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Original Research Article

Effect of age on antral follicle count, AMH levels and pregnancy outcomes in ART

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ABSTRACT

Background: The purpose of this study is to determine if baseline antral follicle assessment and serum AMH level may serve additional information in predicting pregnancy outcome in in-vitro fertilization outcome in women of different age groups.
Methods: A total of 680 cycles of in-vitro fertilization (IVF) in all clinics of Nova IVF fertility all over India from June 2023 to December 2024 were retrospectively analyzed in a cohort study. All the patients recruited in the study will be divided into 2 groups, based on age as-Group 1) Age<35 years (n=335) Group 2) Age>35 years (n=345). Each group will be further subdivided into 3 subgroups, depending upon their AMH (anti mullerian hormone) and AFC (antral follicle count). They were divided into very low AMH group, low-AMH group and high-AMH group. Similarly AFC groups were divided into <5, 5-9 and >10. Primary outcomes included the No of oocytes retrieved, No of mature oocytes, Biochemical Pregnancy rate, Clinical Pregnancy Rate. Secondary outcomes included fertilisation rate, blastulation rate, miscarriage rate.
Results: The pregnancy rates for women below 35 years and above 35 years were not significant in the AMH subgroups but was significant in the AFC subgroup of 5-9, where it was 53.12% and 27.91% respectively. The Area Under the Curve (AUC) for the logistic regression model 0.656, indicating moderate discriminative ability. The distribution of AFC and AMH values by age group illustrates that younger women more frequently fall into higher AFC and AMH categories, supporting their use as age-related biomarkers. However, pregnancy and miscarriage rates are stratified by AMH and AFC groups failed to reach statistical significance, implying limited predictive value for these markers in isolation when it comes to final pregnancy outcome.
Conclusions: Correlation analysis further affirmed that AFC has stronger associations with ovarian and embryological parameters compared to AMH. This aligns with prior research and clinical understanding, which recognizes AFC as a more dynamic and reliable predictor of ovarian reserve and treatment responsiveness. AMH, while useful, showed only moderate correlation with oocyte quantity and did not significantly predict fertilization, blastulation or pregnancy outcomes.

Keywords: AMH, Antral follicle count, In-vitro fertilization, Ovarian reserve, Pregnancy

INTRODUCTION

The world's first test-tube baby born in 1978, assisted reproductive technology (ART) has been carried out for more than 40 years.[1] Controlled ovarian hyperstimulation (COH), is a main aspect of ART, aims to obtain an appropriate number of mature oocyte which results in pregnancy.[2] To achieve that we have to check the ovarian reserve, for which AMH and AFC play a crucial role along with the age of the woman.[3]

Studies have shown that the relationship between AFC/the ovarian and maternal age shows a positive correlation trend before the age of 25 and gradually decreases with the increase of age from around 30 years more so in the Indian women.[4] However, due to individual heterogeneity, a certain number of women have variations between age, AFC and AMH levels (some young women have a lower AMH level, while some older women have a higher AMH level).[5] This paper aims to understand the relation between age, AFC and AMH levels.

METHODS

Study type and period

This was a retrospective cohort study analyzing data from IVF cycles conducted between June 2023 and December 2024.

Study place

The study was conducted across all clinics of Nova IVF Fertility in India.

Selection criteria

A total of 680 IVF/ICSI cycles were included in the analysis.

Inclusion criteria

All couples undergoing IVF at any of the Nova Fertility and IVF centres, with the female partner aged between 21-50 years and the male partner aged between 21 to 55 years.

Exclusion criteria

Male factor infertility, patients undergoing IVF using donor gametes (oocytes or sperms), cycles in which no embryo transfer was performed, women who have undergone surgical intervention or gonadotoxic therapy on their ovaries in the past. Authors have 680 Patients in our dataset. Among them, 335 patients (49.3%) are under the age of 35, while 345 patients (50.7%) are aged 35 years or older.

Clinical protocol

In a fixed protocol for all patients recruited, early follicular phase that is day 2/3 of menses, antral follicle count was done of follicles 6-10 in size were noted and endometrial thickness (EMT) were monitored by transvaginal ultrasound. IVF Stimulation was started for all patients in an Antagonist protocol, using FSH/HMG gonadotrophins, in a daily dose of 225 IU or 300 IU with regard to age, AMH, AFC and BMI of the patients, for 10-11 days (total dose of approximately 3000-3300 IU),with either agonist or dual(agonist +HCG) trigger when 80% of the follicles were above 18 mm. OPU was done after 35 hours of trigger under general anaesthesia. The oocytes were retrieved under the guidance of vaginal ultrasound and fresh semen sample collection was done on the day of OPU after 2 days of abstinence. Depending on the patient's medical history, IVF or ICSI was done. For each patient the number of mature oocytes and fertilisation was noted and was taken ahead for blastocyst culture.

Each patient was prepared for frozen transfer in the next cycle with HRT protocol with Estradiol valerate of 6 mg-10 mg, monitoring by transvaginal ultrasound was done and an endometrial thickness of about 7-10 mm was taken up transfer. Routine blood value of progesterone was done and if found <1, intramuscular progesterone injections with vaginal micronized progesterone was used for all patients.

Follow-up after frozen transfer: Serum β-human chorionic gonadotropin (β-hCG) was done on the 10th day after embryo transfer and the serum β-HCG>50 IU/l was defined as positives; doubling was checked after 48 hours. Transvaginal ultrasound 2 weeks after positive HCG report showed gestational sac with cardiac activity was diagnosed as clinical pregnancy. Live births are defined as those with a 28 weeks gestation and delivery of a neonate with vital signs.

Statistical analysis

All statistical analyses are conducted using Python, leveraging libraries such as pandas, scipy, statsmodels and sklearn. Descriptive statistics are used to summarize key variables including Female Age, Antral Follicle Count (AFC), Anti-Müllerian Hormone AMH), Retrieved Oocytes and Mature MII, with measures such as mean, standard deviation, minimum, maximum and percentiles providing a detailed overview of the cohort's clinical profile.

Comparative analyses are performed by stratifying patients into two age groups (<35 years and ≥35 years) and independent samples t-tests (Welch's t-test) are applied to continuous variables to detect significant differences in oocyte retrieval, maturation, fertilization and blastulation rates. Chi-square tests are used for categorical outcomes such as pregnancy and miscarriage rates. Significance is determined at a threshold of $p<0.05$. Correlation analysis using Pearson's method is employed to explore relationships among ovarian reserve markers (AMH, AFC) and embryological outcomes (retrieved oocytes, mature MII, 2PN), confirming the strength and reliability of AFC and oocyte-related parameters. A multivariate logistic regression model is then constructed to identify independent predictors of pregnancy rate (PR), incorporating variables such as Female Age, AMH, AFC, Retrieved Oocytes, Mature MII, 2PN and Blastocyst formation. To assess model discrimination, a Receiver Operating Characteristic (ROC) curve is plotted.

RESULTS

Authors have 680 Patients in our dataset. Among them, 335 patients (49.3%) are under the age of 35, while 345 patients (50.7%) are aged 35 years or older.

Correlation analysis

Advanced maternal age negatively impacts ovarian reserve and response, causing reductions in AFC, oocyte retrieval, mature oocyte yield and 2PN fertilization rates. Strong correlations between mature oocytes, retrieved oocytes, 2PN fertilization and AFC confirm their reliability as markers of ovarian function and IVF prognosis.

AMH is widely used to estimate ovarian reserve, its moderate correlation with follicle counts and oocyte parameters suggests it should be interpreted alongside other indicators. Together, these markers provide a comprehensive assessment of ovarian health, improving IVF outcome predictions and guiding individualized treatment strategies.

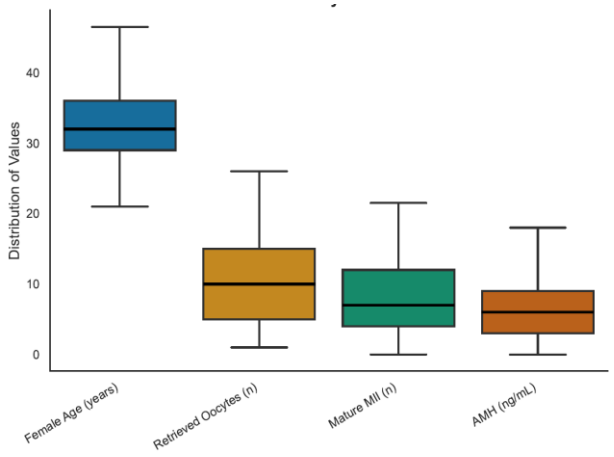

Figure 1: Distribution of key IVF parameters.

Regression analysis

A multivariate logistic regression model is fitted to evaluate the predictors of pregnancy rate (PR). The model includes the following variables: female age, AMH, AFC, retrieved oocytes, mature MII, 2PN (two pronuclei), blastocysts formed (blast new).

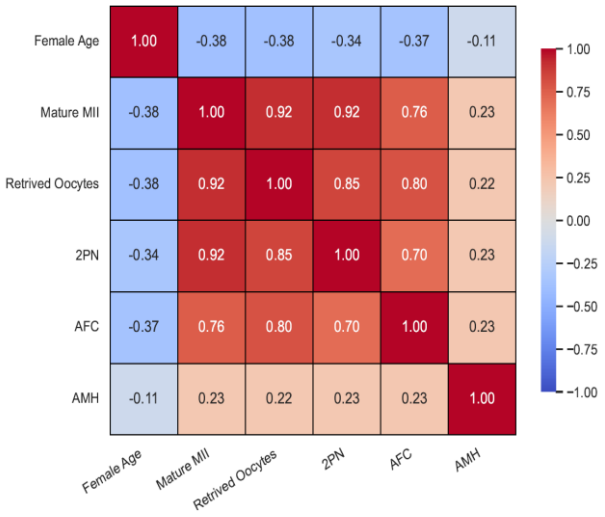

Figure 2: Correlation heatmap illustrating the relationships among key IVF parameters, including female age, mature (MII) oocytes, retrieved oocytes, number of two-pronuclear (2PN) embryos, antral follicle count (AFC), and anti-Müllerian hormone (AMH). The color scale represents the strength and direction of the correlation coefficients, ranging from negative (blue) to positive (red).

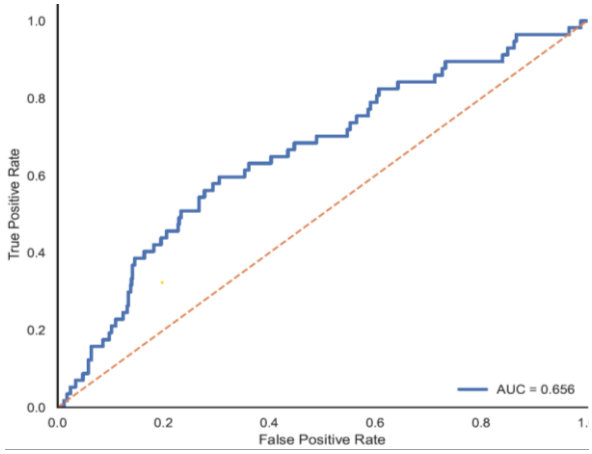

Figure 3: ROC curve for pregnancy prediction model.

Table 1: Demographic details.

Statistic	Female age	Retrieved oocytes	Mature MII	AMH	AFC
Mean	32.78	10.95	8.22	2.16	6.29
Std dev	4.79	6.64	5.30	1.48	3.81
Min	21.00	1.00	0.00	0.01	0.00
25th percentile (Q1)	29.00	6.00	4.00	0.98	3.00
Median (Q2)	33.00	10.00	7.00	1.88	6.00
75th percentile (Q3)	36.00	14.00	11.00	3.18	9.00
Max	46.50	26.00	21.50	6.47	18.00

Table 2: Distribution of AFC and AMH Group.

AFC distribution			
Age group (in years)	<5	5-9	≥10
<35	79	120	136
≥35	137	103	105
AMH distribution			
Age group (in years)	<1	1-2.5	>2.5
<35	56	119	160
≥35	121	122	102

Table 3: Comparative analysis.

Measure	Aged <35	Aged ≥35	Statistical test	P value	Significance
Oocyte retrieved	12.9	9.03	independent samples t-tests (Welch's t-test)	<0.05	significant
Mature MII	9.88	6.66	independent samples t-tests (Welch's t-test)	<0.05	significant
2 PN	8.38	5.59	independent samples t-tests (Welch's t-test)	<0.05	significant
Fertilization rate					
AFC group					
<5	79.80%	82.00%	independent samples t-tests (Welch's t-test)	0.5528	Not significant
05 September	81.90%	89.70%	independent samples t-tests (Welch's t-test)	0.4088	Not significant
≥10	86.60%	88.20%	independent samples t-tests (Welch's t-test)	0.4709	Not significant
AMH group					
<1	83.70%	84.60%	independent samples t-tests (Welch's t-test)	0.7988	Not significant
1-2.5	82.70%	82.90%	independent samples t-tests (Welch's t-test)	0.9228	Not significant
>2.5	83.60%	92.10%	independent samples t-tests (Welch's t-test)	0.3943	Not significant
Blastulation rate					
AFC group					
<5	56.70%	58.70%	independent samples t-tests (Welch's t-test)	0.7394	Not significant
05-September	55.50%	53.80%	independent samples t-tests (Welch's t-test)	0.6749	Not significant
≥10	55.20%	64%	independent samples t-tests (Welch's t-test)	0.01	significant
AMH group					
<1	63.40%	61.00%	independent samples t-tests (Welch's t-test)	0.6386	Not significant
1-2.5	55.10%	60.20%	independent samples t-tests (Welch's t-test)	0.192	Not significant
>2.5	53.30%	53.90%	independent samples t-tests (Welch's t-test)	0.8548	Not significant
Pregnancy rate					
AFC group					
<5	83.33%	54.17%	Chi-Square Test	0.401	Not significant
05-September	53.12%	27.91%	Chi-Square Test	0.0479	significant
≥10	61.11%	61.54%	Chi-Square Test	0.998	Not significant

Continued.

Measure	Aged <35	Aged ≥35	Statistical test	P value	Significance
AMH group					
<1	60%	58.06%	Chi-Square Test	0.9978	Not significant
1-2.5	65.38%	40.54%	Chi-Square Test	0.0918	Not significant
>2.5	55.26%	32.00%	Chi-Square Test	0.12	Not significant
Miscarriage rate					
AFC group					
≥10	2.78%	7.69%	Chi-Square Test	0.778	Not significant
AMH group					
<1	2.63%	0.00%	Chi-Square Test	>0.05	Not significant
1-2.5	0.00%	2.27%	Chi-Square Test	>0.05	Not significant
>2.5	0.00%	3.22%	Chi-Square Test	>0.05	Not significant

No miscarriage are found in the AFC group >5 and 5 to 9.

Table 4: Multivariate logistic regression summary.

Variable	Coefficient (β)	Std. error	Z value	P value	95% CI (Lower)	95% CI (Upper)	Significance
Intercept	-1.936	1.199	-1.614	0.107	-4.287	0.415	Not Significant
Female age	-0.0087	0.032	-0.269	0.788	-0.072	0.055	Not Significant
AMH	-0.091	0.08	-1.137	0.256	-0.248	0.066	Not Significant
AFC	0.184	0.069	2.654	0.008	0.048	0.32	Significant
Retrieved oocytes	-0.1546	0.063	-2.455	0.014	-0.278	-0.031	Significant
Mature MII	0.0455	0.09	0.508	0.611	-0.13	0.221	Not Significant
2PN	0.0806	0.079	1.016	0.31	-0.075	0.236	Not Significant
Blastocyst (Blast)	-0.051	0.072	-0.709	0.478	-0.192	0.09	Not Significant

The model is statistically non-significant overall (LLR p value=0.1152), with a pseudo-R^2=0.031 and log-likelihood=-178.23, indicating low explanatory power.

ROC curve and model discrimination

The AUC for the logistic regression model is 0.656, indicating moderate discriminative ability.

DISCUSSION

The evaluation of ovarian reserve is a cornerstone of infertility assessment and IVF success prediction. Two of the most validated biomarkers in this regard are AMH and AFC. In this study, we aimed to assess their roles in predicting ovarian response and embryological outcomes across different age groups, drawing comparisons with existing literature. Women presenting with elevated AMH demonstrated substantially higher oocyte retrieval numbers compared to those with lower AMH values, mirroring observations from Seifer et al that linked increased AMH concentrations with greater oocyte yield.[7,9] Despite its utility in estimating oocyte numbers, AMH showed limited ability to forecast qualitative outcomes such as fertilization, blastocyst formation or eventual live birth.[4-7]

In the regression analysis, AMH failed to emerge as a significant predictor of pregnancy outcomes, echoing findings from Tal, Mutlu, Broer and others. Conversely, AFC was more consistently associated with ovarian responsiveness and embryological indicators. In our regression model, AFC emerged as a significant positive predictor of pregnancy likelihood (β=0.184, p=0.008).[9,10] Interestingly, while younger women (<35 years) had significantly better ovarian responses in terms of oocyte retrieval, MII oocytes and 2PN fertilizations (Table 1 and 3), blastulation and fertilization rates did not differ significantly across age groups in most AMH/AFC subcategories.

This suggests that embryo competence may not be solely dictated (Ntostis et al) by these biomarkers, but perhaps by other intrinsic and extrinsic factors such as mitochondrial function, as noted by Ntostis et al and corroborated by the effects of oocyte aging on ATP production and embryo viability.

Interestingly, women aged ≥35 years within the high-AFC category demonstrated notably higher blastulation rates (p=0.01), suggesting that adequate follicle numbers may preserve oocyte competence even in advanced maternal age.[10]

In patients with PCOS, high AMH levels were associated with decreased ovarian responsiveness and poor ART outcomes, particularly when AMH exceeded 4.7 ng/ml.[10,11] The findings align with this threshold, highlighting the dual role of AMH as both a diagnostic marker for PCOS and a predictor of potential ovarian hyper-responsiveness or suboptimal oocyte quality. Although both markers showed associations with ovarian and embryological outcomes, neither AMH nor AFC reliably predicted miscarriage risk.[5,12,17] This is in line with prior reports highlighting the restricted sensitivity and specificity of these markers when evaluated independently.[5,12,17]

Finally, while the regression model achieved a moderate AUC of 0.656, it did not reach overall statistical significance (LLR p=0.1152).[6,7]

This underlines the need for comprehensive predictive frameworks that incorporate additional clinical and embryological variables, such as endometrial receptivity, embryo morphology and genetic testing.

Limitations

This study has some limitations. It was retrospective, which may limit generalizability. Subgroup sizes for low AMH and AFC were small, reducing statistical power for certain outcomes.

Future directions

Future research should include prospective, multi-center studies with larger and more diverse populations. Incorporating comprehensive hormonal profiles, genetic embryo testing and endometrial assessments can improve predictive accuracy.

Long-term follow-up including live birth and neonatal outcomes and development of integrated predictive models, will enhance individualized IVF treatment planning.

CONCLUSION

This study highlights the significance of AFC as a reliable and dynamic marker of ovarian reserve and a strong predictor of IVF outcomes. While AMH remains a valuable tool for assessing oocyte quantity, it demonstrates limited predictive power for downstream reproductive events such as fertilization, blastulation and pregnancy success. The findings also reinforce the impact of maternal age on IVF outcomes, particularly in terms of oocyte and embryo quality.

Nonetheless, age-independent markers like AFC provide additional clinical value in assessing reproductive potential. Importantly, while AMH and AFC are essential tools for ART counselling and stimulation planning, they should not be relied upon in isolation to predict live birth or miscarriage outcomes. A more comprehensive approach integrating these biomarkers with broader clinical, embryological and possibly genetic data is needed to optimize patient stratification and personalize treatment strategies for improved reproductive outcomes.

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