted to predict ovarian response using different markers, such as age, FSH, LH, and AFC, but not AMH (15). However, AMH has shown significant advantages over FSH in predicting ovarian response (16). While AFC has been considered as a reliable indicator of the number of follicles, there have been limitations due to variations in measurements among ultrasound administrators (17). Disagreements about acceptable cutoff levels and the lack of standardized AMH tests have also contributed to challenges in clinical settings (14, 18).

Given these complexities, there is a pressing need for further research to establish better study validity and a global standard for AMH measurement, enhancing the clinical utility of this kind of ovarian response biomarker (19). ESHRE has recognized this need and emphasized on the importance of standardization (20, 21).

In conclusion, AMH shows promising potential as a robust marker for ovarian reserve and response to stimulation, but efforts to standardize AMH measurements and establish a global standard are vital for its widespread clinical application. Further research in diverse populations and under various conditions will strengthen its clinical utility and improve the accuracy of predictions in assisted reproductive treatments.

Ovarian response after in vitro fertilization or in vivo oocyte selection can be predicted with the use of serum AMH. The best AMH removal levels found in this study demonstrate the importance of considering context when making predictions about ovarian response.

Conflict of interest: The authors declare there is no conflict of interest.

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References

1.Dokuzeylül Güngör N, Güngör K. Ovarian stimulation drugs alter the metabolite content of the growing follicle: in vivo spectroscopic evaluation of follicle fluid. J Turk Ger Gynecol Assoc. 2021;22(2):132-8.

2.Salama S, Sharaf M, Salem SM, Rasheed MA, Salama E, Elnahas T, Lotfy R. FSH versus AMH: age-related relevance to ICSI results. Middle East Fertil Soc J. 2021;26(1):27.

3.Mamsen LS, Bøtkjær JA, Kristensen SG, Pors SE, Jeppesen JV, Kumar A, et al. High Variability of Molecular Isoforms of AMH in Follicular Fluid and Granulosa Cells From Human Small Antral Follicles. Front Endocrinol (Lausanne). 2021;12:617523.

4.Gholivand K, Rahimzadeh Dashtaki M, Alavinasab Ardebili SA, Mohammadpour M, Ebrahimi Valmoozi AS. E. New graphene oxide-phosphoramide nanocomposites as practical tools for biological applications including anti-bacteria, anti-fungi and anti-protein. J Mol Struct. 2021;1240:130528.

5.Zhou SJ, Sun TC, Song LL, Yang M, Sun XP, Tian L. The status and comparison of ovarian reserve between fertile and infertile healthy Chinese women of reproductive age. Medicine (Baltimore). 2021;100(17):e25361.

6.Yang L, Xu H, Chen Y, Miao C, Zhao Y, Xing Y, et al. Melatonin: Multi-Target Mechanism Against Diminished Ovarian Reserve Based on Network Pharmacology. Front Endocrinol (Lausanne). 2021;12:630504.

7.von Wolff M, Roumet M, Stute P, Liebenthron J. Serum anti-Mullerian hormone (AMH) concentration has limited prognostic value for density of primordial and primary follicles, questioning it as an accurate parameter for the ovarian reserve. Maturitas. 2020;134:34-40.

8.Massarotti C, La Pica V, Sozzi F, Scaruffi P, Remorgida V, Anserini P. Influence of age on response to controlled ovarian stimulation in women with low levels of serum anti-Müllerian hormone. Gynecol Endocrinol. 2020;36(12):1074-8.

9.Cedars MI. Evaluation of Female Fertility-AMH and Ovarian Reserve Testing. J Clin Endocrinol Metab. 2022;107(6):1510-9.

10.Seckin B, Turkcapar F, Ozaksit G. Elevated day 3 FSH/LH ratio: a marker to predict IVF outcome in young and older women. J Assist Reprod Genet. 2012;29(3):231-6.

11.Siddiqui QUA, Anjum S, Zahra F, Yousuf SM. Ovarian reserve parameters and response to controlled ovarian stimulation in infertile patients. Pak J Med Sci. 2019;35(4):958-62.

12.Nelson SM, Klein BM, Arce JC. Comparison of antimüllerian hormone levels and antral follicle count as predictor of ovarian response to controlled ovarian stimulation in good-prognosis patients at individual fertility clinics in two multicenter trials. Fertil Steril. 2015;103(4):923-30.

13.Hamdine O, Eijkemans MJ, Lentjes EW, Torrance HL, Macklon NS, Fauser BC, et al. Ovarian response prediction in GnRH antagonist treatment for IVF using anti-Müllerian hormone. Hum Reprod. 2015;30(1):170-8.

14.Iliodromiti S, Anderson RA, Nelson SM. Technical and performance characteristics of anti-Müllerian hormone and antral follicle count as biomarkers of ovarian response. Hum Reprod Update. 2015;21(6):698-710.

15.Broekmans FJ, Verweij PJ, Eijkemans MJ, Mannaerts BM, Witjes H. Prognostic models for high and low ovarian responses in controlled ovarian stimulation using a GnRH antagonist protocol. Hum Reprod. 2014;29(8):1688-97.

16.Andersen AN, Witjes H, Gordon K, Mannaerts B; Xpect investigators. Predictive factors of ovarian response and clinical outcome after IVF/ICSI following a rFSH/GnRH antagonist protocol with or without oral contraceptive pre-treatment. Hum Reprod. 2011;26(12):3413-23.

17.Arce JC, La Marca A, Mirner Klein B, Nyboe Andersen A, Fleming R. Antimüllerian hormone in gonadotropin releasing-hormone antagonist cycles: prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. Fertil Steril. 2013;99(6):1644-53.

[DOI: 10.22088/jbums.26.1.25]

18.Alson SSE, Bungum LJ, Giwercman A, Henic E. Anti-müllerian hormone levels are associated with live birth rates in ART, but the predictive ability of anti-müllerian hormone is modest. Eur J Obstet Gynecol Reprod Biol. 2018;225:199-204.

19.Movahed F, Chegini V, Mohammad Khaniha F. Simultaneous Occurrence of Ectopic Pregnancy in Both Fallopian Tubes and Live Intrauterine Fetus Following the Use of Ovulation Induction Medications: A Case Report. J Babol Univ Med Sci. 2023;25(1):20-5. [In Persian]

20.Farhud DD, Mojahed N. SARS-COV-2 Notable Mutations and Variants: A Review Article. Iran J Public Health. 2022;51(7):1494-501.

21.Sun XY, Lan YZ, Liu S, Long XP, Mao XG, Liu L. Relationship Between Anti-Müllerian Hormone and *In Vitro* Fertilization-Embryo Transfer in Clinical Pregnancy. Front Endocrinol (Lausanne). 2020;11:595448.

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