International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Bose S et al. Int J Reprod Contracept Obstet Gynecol. 2024 Dec;13(12):3530-3535
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20243434

Original Research Article

Assessing the predictive limitations of anti-Müllerian hormone in ovarian response: insights from controlled ovarian stimulation

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Received: 29 September 2024
Accepted: 07 November 2024

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ABSTRACT

Background: Anti-Müllerian hormone (AMH) is widely used to assess ovarian reserve and predict ovarian response during controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF) cycles. This study aims to evaluate the accuracy of AMH in predicting ovarian response, embryo quality, and clinical outcomes in IVF/ICSI cycles using a gonadotropin-releasing hormone (GnRH) antagonist protocol with a standardized gonadotropin dose of 150 IU for high and suboptimal responders. Additionally, the study examines the predictive value of alternative markers, such as age and antral follicle count (AFC), especially for suboptimal responders.
Methods: This retrospective, single-centre study analysed data from 158 women aged 21-35 years with AMH ≥1.5 ng/ml undergoing their first IVF cycle from July 2022 to July 2023. Patients were categorized into poor responders (<4 oocytes), suboptimal responders (4-9 oocytes), and poor responders , suboptimal responders and high responders ≥10-15 normal responders, >15 hyper responders based on oocyte retrieval. AMH levels, AFC, and age were assessed as predictors of ovarian response, embryo quality, and implantation rates. Statistical analyses included linear and logistic regression, and receiver operating characteristic (ROC) curves were used to evaluate predictive accuracy.
Results: Among 158 patients meeting the inclusion criteria, AMH showed a significant correlation with the number of oocytes retrieved (p=0.0036). High responders had better embryo quality and clinical pregnancy rates compared to suboptimal responders. AMH had a higher predictive value for high response (AUC=0.682) compared to suboptimal response (AUC=0.378), where age was a better predictor (AUC=0.522).
Conclusions: AMH is a reliable predictor of high ovarian response in GnRH antagonist protocols but is less effective for suboptimal responders. Comprehensive evaluations incorporating AMH, age, and AFC are crucial for individualized COS strategies to optimize outcomes in assisted reproductive technologies.

INTRODUCTION

Anti-Müllerian hormone (AMH) is acknowledged as an important guide for evaluating ovarian reserve and predicting how the ovaries will respond during controlled ovarian stimulation (COS) in *in vitro* fertilization (IVF).[1,2] While AMH is effective at indicating a high ovarian response, its reliability in predicting low responses and its connection to embryo quality and pregnancy rates are still unclear.[2] Additionally, AMH's effectiveness as a standalone predictor needs to be reassessed alongside other indicators like age and antral follicle count (AFC).[3]

This study aims to evaluate how accurately AMH predicts ovarian response, embryo quality, and clinical outcomes using a gonadotropin-releasing hormone (GnRH) antagonist protocol with a conventional initial dosage of 150 IU of gonadotropins. It will also consider alternative markers such as age and AFC, and propose management strategies for patients with suboptimal responses.[4]

In assisted reproductive technology (ART), customizing COS is essential for improving success rates, which in turn relies on reliable predictions of ovarian response.[5] It is crucial to understand the protocols which affect follicular development and hormone levels, as this may influence the accuracy of tests of ovarian reserve.[6] This is even more relevant, as clinicians are increasingly using GnRH antagonist protocol. AMH has been shown to be a dependable predictor of both high and low ovarian responses in GnRH agonist cycles.[7] However, its accuracy in predicting responses in oocyte donor cycles with antagonist protocols is only slight.[8] In 2015, a new category called "suboptimal responders" was introduced. It is defined as patients who have 4–9 oocytes retrieved after conventional stimulation.[9] This group is important to study, as the number of embryos available for transfer is directly related to the number of oocytes retrieved.[10]

Therefore, further research is needed to assess AMH's predictive accuracy for ovarian response in GnRH antagonist cycles.[11] This study aims to evaluate the predictive limitations of AMH in predicting ovarian responses in IVF cycles following GnRH antagonist stimulation protocol.

Aim

The aim of this study was to determine the predictive limitations of AMH in ovarian response in antagonist cycles.

METHODS

This retrospective, single-centre study was conducted at a tertiary reproductive medicine centre from July 2022 to July 2023. A total of 158 women aged 21-35 years with regular menstrual cycles and AMH levels more than 1.5 ng/ml, undergoing their first IVF cycle, were included. All participants received a standardized GnRH antagonist protocol with a starting dose of 150 IU of gonadotropins. Patients were stratified into three distinct groups based on their ovarian response: suboptimal responders (>4-9 oocytes retrieved), normal responders (10-15 oocytes retrieved), and hyper-responders (≥15 oocytes retrieved). Patients with severe endometriosis, polycystic ovary syndrome (PCOS), ovarian surgery, or hormonal disorders such as hyperprolactinemia or untreated hypothyroidism were excluded from the study.

Inclusion criteria

Women aged 21-35 years, AMH ≥1.5 ng/ml, regular menstrual cycles, first IVF cycle, and GnRH antagonist protocol with 150 IU of gonadotropins were included.

Exclusion criteria

Women with PCOS, diminished ovarian reserve (AMH <1.5 ng/ml), severe endometriosis, genetic conditions affecting fertility, ovarian surgery, hyperprolactinemia, and patients with untreated hypothyroidism were excluded.

Controlled ovarian stimulation

All patients underwent controlled ovarian stimulation using recombinant follicle-stimulating hormone (FSH) (Gonal F, Merck Serono) or hp-HMG (Materna, EMCURE) on day 1 or day 2 of menses, starting at a dose of 150 IU. The dose was adjusted during the stimulation period based on the patient's ovarian response. A GnRH antagonist (0.25 mg cetrorelix) was initiated when the lead follicle reached 14 mm in diameter and was continued throughout the gonadotropin treatment period. Final oocyte maturation was triggered using either a GnRH agonist (1 mg leuprolide) to prevent OHSS or recombinant hCG (250 mcg ovitrelle) to achieve optimal oocyte yield. Oocyte retrieval was performed 35 to 38 hours after the trigger injection. All embryos were cryopreserved using the vitrification technique following our institute's 'freeze-all' policy. Frozen embryos were subsequently transferred in a hormone replacement cycle.

AMH assay and AFC

AMH levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit. Blood samples were collected on day 2 or 3 of the menstrual cycle. The assay sensitivity was 0.01 ng/ml, with an intra-assay coefficient of variation (CV) of <5%.[12] The AFC was calculated during the early follicular phase via two-dimensional transvaginal ultrasonography which is in line with practical recommendations for standardized measurement of AFC.[13]

Study parameters

Primary outcome was ovarian response (number of oocytes retrieved after controlled ovarian stimulation).

Secondary outcomes were stimulation characteristics, no of mature oocytes, number of embryos for cryopreservation and implantation rate in the frozen-thawed hormone replacement cycle.

Statistical analysis

Analysis of data was done using statistical package for the social sciences (SPSS) version 26.0 with chi-square test for categorical parameters. Descriptive statistics were used to summarize patient demographics and baseline characteristics. The relationship between AMH levels and ovarian response was evaluated using linear regression analysis. To determine the sensitivity and specificity of AMH in predicting suboptimal and hyper-response, receiver operating characteristic (ROC) curves were generated. Analysis of variance (ANOVA) test was applied for AMH, the number of 2PN oocytes and the number of cryopreserved embryos using SAS software. The statistical significance was set at $p<0.05$.

RESULTS

The study involved 158 patients diagnosed primarily with infertility, all of whom were scheduled for IVF treatment. Patients were categorized into three subgroups based on their ovarian response: high response (n=38), optimal response (n=81), and suboptimal response (n=39). Baseline profile, such as age, weight, and AMH levels, were compared across these groups which were similar and are presented in Table 1.

Among the patients, AMH distribution revealed that 56 women (35.4%) had AMH levels above 3.5, while 52 women (32.9%) had AMH levels between 1.5 ng/ml and 3.4 ng/ml. The three subgroups showed a significant difference in mean AMH values (p=0.0036). Additionally, the Chi-square test indicated a significant positive correlation (p<0.05) between AMH levels and the number of oocytes retrieved.

Predictive value of AMH and age

The predictive ability of AMH and age for high and sub-optimal responses was assessed using receiver operating characteristic (ROC) curve analysis, comparing the areas under the curve (AUC). For high response prediction, the ROC analysis revealed that AMH had a greater AUC (0.682, 95% CI: 0.579-0.785) than age (AUC: 0.604, 95% CI: 0.499-0.709), indicating that AMH provides better accuracy for this prediction.

In contrast, AMH demonstrated limited efficacy in predicting sub-optimal responses compared to age. For sub-optimal response prediction, AMH had an AUC of 0.378 (95% CI: 0.281-0.476), while age yielded a higher AUC of 0.522 (95% CI: 0.422-0.621).

Figures 1 and 2 shows ROC for AMH and age indicating a high and sub-optimal response.

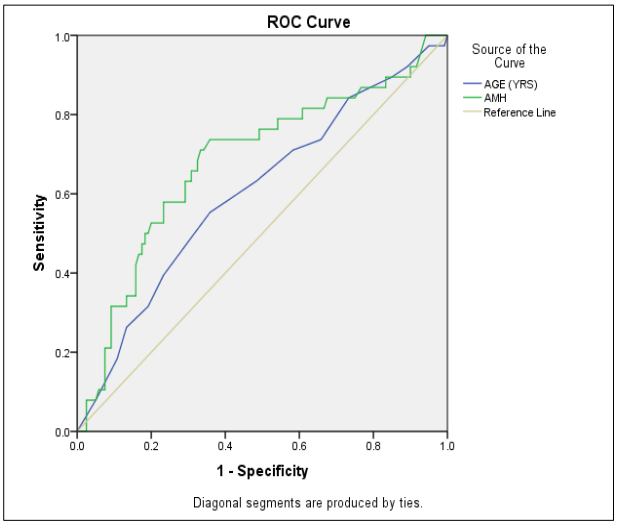

Figure 1: ROC curve analysis of age and AMH for predicting high ovarian response.

Predictive value of AFC and age

AFC showed strong predictive value for a high ovarian response (≥15 oocytes), with an AUC of 0.759 (95% CI: 0.692-0.826), meaning that a higher AFC is closely linked to retrieving more oocytes. This makes AFC a reliable indicator of a strong ovarian response. However, for suboptimal response (4-9 oocytes), AFC had a lower AUC of 0.432 (95% CI 0.355-0.509), indicating it is less effective at predicting this outcome.

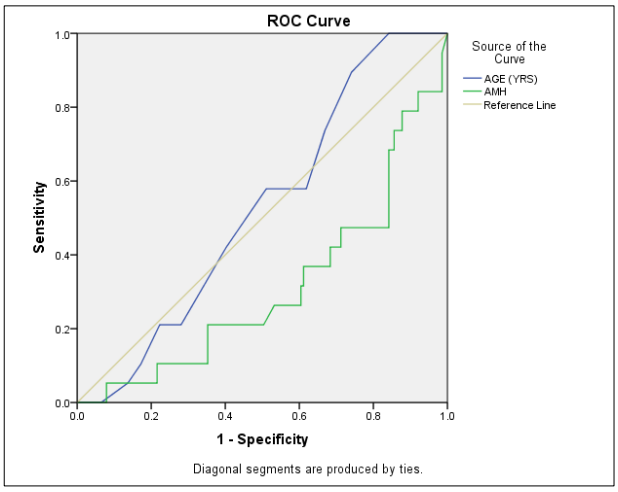

Figure 2: ROC curve analysis of age and AMH for predicting suboptimal ovarian response.

The subgroup population was analysed using the ANOVA procedure in SAS software, stratified by the 25th, 50th, and 75th percentiles of serum AMH, total oocytes retrieved, number of 2PN oocytes, and embryos available for cryopreservation. Box plot analysis revealed a significant decline in median values as ovarian response decreased from high to low (Figures 3 and 4). Also, a statistically significant difference was noted among the three subgroups for all of these outcome parameters as shown in Table 2.

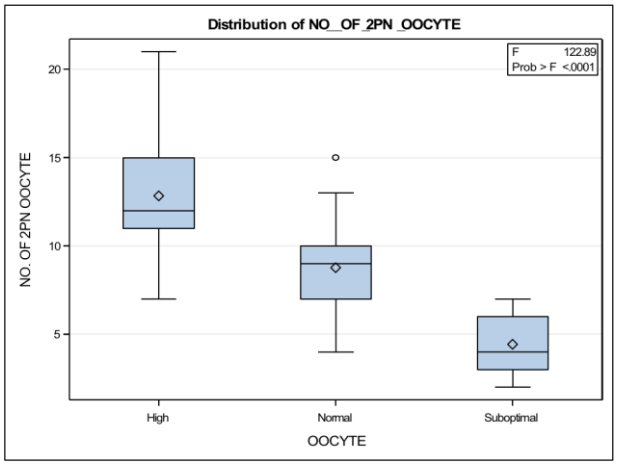

Figure 3: Distribution of the number of 2 PN oocytes retrieved among the three subgroups.

Stimulation characteristics

The duration of stimulation, total gonadotropin dosage, and number of oocytes retrieved differed across response groups. High responders required less gonadotropins and produced more oocytes compared to optimal and suboptimal responders. Moreover, the high response group showed a significantly higher number of cryopreserved embryos (p<0.0001).

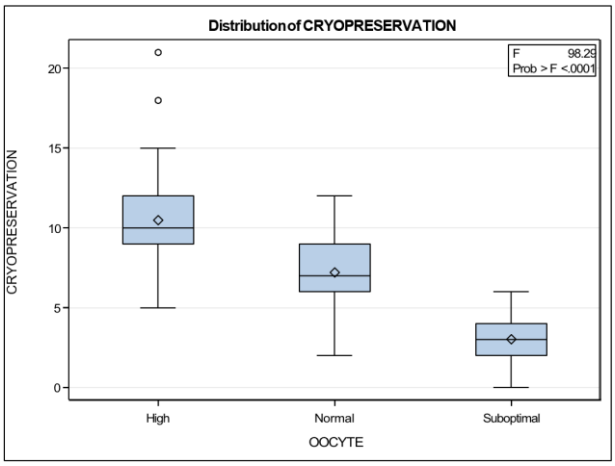

Figure 4: Distribution of the number of cryopreserved embryos among three subgroups.

Embryo quality and fertilization rates

High responders produced significantly more good-quality blastocysts (grade A and B) than optimal and suboptimal responders (p<0.05). While embryo quality correlated positively with AMH (r=0.41) **(**95% CI 0.271-0.532), fertilization rates were similar across the AMH groups, indicating that while AMH predicts ovarian reserve, its role in fertilization potential is limited.

Implantation rates

Suboptimal responders showed significantly lower implantation rates compared to both high and optimal responders (p<0.05). The optimal response group had the highest implantation rate at 52%, followed by the high response group at 48%, while the suboptimal group had the lowest rate at 21% (p<0.05).

Suboptimal responders

Among suboptimal responders, AMH was a weaker predictor of clinical outcomes. Alternative strategies, such as dual triggering and higher gonadotropin doses, were used to optimize oocyte yield, yet these patients had lower clinical pregnancy rates (18%) compared to optimal responders (45%).

Table 1: Baseline profile of women undergoing IVF in GnRH antagonist protocols.

Baseline profile	High response (≥15 oocytes)	Optimal response (10-14 oocytes)	Suboptimal response (>4-9 oocytes)	P value
Age (years)	29.47±3.74	27.96±3.67	28.70±3.31	0.09
Weight (kg)	58.24±10.04	58.74±10.40	60.63±9.60	0.53
AMH (ng/ml)	6.07±2.29	4.96±2.64	4.23±1.78	0.0036
AFC	≥20	17 (13-20)	9 (7-12)	0.0011
Primary subfertility (%)	16.46	37.34	15.82	0.6114
Secondary subfertility (%)	7.59	13.92	8.84	0.916
Subfertility duration (years)	4.63±3.08	4.54±2.72	4.32±2.21	

Table 2: Stimulation characteristics and clinical outcome.

Stimulation characteristics	High response (≥15 oocytes)	Optimal response (10-14 oocytes)	Suboptimal response (4-9 oocytes)	P value
Total dose of FSH (IU)	2350±395	2926.23±1060.36	3298.08±994.01	0.578
Duration of stimulation (days)	10.08±1.23	10.25±1.4	11.79±1.65	0.19
Total number of oocytes retrieved	18.05±2.59	12.61±1.88	7.33±1.52	0.0001
Number of 2PN oocytes	12.84±3.09	8.77±2.29	4.43±1.48	0.0001
Number of cryopreserved embryos	10.47±3.02	7.209±2.31	3.97±1.62	0.0001
Implantation rates (%)	48	52	21	

DISCUSSION

This study reinforces the role of AMH as a dependable guide for predicting ovarian response. This is particularly so in high responders undergoing controlled ovarian stimulation with GnRH antagonist protocol. The main advantage of AMH lies in its minimal fluctuation during the menstrual cycle, reduced inter-observer variation, and the decreased need for additional ultrasounds. The studies by Choi et al and Hamdine et al, support the role of AMH as a more accurate predictor of excessive ovarian response

compared to age.[1,2] It is further supported with studies from Arce et al and Broer et al, who identified AMH as a reliable marker for identifying patients at risk of ovarian hyperstimulation syndrome (OHSS).[3,4] However, while AMH is strong in predicting high responders, its limitations, particularly in predicting suboptimal responses, need to be acknowledged. Suboptimal responders (those retrieving 4-9 oocytes) represent a challenging group in IVF due to their lower clinical pregnancy and live birth rates, and AMH alone may not suffice in guiding treatment for this group.[14]

In line with earlier studies like those by Broer et al and Joan-Calles et al, our findings confirm that AMH is superior to age in predicting ovarian response, particularly in high responders.[4,5] However, this study adds to existing literature by critically addressing the limitations of AMH with respect to suboptimal responders. The heterogeneous nature of this group makes it more difficult to manage. Existing literature provides limited guidance on how best to optimize stimulation protocols for suboptimal responders.

Our results showed that AMH had a strong correlation with high response, particularly in women with increased AMH levels, who tend to retrieve a higher number of oocytes. This can help adjust gonadotropin doses to reduce the risk of OHSS while still maximizing embryo yield.[4] However, AMH was less accurate in predicting suboptimal responses, highlighting the need for a more individualized approach that considers integrating other markers such as AFC, age, FSH and oestradiol.[15]

Suboptimal responders: a clinical challenge

Suboptimal responders represent a distinct and challenging group in ART. While they do not perform as poorly as low responders, their outcomes are far from those of high responders. Current literature, including our study, shows wide variation in ovarian response even among women of similar age and AMH levels. These patients often require higher gonadotropin doses or alternative protocols like dual-trigger or mild stimulation strategies to optimize outcomes. However, our study indicates that AMH alone is insufficient for predicting response in this group. Studies like those by Nelson et al and Hochebarg et al emphasize the benefit of a multi-marker approach, particularly in patients with less predictable ovarian reserves.[12,16] This approach integrating AMH with AFC, age, and possibly emerging biomarkers such as follicle-stimulating hormone receptor (FSHR) gene polymorphisms and insulin-like growth factors (IGF) could enhance predictive accuracy and treatment customization for suboptimal responders.[17]

The individualized approach to ovarian stimulation is now recognized as essential, particularly for managing suboptimal responders. In our study, AMH proved useful in adjusting gonadotrophin doses to minimize OHSS in high responders. However, for suboptimal responders, AMH's predictive limitations highlight the need for a more nuanced approach. Clinicians should consider other factors such as AFC and patient-specific characteristics like metabolic health and genetic predispositions. This personalized approach ensures that ovarian stimulation is optimized for each patient, improving both safety and reproductive outcomes.[18]

Future directions and research gaps

This study highlights the need for further exploration into managing suboptimal responders, an area that remains under-researched. Investigations into novel biomarkers and personalized stimulation strategies could significantly contribute to refining treatment protocols. Future studies should include larger sample sizes and more diverse populations to enhance generalizability. Additionally, long-term outcomes such as cumulative pregnancy and live birth rates from subsequent frozen embryo transfers (FET) were not analysed in this study, representing another area for future research. Prospective studies focusing on the interplay between various markers would provide stronger evidence for personalized IVF protocols, especially in complex cases like recurrent implantation failure.

Limitations

While this study provides valuable insights, it is important to recognize its limitations. The small sample size and retrospective design introduces potential selection bias, and the relatively homogenous study population may limit generalizability to more diverse groups. Additionally, we did not analyse long-term reproductive success, such as cumulative live birth rates, which would have provided a more comprehensive understanding of AMH's role in predicting overall IVF success.

CONCLUSION

In conclusion, while AMH is a valuable predictor for high ovarian response, its predictive value is limited for suboptimal responders, necessitating the integration of additional markers like AFC and age. A tailored, patient-specific approach that incorporates these factors is essential for optimizing IVF/ICSI outcomes. Suboptimal responders, in particular, require focused clinical guidelines and personalized treatment strategies. Future studies should explore the impact of genetic and lifestyle factors on AMH's predictive value and consider longitudinal tracking of AMH levels across multiple IVF cycles for a deeper understanding of ovarian reserve dynamics.

ACKNOWLEDGEMENTS

The authors would like to thank the embryology team and Dr. Arijit Ganguly for their cooperation in the completion of the study.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Bose S, Konar L. Assessing the predictive limitations of anti-Müllerian hormone in ovarian response: insights from controlled ovarian stimulation. Int J Reprod Contracept Obstet Gynecol 2024;13:3530-5.