



Association Between AMH Levels and Fertility/Reproductive Outcomes Among Women Undergoing IVF: A Retrospective Study

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Abstract

Background: Anti-mullerian hormone (AMH) is a marker for predicting ovarian response to gonadotropin stimulation. It plays an important role in ovarian primordial follicle recruitment and dominant follicle selection. Therefore, the present study evaluated the AMH levels and their association with fertility/reproductive outcomes among women undergoing IVF.

Methods: A retrospective study was conducted on 665 women in GarbhaGudi Institute of Reproductive Health and Research in India from October 2018 to 2019. Subjects were divided into ≥ 1.1 and ≤ 1.1 AMH level groups. Data on age, luteinizing hormone; LH (*mIU/L*), follicle-stimulating hormone values; FSH (*mIU/ml*), LH value, oocytes retrieved, and oocytes fertilization were collected. AMH category was considered as the primary explanatory variable. Independent sample t-test and chi-square tests were performed. The $p < 0.05$ was considered statistically significant.

Results: Couple's age, FSH values (*mIU/ml*), number of large follicles, matured oocytes, fertilized oocytes, and cleaved embryos were statistically significant ($p < 0.001$) among subjects with ≥ 1.1 AMH values. Percentage of women with successful embryo transfer was slightly higher among AMH category 1.1 ($p = 0.09$). Fertilization rate (86.67 ± 20.08 vs. 83.64 ± 21.39 , $p = 0.18$) and clinical pregnancy rate (43.38% vs. 36.36% , $p = 0.19$) were slightly higher among women with AMH level of ≥ 1.1 as compared to AMH of < 1.1 . Live birth rate was slightly higher among women with AMH level of 1.1 (25.85% vs. 22.22% , $p = 0.45$). Also, the number of fertilized oocytes was associated with clinical pregnancy rate (aOR=1.20, 95% CI 1.09-1.33).

Conclusion: Women with ≥ 1.10 serum AMH levels had more number of retrieved oocytes, good oocyte quality, increased embryo transfer, and fertilization rates.

Keywords: Anti-mullerian hormone, Fertilization, Pregnancy rate.

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Introduction

Conventionally, embryo morphology was used as a guideline for embryo selection, and other methods include oocyte and zygote mor-

phology, blastocyst culture, and blastomere symmetry (1). Serum levels of many important key hormones are used to monitor the growth of the

gonadotropin-stimulated follicle and ovarian reserve in assisted reproductive technology (ART). Measurement of basal serum hormone concentrations such as luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, inhibin, and ultrasonographic indices on the third day of the cycle such as several early antral follicles and pretreatment ovarian volume were used as classic methods to predict the response of ovarian stimulation (2). Recently, anti-mullerian hormone (AMH) has been proposed for predicting ovarian response to gonadotropin stimulation.

AMH is a dimeric glycoprotein that belongs to the transforming growth factor β (TGF β) superfamily (3). AMH expression was first noted in the proliferating granulosa cells in primary follicles of the ovary with the greatest levels of expression in pre-antral and early antral follicles and the concentrations gradually fall in mural granulosa cells of large antral follicles (4). It was proposed that the size of the growing small follicles reflects the serum AMH concentrations, which in turn correlated with the ovarian reserve or the number of residual primordial follicles. Several investigators have observed a fall in serum AMH with increasing age, reflecting a drop in the number of growing follicles available for recruitment (5, 6). This fall in AMH precedes the fall observed in more traditional markers of ovarian reserve such as FSH and inhibin B. Poor response was observed in serum AMH levels of <1 ng/ml, the normal response in 1–4 ng/ml, and good response in >4 ng/ml (7).

According to previous studies, Irez et al. (8) reported that pregnancy rate (PR) was minimum in subjects with low AMH values $<10\%$ (9.5%) ($p=0.040$) and AMH levels may predict the presence of post maturity and nucleoli Z score, quality of oocytes, early cleavage and ICSI outcomes. Leka-mge et al. (9) proposed that patients had lower fertilization rates, fewer oocytes, fewer embryos, and had a higher incidence of miscarriage with low AMH levels. Nardo et al. (10) reported that the AMH area under the receiver operating characteristic curve (ROC AUC) value of 0.81 was a good predictor of excessive response to ovarian stimulation.

Measurement of anti-mullerian hormone is a reliable predictor of in vitro fertilization (IVF) outcomes. It is a more precise measure of the ovarian reserve than the serum levels of hormones such as FSH, LH, and estrogen (E_2). There is only limited research available in India that conducted retro-

spectively to evaluate the basal AMH levels accurately in order to reflect the total developing follicular cohort and ovarian response to gonadotropins in ART cycles. The present study was carried out to assess the AMH levels, and its association with oocyte morphology, embryo quality, cleavage rate, clinical pregnancy rate, and fertility outcome among women undergoing IVF.

Methods

A retrospective study was conducted in the department of embryology in the GarbhaGudi Institute of Reproductive Health and Research in Bangalore, India for a period of one year from October 2018 to October 2019 among women undergoing IVF cycles. Women <40 years of age, with both ovaries present on transvaginal ultrasound scan, no previous history of ovarian surgery, no exposure to cytotoxic drugs or pelvic radiation therapy, no hormonal therapy in the six months before entering the study, and body mass index ≥ 19 <30 kg/m^2 were included in the study.

Women with a history of previous IVF failure, donated sperm or oocyte, endometriosis, recurrent abortion, immune diseases, uterine abnormality, ovarian cyst or previous ovarian surgery, and allergy or contraindications to oral estradiol or progesterone treatment were excluded from the study. A priori sample size calculation was done with 665 samples. AMH measurement was performed in a single accredited laboratory situated within the study setting, considering the retrospective nature of the study. But as per the study findings, the ratio of the study participants in high and low AMH groups was 4.40. With the observed mean and standard deviation of the number of mature oocytes in the study and alpha error of 0.05, the study had yielded more than 99% power.

Outcome and explanatory variables in the study were considered to compile the different statistics. The $p<0.05$ was considered statistically significant. A daily dose of recombinant FSH (Gonal-F, Merck Serono, Germany), Folisurge (Intas Pharmaceuticals Ltd, India), Briogyn (Cadila Healthcare Ltd, India), hMG (Menopur, Ferring Pharmaceuticals, USA), Zyhmg (Zydus Takeda Healthcare Ltd, India), and antagonist cetrorelix (Merck Serono, Germany) was given for controlled ovarian stimulation. The medicine used to prevent early LH surge is Ovucet (German Remedies, India) and leuprolide (Lupron Depot) in antagonist protocol and in GnRH agonist protocol, respectively.

The oocytes were collected through transvaginal

ultrasound-guided ovum pick-up at 35/34 hr after hCG administration. Before the removal of cumulus cells, the retrieved oocytes were kept in a culture medium for 2 to 3 hr. After exposure to a HEPES-buffered medium containing hyaluronidase, the surrounding cumulus cells were separated. For ICSI, mature oocytes releasing the first polar body were used. In evaluating embryo quality, the embryos were graded from day one until day five. Fertilization was affirmed by the presence of two pronuclei and the extrusion of the second polar body approximately 16-18 hr after ICSI.

The data was collected about couple's age and BMI, LH (mIU/L), prolactin (PRL, ng/ml), FSH values (mIU/ml), large follicles, LH value on the day of hCG (ng/ml), retrieved oocytes, matured oocytes, fertilized oocytes, and cleaved embryos between AMH categories. Waiver of the participants' consent in utilizing the data for the study was obtained from the ethics committee, and the study was approved by them (No: GEC/GGIRH19_8/26052020, dated: 26.5. 2020).

AMH category was considered as the primary explanatory variable. Clinical pregnancy, fertilization rate, live birth rate, abortion rate, and biochemical pregnancy rate were outcome variables. Independent sample T-test was used for comparison of continuous outcome parameters with bina-

ry explanatory variables. The chi-square test was used for comparison of qualitative explanatory and outcome parameters. SPSS vs. 22 (IBM, USA) was used for statistical analysis. The p<0.05 was considered statistically significant.

Binary logistic regression with stepwise backward elimination was performed to build the optimal model to assess the determinants of clinical pregnancy. The model with the lowest Akaike Information Criterion (AIC) was considered the optimal model. AIC is a single number score that can be used to determine which of the multiple models is most likely to be the best for a given dataset. When AIC value is lower, better model can be selected. At the beginning, a total of 12 predictors were entered, making it a complete model, and at each step, one predictor was removed that lowered the AIC value the most. This procedure was stopped when the AIC value could not be lowered with the removal of any predictor.

Results

The mean baseline parameters like couple's age and FSH values (mIU/ml) were found significantly higher in the AMH category <1.10 whereas parameters like large follicles, matured oocytes, oocytes fertilized and cleaved embryos were found significantly higher in the AMH category ≥1.10 (Table 1).

Table 1. Comparison of mean baseline parameters between AMH categories (n=665)

Parameters	AMH category (Mean±SD)		p-value
	<1.10 (n=123)	≥1.10 (n=542)	
Wife's age (year)	34.3±5.53	30.2±4.48	<0.001
Husband's age (year)	38.33±5.28	35.45±4.68	<0.001
BMI of wife (kg/m ²)	27.41±5.3	26.39±4.7	0.036
BMI of husband	27.04±4.25	27.25±4.21	0.629
LH (mIU/L)	4.18±4.03	4.39±4.12	0.599
PRL (ng/ml)	16.38±7.91	16.95±9.01	0.529
FSH (mIU/ml)	10.32±9.52	5.94±2.89	<0.001
No. of large follicles	8.22±6.44	12.03±6.12	<0.001
No. of oocytes retrieved	8.37±4.52	12.14±6.48	<0.001
Zona abnormalities	30 (24.39%)	150 (27.68%)	0.450
Polar body abnormalities (%)	88 (71.55%)	446 (82.29%)	0.006
No. of matured oocytes	6.93±3.73	9.44±4.93	<0.001
No. of oocytes fertilized	5.76±3.43	8.12±4.52	<0.001
No. of cleaved embryos	5.82±3.41	8.13±4.53	<0.001
The endometrial thickness on the day of HCG (mm)	8.91±1.39	8.79±1.29	0.443
No. of women for whom embryo transfer was done	99 (80.49%)	468 (86.35%)	0.090

The clinical pregnancy rate was relatively higher in the AMH category ≥ 1.10 (43.38% vs. 36.36%), but the difference was not statistically significant ($p=0.199$). The other outcomes, including fertilization rate (86.67 ± 20.08 vs. 83.64 ± 21.39) and live birth rate (25.85% vs. 22.22%, $p=0.45$) were also higher among the AMH category ≥ 1.10 group. However, none of the differences were statistically significant. When comparing the final pregnancy outcome, there was not much difference in the proportion between the categories (Table 2).

Table 2. Comparison of fertility outcomes between AMH categories among women for whom embryo transfer was done ($n=567$)

Parameters (%)	AMH category (Mean \pm SD)		P
	<1.10 (n=99)	≥ 1.10 (n=468)	
Fertilization	83.64 \pm 21.39	86.67 \pm 20.08	0.180
Biochemical pregnancy	3 (3.03%)	18 (3.85%)	1.000
Clinical pregnancy	36 (36.36%)	203 (43.38%)	0.199
Abortion	6 (6.06%)	26 (5.56%)	0.843
Live birth	22 (22.22%)	121 (25.85%)	0.450
Final pregnancy outcome			
Singleton	18 (62.07%)	110 (66.67%)	
Twins	11 (37.93%)	52 (31.52%)	0.727 *
Triplets	0 (0%)	3 (1.82%)	

* Simulated p-value was used due to lack of subjects in one of the cells

In the complete model, the AIC value was 763.12. None of the predictors were found to be significant in the complete model ($p>0.05$). The optimal model was formed with two predictors (number of matured oocytes and fertilized oocytes). In the optimal model, the AIC value was 746.79. The number of oocytes fertilized was found to be a significant predictor in the optimal model. Regarding optimal model, for each number increase in fertilized oocytes, the probability of having clinical pregnancy is increased 1.20 times (aOR=1.20, 95%CI 1.09-1.33; $p<0.001$) (Table 3).

Discussion

In IVF, embryologists are very keen on the assessment of oocyte and embryo quality. An oocyte ideally should have a clear, moderately granular cytoplasm, an intact first polar body, a small perivitelline space, and transparent zona pellucida. Prediction of oocyte competence and the selection methods proposed are still controversial and ineffective (7).

AMH is likely to be the best marker reflecting the decrease of ovarian reserve in the reproductive period compared to other ovarian tests. Hence, AMH in serum was considered to be a marker for embryo quality. Also, during IVF cycles, basal serum AMH concentrations can be used to detect ovarian response (11).

Table 3. Determinants of clinical pregnancy ($n=567$)

Model	Independent variables	AIC	aOR (95% CI)	p-value
Complete model				
	Wife's age (year)		0.98 (0.93-1.03)	0.453
	Husband's age (year)		1.01 (0.96-1.06)	0.643
	BMI of wife		0.98 (0.94-1.02)	0.291
	BMI of husband		1.01 (0.97-1.06)	0.649
	LH (mIU/L)		1.02 (0.98-1.07)	0.344
	FSH (mIU/ml)	763.12	1.00 (0.96-1.05)	0.834
	No. of large follicles		1.01 (0.98-1.04)	0.556
	No. of oocytes retrieved		0.99 (0.93-1.05)	0.699
	No. of matured oocytes		0.93 (0.83-1.04)	0.213
	No. of fertilized oocytes		1.26 (0.89-1.78)	0.201
	No. of cleaved embryos		0.96 (0.68-1.36)	0.812
	AMH ≥ 1.10		1.00 (0.59-1.68)	0.986
Optimal model (lowest AIC value)				
	No. of matured oocytes	746.79	0.92 (0.84-1.01)	0.098
	No. of fertilized oocytes		1.20 (1.09-1.33)	<0.001

In the present study, wives' age (30.2 ± 4.48) and husbands' age (35.45 ± 4.68) were significantly less in >1.1 AMH group ($p<0.001$). Sowers et al. (12), in their study, reported that serum AMH levels decline with age. Similarly, Revelli et al. (13) confirmed that the probability of pregnancy was significantly affected by age and not by small differences in AMH level. When comparing the mean baseline parameters such as follicle-stimulating hormone values, number of large follicles, matured oocytes, fertilized oocytes, and cleaved embryos were statistically significant ($p<0.001$) among subjects with >1.1 AMH values. Weenen et al., in their study, reported that AMH was a superior marker for ovarian aging (4). In regularly cycling women, serum levels of AMH decrease over time, and there is a strong correlation between the number of antral follicles and AMH levels (5, 14). It is likely that the size of the recruited cohort of follicles is closely associated with the remaining primordial follicle pool. Due to depletion of the primordial follicle pool, postmenopausal women face decreased fertility. A similar study was reported by Irez et al. that AMH had a significantly negative correlation with woman age ($p=0.018$) and FSH ($p=0.006$) (8).

In the current study, assessment of AMH as a marker of ovarian response to FSH, the number of oocytes retrieved (12.14 ± 6.48), number of matured oocytes (9.44 ± 4.93), and number of fertilized oocytes (8.12 ± 4.52) were greater in high-level AMH group ($p<0.001$). A study conducted by Seifer et al. demonstrated that higher AMH on day three of the stimulation protocol was associated with a higher number of retrieved oocytes. In particular, AMH levels were 2.5-fold higher in patients with at least 11 oocytes compared with those with six or fewer retrieved oocytes (1.0 ± 0.4 vs. 2.5 ± 0.3 ng/ml, $p<0.0001$) (15). Majumder et al. reported that AMH levels correlated with the number of good quality embryos available for transfer and the number of frozen embryos, but not with failed cleavage and fertilization rates (16). The similar results reported by Takahashi et al. showed that oocytes were more probable to be fertilized at high levels of AMH, as AMH levels in follicular fluid with fertilized oocytes were more than three times greater than the non-fertilized oocytes in follicles (17).

The number of cleaved embryos was higher in the >1.1 AMH group in the present study. In a study done by Lundin et al., a significantly higher proportion of good quality embryos resulted in

early cleavage when compared with late cleavage (62.5 vs. 33.4% , $p<0.0001$). When examining day two and day three transfers, early-cleaving embryos (306 transfers) resulted in significantly greater rates of pregnancy/transfer (40.5 vs. 31.3% , $p=0.0049$), implantation (28.0 vs. 19.5% , $p<0.0001$), and birth/ongoing pregnancy rate (34.3 vs. 24.0% , $p=0.0009$) than late-cleaving embryos (18). This finding supports the hypothesis that implantation rates were associated with AMH levels.

Fertilization and clinical and biochemical pregnancy rates were 86.67 ± 20.08 , 18 (3.85%), and 203 (43.38%), respectively which are comparatively higher in subjects with >1.1 AMH levels but were not statistically significant in the present study. In line with the study of Hazout et al., greater AMH concentrations were associated with a higher number of mature oocytes, a higher number of embryos, followed by a greater clinical pregnancy rate (19). However, Cupisti et al. reported converse results stating that AMH levels in individual follicles were negatively correlated with developing and matured oocytes (20). This can be elucidated by the fact that in IVF, to achieve a successful pregnancy, several additional factors, such as sperm parameters, male factor, endometrial receptivity, embryo development, and quality should be considered (21).

In the present study, embryo transfer was successful in 86.35% of cases at AMH levels greater than 1.1. When comparing the final pregnancy outcomes, singleton (66.67%) and triplets (1.82%) were more in ≥ 1.10 AMH level group, whereas more twins (37.93%) were reported in ≤ 1.10 AMH level group. Honnma et al. observed a significant association between serum AMH and day five embryo transfer (22). Kissin et al. reported that the number of transferred embryos, age, and stage of embryo transfer are important predictors of multiple gestations in assisted cycles (23). However, Tal et al. reported that serum AMH concentration is an independent predictor of twin pregnancy in fresh cycles in women aged 34 years and older (24).

The current study showed that with an increase in follicle-stimulating hormone, the probability of having AMH levels ≥ 1.10 decreased (aOR=0.76, 95%CI 0.58-0.99; $p=0.044$), and for each number increase in matured oocytes, the probability of AMH level ≥ 1.10 increased 2.44 times (aOR=2.44, 95%CI 1.01-5.87; $p=0.047$). The present study was in line with Singer et al.' (25) research

who reported a negative association between FSH and AMH serum levels in women undergoing IVF, concluding that the AMH level is highly predictive of the FSH level and can be used as an independent indicator of ovarian reserve. Furthermore, Dumesic et al. (26) also reported that intrafollicular AMH levels are negatively correlated with FSH in follicles of normoandrogenic ovulatory women undergoing IVF, concluding that intrafollicular AMH levels reflect the follicular sensitivity to FSH.

One of the main limitations of the study was that study population were all collected from a single center and only those visiting the fertility clinic were included which limits the generalizability of study findings. In fact, further research on the implication of varying AMH levels within the follicular fluid and other parameters like embryo quality, transfer technique, and endometrial receptivity on pregnancy outcomes needs to be investigated.

Conclusion

In conclusion, our results demonstrated that ≥ 1.10 serum AMH levels had a statistically significant more number of retrieved oocytes and good oocyte quality increased fertilization rates ($p=0.180$) and embryo transfer ($p=0.09$). AMH serum levels can serve as a novel marker for ovarian reserve and are associated with ovarian response in ART cycles. Further research should be carried out to produce multifactor prediction models by collecting larger datasets on AMH, FSH, AFC, and chronological age which can be used clinically together to estimate pregnancy prospects.

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Conflict of Interest

There is no conflict of interest.

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