Original Article

To assess serum Anti Mullerian Hormone (AMH) level as a biomarker for oocyte quality

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Abstract

Introduction: Oocyte competence is a factor that affects ART outcomes. Oocyte morphology assessment and scoring are laborious, have inter-observer variations and lack consensus on parameters to be assessed. Role of anti mullerian hormone (AMH) as a biomarker for oocyte quality and competence is investigated. **Aim and Objectives:** To evaluate day 2/3 serum AMH as a predictor of oocyte quality, embryo quality and fertilization rate in a stimulated ART cycle. **Methods:** Single centre, prospective observational study. All patients undergoing IVF-ICSI between August 2018 and February 2019 included. Total Oocyte Score calculated for all oocytes. and subsequently assessed for fertilization, cleavage and day 3 embryo grading. Correlations between AMH levels and TOS, PTOS, Fertilization and Cleavage rates assessed using appropriate statistical methods. **Results:** Of 86 patients and 780 oocytes studied,639 underwent ICSI with fertilization rate 86% (550/639) and cleavage rate 90.7%(499/550). Embryo grades were A-50.6%, B-38.9% and C-10.5%. Mean TOS score and PTOS scores were 0.3±1.99 and 0.44±1.21.TOS was predictive of fertilization [ROC-AUC=0.598($P=0.004$)]. Mean AMH levels were 3.66 ± 3.96 ng/ml [0.69±0.21ng/ml<25th centile, 2.74±1.14 ng/ml, 25th–75th centile, 8.27±5.08ng/ml>75th centile] and decreased with increasing age. AMH levels correlated with TOS, PTOS, fertilization and cleavage rates. The r^2 values were low suggesting poor clinical significance. AMH levels were not predictive of fertilization and embryo grade. **Conclusion:** AMH levels show weak correlation with oocyte quality and are poor predictor of oocyte competence.

Keywords: Anti-mullerian hormone (AMH), cleavage rate, embryo grade, fertilization rate, intra cytoplasmic sperm injection (ICSI), oocyte quality, patient-specific total oocyte score (PTOS), total oocyte score (tos), **6**

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INTRODUCTION

AMH is a dimeric glycoprotein belonging to the transforming growth factor β (TGF-β) family. It is secreted by the granulosa cells of the primary, secondary, pre-antral and early antral follicles.[1] It inhibits the initiation of primordial follicle growth and regulates recruitment of cohorts of antral follicles. AMH levels reflect the primordial follicle pool and are a predictor of ovarian response to hormonal stimulation in ART cycles.[2] AMH levels decline with increasing age and is a marker of ovarian ageing and ovarian reserve.

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Success of ART depends upon getting adequate number of good quality oocytes, the embryo's developmental competence and their implantation potential. Currently, only a small percentage of the total oocytes retrieved in an ART cycle leads to a successful child birth.[3] An assessment of oocyte quality may help predict which oocytes may lead to successful implantation.

Oocyte quality is usually assessed at the time of insemination. Up to 60% of the oocytes post denudation of cumulus cells are found to have intra cytoplasmic and extra cytoplasmic abnormalities.[4] Intracytoplasmic parameters commonly assessed are dark or granular cytoplasm and cytoplasmic fragments like vacuoles, SER discs. The extra cytoplasmic parameters commonly assessed include zona pellucida colour, perivitelline space size and granularity, first polar body morphology and oocyte shape.

There is a debate whether AMH levels can predict oocyte quality. Low AMH levels have been associated with dark central granulations, while aggregations of SER are seen in patients with high AMH levels, both correlate with poor oocyte quality.[2]

Lazzaroni-Tealdi *et al.*[5] validated patient-specific total oocyte score (PTOS) for oocyte quality. PTOS scores directly correlated with increased embryo cell number, embryo grade and clinical pregnancy rates and added to the prediction of the success of the ART cycle over and above routine embryo morphology assessment.

In this study we sought correlations between AMH levels with oocyte quality parameters using TOS and PTOS scores.

MATERIALS AND METHOD

The study was conducted at Southend Fertility & IVF Centre, Vasant Vihar, New Delhi after obtaining ethical clearance from institute ethics committee. It was a prospective observational study conducted between August 2018 and February 2019. All infertile couples undergoing IVF-ICSI, irrespective of their cause of infertility and ovarian stimulation protocol were included.

Oocyte quality was assessed and PTOS were calculated. Correlation between AMH levels and oocyte score with embryo grade, fertilization rate and cleavage rate were assessed.

Women undergoing controlled ovarian stimulation (COS), irrespective of COS protocol used and between age group 20 and 45 years were included. Those with severe male factor infertility or where TESA sample was used, those who did not undergo IVF-ICSI and those with cycle cancellation were excluded.

Day 2/3 Baseline Serum AMH levels were measured using enzyme-linked fluorescent assay.

Oocyte retrieval was done 34 hours after the ovulation trigger. The oocytes were incubated for 2 hours after retrieval to facilitate their final maturation and then denuded for ICSI.

Oocyte morphology was evaluated at the time of ICSI and scored according to the scoring system described by Lazzaroni-Tealdi *et al.*[5] except that oocyte size was judged by comparison only. The total oocyte score (TOS) was calculated for each oocyte and PTOS was calculated for each patient by averaging the TOS. The inseminated oocytes were cultured according to standard protocol. Fertilization was assessed at 17–18 hours and cleavage was assessed at 26 hours after insemination respectively. Embryo grading was done on Day 3 using the Istanbul Consensus (2010) for Embryo Grading.[6]

STATISTICAL METHODS

The patients were divided into three groups based on the AMH levels taking the 25th and 75th percentile as the cutoffs. Correlations with serum AMH in the entire study group and each subgroup were assessed with oocyte quality, fertilization rate, cleavage rate and embryo quality. Appropriate statistical methods for parametric and non-parametric data including Chi-square test, Student's t-test, Krusker-Wallis etc. were used. ROC curves for finding cutoffs were generated. A *P*-value of <0.05 was considered as significant.

RESULTS

A total of 86 patients undergoing IVF-ICSI were recruited. The demographic profile of the patients is presented below in Table 1.

The mean age of the study population was 33.68 ± 4.14 years and the mean BMI was $24.81 \pm 3.75\ kg/m^2$. Majority (69.8%) of the study population presented with Primary infertility. The most common cause of infertility was determined to be male factor. A total of 780 oocytes were retrieved with a median of 8.5 oocytes per patient.

Table 1: Demographic profile in study population

Study characteristics	Mean or %
Age (years)	33.68 (±4.14)
BMI (Kg/m2)	24.81 (± 3.75)
Baseline hormones	
D2 FSH (mIU/ml)	6.16 (±2.67)
D2 LH (mIU/ml)	4.10 (±2.55)
D2 E2 (pg/ml)	37.09 (±16.6)
D2 P4 (ng/ml)	0.49 (±0.33)
D2 AMH (ng/ml)	3.66 (±3.96)
Diagnosis	
Primary infertility	69.8%
Secondary infertility	30.2%
Cause of infertility	
Unexplained	3.4%
DOR	24.4%
Tubal	23.2%
Endometriosis	17.4%
PCOS	23.2%
Male	45.3%
RPL	3.4%
AMA	12.8%
COS protocols	
Antagonist	66.3%
Long	10.4%
Stop	9.3%
Minimal	1.1%
Mean gonadotropin dose for stimulation (IU)	3141 (±1062)
Trigger	
GnRH agoinist	29%
rHCG	71%
Total oocytes retrieved	*N* = 780, median 8.6 (IQR 7)
M-II	645 (82.69%)
M-I	86 (11.02%)
GV	43 (5.51%)
Total oocytes inseminated	639
Total oocytes fertilized (fertilization rate)	550 (86.07%)
Total embryos cleaved (cleavage rate)	499 (90.7%)
Grade of embryos	
Grade A	251(50.6%)
Grade B	193 (38.9%)
Grade C	52 (10.5%)
Mean TOS	0.30 (± 1.99)
Mean PTOS	0.44 (± 1.21)

Among these, 43 (5.51%) were in the Germinal Vesicle (GV) stage, 86 (11.02%) were in Metaphase I(M-I) stage and 645 (82.69%) were Metaphase II (M-II) stage oocytes. All the mature M-II oocytes ($N = 645$) which were normal in size were analyzed for their morphological characteristics. Those oocytes which were significantly larger in size as compared to their sibling oocytes were excluded ($N = 12$).

Out of the 645 M-II oocytes obtained, 639 oocytes were inseminated, the remaining were discarded due to abnormality of size. Of the 639 inseminated oocytes, 550 got fertilized giving a fertilization rate of 86.0% (550/639). The fertilized oocytes were further cultured and assessed at 26± 1 hour post insemination for first cleavage into two cells. Out of the 550 fertilized oocytes,

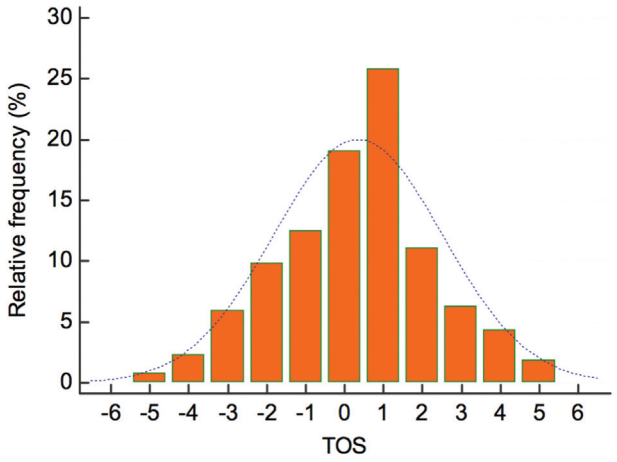

Figure 1: Distribution of total oocyte score in the study population.

Table 2: Oocyte morphology characteristics

	−1	0	+1
PB	94 (14.7%)	390 (61.0%)	155 (24.2%)
ZP	114 (17.8%)	394 (61.6%)	131 (20.5%)
PVS	183 (28.6%)	390 (61.0%)	65 (10.2%)
Cytoplasm	257 (40.2%)	330 (51.6%)	52 (8.1%)
Shape	70 (10.9%)	67 (10.5%)	502 (78.6%)

cleavage was seen in 499 giving a cleavage rate of 90.7% (499/550). On day 3, a total of 496 embryos were formed while 3 embryos got arrested at two cell stage. Out of these, 251(50.6%) were of Grade A, 193(38.9%) were of Grade B and 52 (10.5%) were of Grade C.

Out of the 86 patients included in the study, embryo transfer was cancelled in five patients in view of either non availability or poor quality of embryos(failed cycle rate of 5.8%). No significant correlation of TOS and AMH was seen with failed cycles.

OOCYTE SCORE AND CORRELATES

Oocyte morphology assessment was done for all oocytes. Each parameter was scored with: "−1" representing poor quality, "0" average quality and "+1" good quality. Oocyte size was not scored as large oocytes were excluded. Total Oocyte Scores (TOS) were calculated by summing the parameters (range −5 to +5). PTOS were calculated by averaging TOS of all oocytes divided by number of oocytes of an individual patient. The mean TOS and mean PTOS for the study population were 0.30±1.99 and 0.44±1.21 respectively. The distribution of the TOS in the study population is depicted in Figure 1 and Table 2.

OOCYTE SCORE AND FERTILIZATION

Among the 11 subgroups of TOS scores (−5 to +5), no correlation with fertilization rate could be established

(chi-square, $P = 0.2414$). Similarly, PTOS scores also did not correlate with fertilization. However, individual TOS score correlated with and was a predictor of fertilization (*P*-value = 0.0044) [Figure 2].

ASSESSMENT OF AMH AS A BIOMARKER

The mean AMH level of the population was 3.66 (±3.96). AMH levels were also assessed as per centiles. Group I: AMH<25th percentile (<1.09ng/ml), mean AMH: 0.69 ±0.21ng/ml, Group II: AMH between 25th and 75th percentile (1.09–4.9ngm/ml), mean AMH: 2.74±1.14ng/ml, Group III: AMH > 75th percentile (4.9 ngm/ml), mean AMH: 8.27±5.08ng/ml.

AMH levels tended to significantly correlate with age, TOS, PTOS, fertilization and cleavage rates. The serum AMH showed a declining trend with increasing age [Graph 'M' Figure 3]. Table 3 gives correlates of AMH as percentiles with PTOS, fertilization and cleavage rates.

The correlations of AMH with various parameters are depicted in graphs "A" to "O" in Figure 3.

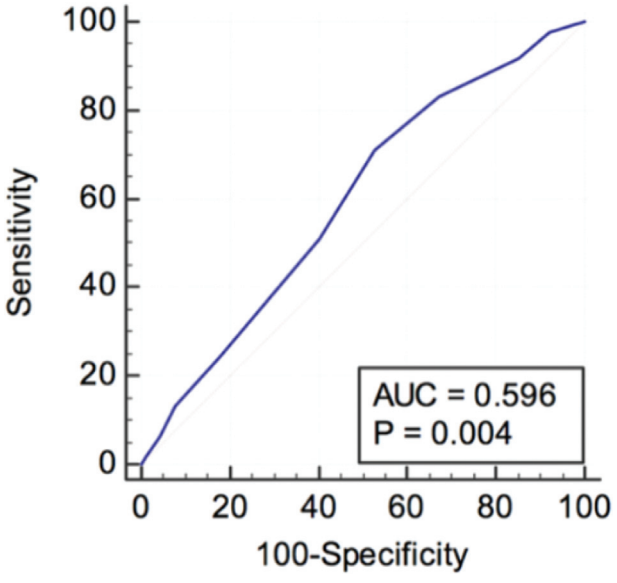

Figure 2: TOS as a predictor of fertilization.

AMH AND OOCYTE SCORE

Graphs A, B, C, D [Figure 3] show correlates of AMH with PTOS. A decreasing trend in the PTOS was seen with increasing AMH levels. AMH levels correlated with PTOS scores across all AMH subgroups. A decreasing trend in PTOS scores was most significant in <25 centile AMH group [graph B] and opposite trend was seen in AMH > 75 centile group [graph D]. However, the correlates were weak as both had low r^2values. Also, the overall PTOS scores didn't differ amongst the three AMH centile groups [graph N].

AMH AND FERTILIZATION

Graphs E, F, G, H [Figure 3] show correlates of AMH with fertilization. Decrease in AMH levels was associated with decreasing fertilization rates [graph E]. This trend was also seen in two AMH subgroups, group with AMH<25th centile and that between 25th and75th centile [graphs F, G]. At AMH values beyond 75th centile, an increase in the fertilization rate was seen but was not statistically significant($P = 0.219$). All these however had poor r^2 values suggesting poor clinical correlations. This is also borne out as the ROC curve to evaluate use of AMH as a predictor of fertilization showed poor predictability with no significance (*P*-value = 0.835) [Figure 4].

AMH AND CLEAVAGE

Graphs I, J, K, L (Figure 3] show correlates of AMH with cleavage. A decreasing trend in the cleavage rate with a decrease in AMH was observed [Figure 3]. This trend was consistently seen in all AMH subgroups [graphs J, K, L] [Figure 3]. The trends were statistically significant in $<25^{th}$ centile and 25^{th}–75^{th} centile groups and nonsignificant in $>75^{th}$ centile group ($P = 0.368$). However, r^2values were low, again suggesting poor clinical significance.

AMH AND EMBRYO GRADE

Graph O [Figure 3] depicts the correlates of AMH with embryo grade. Kruskal-Wallis Test was used to assess the

Table 3: Correlation of AMH

	AMH	PTOS		Fertilization		Cleavage	
	Mean	Mean	*P*-value	Mean	*P*-value	Mean	*P*-value
Total AMH	3.68±3.94	0.44±1.21	**0.000**	6.49±4.31	**0.000000487**	6.05±4.53	**0.00021**
<25th percentile	0.69±0.21	0.42±1.48	0.42	3.33±1.75	**0.0000023**	2.75±1.81	**0.00016**
25th–75th percentile	2.74±1.14	0.46±1.24	**0.000**	5.74±3.71	**0.0000125**	5.12±3.95	**0.00068**
>75th percentile	8.27±5.08	0.38±0.89	**0.000**	9.39±4.49	0.219	9.12±4.98	0.368

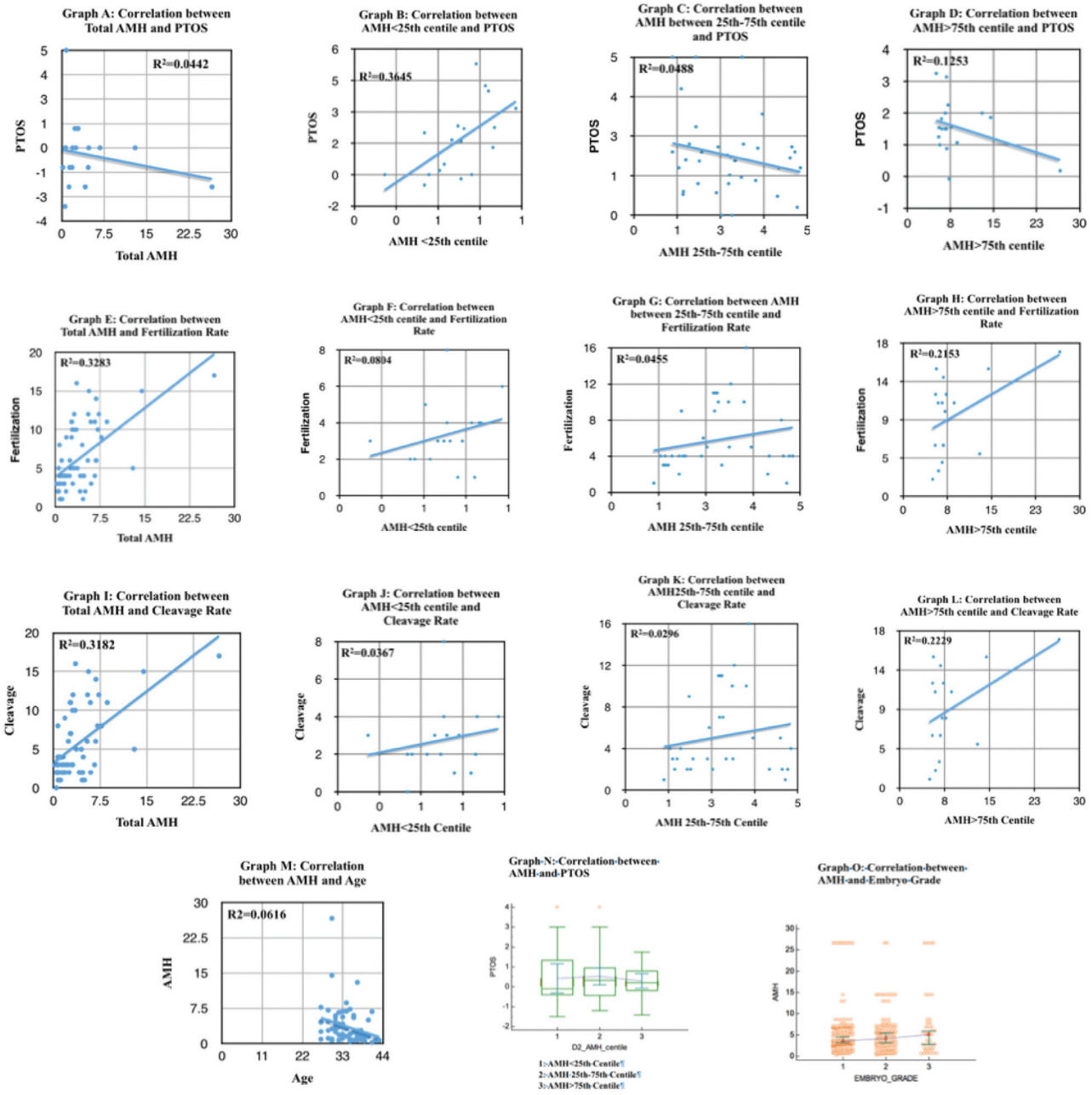

Figure 3: AMH correlates.

relation and it was found that AMH levels and centiles did not predict the embryo grade (*P*-value = 0.8645).

DISCUSSION

The current study assessed the role of AMH as a biomarker for oocyte competence by assessing its role in predicting oocyte quality, fertilization, cleavage and embryo grade. The population evaluated is representative of the one visiting a tertiary infertility center with a mixed bag of various causes of infertility managed with appropriate COS protocols. Our study showed a fertilization rate of 86.07% and a cleavage rate of 90.7%. This is consistent with the fertilization and cleavage rates reported across literature.

The AMH levels were found to show a decreasing trend with age which is consistent with observations in literature as AMH is a marker for ovarian reserve that declines with increasing age.[7] AMH levels show variation amongst different races and ethnicities and hence it is recommended to develop population and age-specific AMH levels.[8] Our study population was divided into three groups based upon the AMH levels, those with low AMH levels that is <25th percentile, those with normal AMH levels i.e. between 25th and 75th percentile and

those with high AMH levels that is >75th percentile. The cut-off for low AMH group was <1.09ng/ml and cut-off for high AMH group was 4.9ng/ml. Even though the sample size was small, our cutoffs for AMH levels were consistent with what is described in literature.[9] However, because of the small sample size, subdivision according to age was not done.

Oocyte quality is considered as a predictor of oocyte competence.[10] Currently, oocyte quality assessment is done by evaluating the oocyte morphology for many intracytoplasmic and extracytoplasmic features. Many composite scores based on these parameters are described and validated.[3,5,11,12] We used PTOS scoring system with some modification (omission of exact size in assessment) for assessing oocyte quality. Oocyte morphology score was assigned according to the Lazzaroni-Tealdi scoring system which has a range from (−6) to (+6), with a score of (−6) being the worst quality and (+6) being the best quality. In our study we had excluded oocytes with abnormal size and so did not the score size thus. TOS scores therefore ranged between (−5) and (+5). The mean TOS score in our study was 0.30 ± 1.99. The mean PTOS score was 0.44± 1.21.The oocyte score distribution among the study population was normal [Figure 1]. This is similar to the distribution pattern described in literature.[5] It was also seen that TOS scores were predictive of fertilization [Figure 2]. Although, no specific PTOS cutoff score was predictive of fertilization, and PTOS scores didn't predict cleavage and embryo grade in the current study, these may be due to a smaller sample size for such assessment as evaluation of PTOS was not the primary objective of the study. A normal distribution of TOS values and its ability to predict fertilization suggests it to be a valid score for oocyte morphology assessment.

AMH levels correlated with TOS and PTOS scores. A decreasing oocyte quality was seen with decreasing AMH levels. This was best seen at <25th centile AMH values [graph B in Figure 3] while a high AMH level (>75th centile) tended to show better quality. Findings are consistent with those by Ebner at al who found worsening of oocyte score with AMH levels either below the lower cut-off or increase beyond the upper cut-off.[9]

Similarly, decreasing AMH levels were associated with decreasing fertilization [graph E in Figure 3]. This was consistent across all AMH centile groups [graphs F, G, H in Figure 3]. Similar correlations were seen between AMH levels and cleavage rates [graphs I, J, K, L in Figure 3]. Although statistically significant the, r^2values for all these

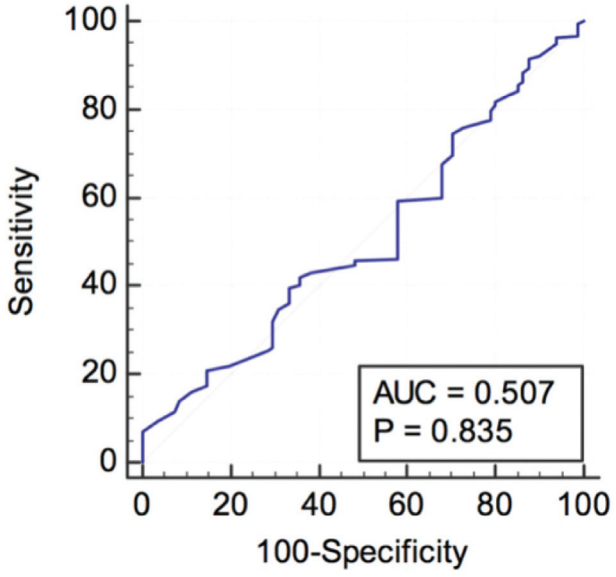

Figure 4: AMH as a predictor of fertilization.

correlations were very low and suggested poor clinical correlation. ROC curve was plotted between AMH levels and fertilization and failed to show any significant predictive value of AMH for fertilization. AMH levels also could not predict embryo grades [graph O in Figure 3].

AMH is considered to be a good marker of ovarian reserve. It is also considered a good predictor of ovarian response to stimulation.[13] Low AMH levels have been associated with dark central granulations, while aggregations of sER are seen in patients with high AMH levels, both correlate with poor oocyte quality.[9,13] All these suggest biological plausibility for development of AMH as a biomarker for oocyte competence. Further, its assays are standardized and readily available and levels are routinely assessed pre-COS. On the other hand oocyte quality assessment is a laborious process as each stimulation cycle can often produce multiple oocytes and each oocyte has to be assessed individually and is subject to inter-observer variations. Also, there is no consensus on which parameters of oocyte quality are best predictors of oocyte competence.The study did find statistically significant correlations between AMH levels and oocyte quality, fertilization and cleavage rates. However, the correlations turned out to be clinically weak. This can be explained by the fact that oocyte competence is a complex phenomenon involving other factors which may play a more significant role than AMH.

Though the current study shows that AMH may be a poor predictor of oocyte competence, there are some

limitations. We could not divide patients according to age due to limited sample size. In a previous study by Gupta S et al, they found a correlation of AMH with oocyte quality in advanced age.[14] However, that study could also have a bias as advanced age in itself is a predictor of poor outcomes. Role of AMH in patients with advanced age needs further studies and evaluation to elicit its role in that subgroup.

CONCLUSIONS

AMH levels showed correlations with oocyte quality, fertilization rates, cleavage rates. However these associations were clinically very weak. AMH showed little predictive value as a marker for oocyte fertilization. Thus, the role of serum AMH as a biomarker for oocyte quality and competence is questionable. A much larger sample size would be needed to draw any definitive conclusions.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Kamil AN, Saleh BOM, Hassan NF. Serum anti-müllerian hormone (AMH) in prediction of oocyte quality and in vitro fertilization (IVF) outcome. The Iraqi Postgraduate Medical Journal 2013;12:551-61.
2. Borges E, Braga DPAF, Setti A, Figueira RC, Iaconelli A Jr. The predictive value of serum concentrations of anti-Müllerian hormone for oocyte quality, fertilization, and implantation. JBRA Assisted Reproduction 2017;21(3):176-182.
3. Wiweko B, Anggraheni U, Mansyur E, Yuningsih T, Harzief AK, Pratama G, Sumapraja K, Natadisastra M, Hestiantoro A. Serum AMH level predicts oocytes quality better than follicular fluid AMH level. Asian Pacific Journal of Reproduction 2016;5:361-4.
4. Braga DP, Setti AS, Figueira Rde C, Machado RB, Iaconelli A Jr, Borges E Jr. Influence of oocyte dysmorphisms on blastocyst formation and quality. Fertil Steril 2013;100:748-54.
5. Lazzaroni-Tealdi E, Barad DH, Albertini DF, Yu Y, Kushnir VA, Russell H, Wu YG, Gleicher N. Oocyte scoring enhances embryo-scoring in predicting pregnancy chances with IVF where it counts most. PLoS One 2015;10:e 0143632.
6. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Human Reproduction 2011;26:1270-83.
7. Broer SL, Broekmans FJM, Laven JSE, Fauser BCJM, Anti-müllerian hormone: ovarian reserve testing and its potential clinical implications. Human Reproduction Update 2014;20:688-701.
8. Lehmann P, Vélez MP, Saumet J, Lapensée L, Jamal W, Bissonnette F, Phillips S, Kadoch IJ. Anti-Müllerian hormone (AMH): a reliable biomarker of oocyte quality in IVF. J Assist Reprod Genet 2014;31:493-8.
9. Ebner T, Sommergruber M, Moser M, Shebl O, Schreier-Lechner E, Tew G. Basal level of anti-Müllerian hormone is associated with oocyte quality in stimulated cycles. Human Reproduction 2006;21:2022-6.
10. Mohammad A, Khalili MM, Sultan Abdul-Munaf. Role of oocyte morphology on fertilization and embryo formation in assisted reproductive techniques. Middle East Fertil Soc 2005;10:72-77.
11. Lasienë K, Vitkus A, ValanèiÛtë A, Lasys V. Morphological criteria of oocyte quality. Medicina (Kaunas, Lithuania), February 2009.
12. Rienzi L, Vajta G, Ubaldi F. Predictive value of oocyte morphology in human IVF: a systematic review of the literature. Human Reproduction Update 2011;17:34-45.
13. Irez T, Ocal P, Guralp O, Cetin M, Aydogan B, Sahmay S. Different serum anti-Mullerian hormone concentrations are associated with oocyte quality, embryo development parameters and IVF-ICSI outcomes. Arch Gynecol Obstet 2011;284:1295-1301.
14. Gupta S, Karuputhula N, Kumar N, Srivastava A, Singh B, Dubey K. Correlation of basal serum anti-Mullerian hormone level with oocyte quality and embryo development potential in women undergoing IVF-ICSI. Fertil Sci Res 2017;4:112-6.