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Association of Oocyte recovery with Antimullerian Hormone, Antral Follicle Count and Final Follicle Count in different age group of IVF patients

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Abstract
Background*: Predication the number of oocytes prior to oocyte retrieval is long-term process in IVF treatment. But AMH level, AFC and number of final follicles countare major important biomarker, which can predict the quantity of oocytes in ovary.*
Aim*: The aim of the present study was to correlate the quantity of oocyte recovery with AMH Level, AFC and final follicles count in different age of IVF women.*
Subject and method*: Total 147 number of IVF/ICSI patients were involved in this retrospective study and categorized in four age groups. All patients' AMH hormone level, AFC, final follicles count, duration of infertility, number of total oocytes and matured (M2) oocytes data were taken for study.*
Statistical analysis: *Significance analyses of data were analyzed by one way anova post hoc Turkey HSD test and associations analysis were done by correlation coefficients.*
Results*: After statistical analysis it was found that AMH level, AFC, final follicles count, total number of oocytes and M2 oocytes were significantly decreased in older age group. The correlation analysis of AMH level, AFC and Final Follicle Countwere found to be negative with age but positive with total number of oocytes and M2 oocytes.*
Conclusion: *Our study confirms that quantity of oocyte recovery decline with lower AMH level, less AFC and final follicle count. It was also found to be decreased with older age. It is confirm that AMH level, AFC along with Final Follicle Count provides better prediction the number of oocyte recovery prior to oocyte retrieval.*
Keywords*: AMH, AFC, Follicles, Oocyte, Age*

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I. Introduction

Prediction of IVF outcomes in patients at different ages has been a longstanding goal in reproductive treatment.There are various known parameters for assessing oocyte retrieval and IVF outcomes, but primarily ovarian reserve, including ovarian volume, antral follicle count(AFC), Anti-Mullerian-Hormone (AMH) and follicle stimulating hormone (FSH) at the starting days of the menstrual cycle is most predictable.[1,2]AMH, also known as Mullerian inhibiting substance, is one of thebest markers of ovarian reserve.[3,4] The AMH concentrations are low after birth and during the pre-pubertal phase in women's life. At puberty stage, concentration of AMH in the blood increases, peaking at approximately at age of 20-25 years. After this time, hormone concentrations decrease to undetectable levels following menopause.[5]Whereas AFC is ultra-sonographic marker,directly represents the follicle cohort in the ovaries, which is associated with the number of oocytes retrieved in IVF. At present, AFC is generally accepted as a good predictor of ovarian response to Controlled ovarian hyperstimulation (COH) and has been shown to decline with age.[6]

AMH is a dimeric glycoprotein, a member of the transforming growth factor-beta super family,[7]which is produced by the granulosa cells of small antral and pre-antral follicles, and the quantity of AMH corresponds to the size of the pool of these ovarian follicles.[8,9,10]Furthermore, AMH inhibits the initiation of follicle growth

and the FSH-dependent selection process.[11,12]It has also been suggested that a single AMH measurement may be a good predictor of the onset of menopause in aging women.[4]

The study suggested that number of oocytes retrieved in IVF protocols strongly correlated with the ovarian reserve, and AMH predicted more precisely the quantity of oocyte retrieval than other factors.[13]It has been also reported that the level of AMH before ovarian stimulation correlated with the number of oocytes, which led to the acceptance of low AMH levels as a predictor of poor ovarian response. The final follicles count on day of hCG trigger has greatest tendency to yield oocytes. [14] Shapiro introduced the concept of an "oocyte yield," whereby the numbers of oocytes collected were correlated for the number of final follicles on the day of trigger.[15] Other authors have reported both number of follicles ≥14mm and the number of follicles ≥10mm to allow the reader to account for different estimations of oocyte yield.[16]

In many studies, the ovarian function markers AMH and AFC have been proven to predict oocyte yield in IVF cycle. But very little studieshave been reported prediction of oocyte yield on the basis of final follicle count onhCG trigger day along with AFC and AMH level. Therefore, our aim of the present study was to find out the association of oocyte recovery with AMH level,AFC and number of final follicles count in different age group of IVF/ICSI women Patients.

II. Subjects And Method

The presentretrospective study,total 147 IVF/ICSIwomen patients from January 2017 to December 2018 were included. All patients were stratified into four age groups, groupA(22-27 year), group B (28-31 year) groupC (32-35 year) and groupD (36-46 year). Inclusion criteria: women with age of >22 and <46 years.Exclusion criteria: women with PCOD and high AMH Value. Before starting IVF treatment written informed consent were obtained and blood sample were taken for hormonal test. AFC were done by transvaginal ultrasonography.AMH testwere measured by outsourcedpathological investigation laboratory using Chemiluminescence method.

In the present study, all patient were underwent controlled ovarian stimulation by gonadotropin-releasing hormone (GnRH) agonist long protocol. Pituitary suppression started with Oral contraceptive pillsand lupride-4mg/4ml20U and 10U. The ovarian stimulationswere done by recombinant follicle stimulating hormone Folisurge150U and human menopausal gonadotropins (HMG). All women patientswere followed for follicle monitoring by Transvaginal Ultrasonography (TVS).Day of hCGtrigger were decided when at least 3 follicles reached>17mm diameter,and number of final follicle countwere done on theday the of hCG trigger.Final maturations of follicles weredone by intramuscular of hCG.The Ovum pick-ups wereaccomplished by transvaginal USG after 36 hours of trigger.Retrieved oocytes from left and right ovary maintained in culture media, and cumulus cell were removed after exposure to HEPES-buffered medium withhyaluronidaseby gently denuding pipette.Total number of germinal vesicle (GV), metaphase I (M1) and metaphase II(M2) oocyteswere identified.

The Medical record files of IVF patients were used to obtain the data. Age, duration of Infertility, AMH level, AFC,number of final folliclecount on day ofhCGtrigger, total retrieved oocytesand M2 oocytes datawere analyzed by online available one way anova post hoc Turkey HSD test [Table 1]. The associations of total retrieved Oocytes and M2 oocytes with AMH level, AFC, final follicles count,Age and Duration of infertility were analyzed by correlation coefficients tool of Microsoft excel [Table 2]. The differences were considered statistically significant at $p<0.05$ level.

III. Results

All the 147women patients were grouped into four age groups (A,B,C and D) and each groupspatients'duration of infertility, AMH level, AFC, finalfollicles count, numberof total retrieved oocytes and M2 oocytesrecord were usedfor statistical analysis [Table 1].After statistical analysis, AHM level was found to be less in older age group B(2.68±1.06ng/µl),C (1.99±1.21ng/µl) and D(1.01±0.82ng/µl) as compare to younger age group A(2.82±0.95ng/µl).However, it was significant with group C and D but not significant with group B. The comparison between two group B vs C and C vs D,the AMH level was found to be statistically significant.

The comparison of mean number of AFC in all four groups, it was found to be significantly less in group B (11.48±4.95), C (8.55±5.47) and D (5.16±4.83) as compare to group A (18.03±7.35). However, AFC was found to be less in group C as compare to group B but it was not statistically significant.

The comparison of mean number of final follicle fount in all four groups, it was found to be significantly ($p<0.05$) less in group B(10.10±5.20), C(7.47±6.76) and D(3.27±4.49) as compare to group A(14.19±5.35). However, the mean number of final follicle count wereless in group C as compare to group B but it was not statistically significant.

The statistical analysis of mean number of total oocyte retrieval between all the groups, it was found to be significantly ($p<0.05$) less in group B(7.43±5.73),C(5.13±5.55) andD(3.11± 4.05) as compare to group

A(11.19± 5.10) patients. The group B patient retrieved higher number of oocytes as compare to group C and Dhowever, it was not significant with group C but significantwith D. The comparison between group C and D, group C retrieved higher number of total oocyte as compare to group D but, it was not statistically significant.

The analysis of mean number of total M2 oocytes between all the groups, it was found to be significantly ($p<0.05$) less in group B(5.676 ±3.89), C(4.08 ± 4.51) and D(2.66±3.65) as compare to group A(8.88 ± 5.32). The group B patient retrieved higher number of M2 oocytes as compare to group C and D but it was not statistically significant with group C. The comparison between group C and D, group C retrieved higher number of M2 oocytes but it was not statistically significant.

Table 1: Patients data with significance analysis by one way ANOVA

Groups	**A**	**B**	**C**	**D**	**Significance**
Number of total patients	26	37	40	44	-
Age in years	22-27 (24.96±1.82)	28-31 (29.89±1.02)	32-35 (33.24±1.22)	36-46 (38.29±2.16)	-
Duration of infertility	(4.58±2.58)[a]	(7.09±2.98)[a]	(8.89±3.52)[b]	(10.06±4.98)[c]	p<0.05
AMH level	(2.82±0.95)[a]	(2.68±1.06)[a]	(1.99±1.21)[b]	(1.01±0.82)[c]	p<0.05
AFC	469 (18.03±7.35)[a]	425 (11.48±4.95)[b]	342 (8.55±5.47)[b]	225 (5.16±4.83)[c]	p<0.05
Final follicles count (on day of hCG)	369 (14.19±5.35)[a]	374 (10.10±5.20)[b]	299 (7.47±6.76)[b]	144 (3.27±4.49)[c]	p<0.05
Total Oocyte	291 (11.19± 5.10)[a]	275 (7.43±5.73)[b]	205 (5.13±5.55)[b]	137 (3.11± 4.05)[c]	p<0.05
Matured(M2) oocyte	231 (8.88 ± 5.32)[a]	210 (5.676 ±3.89)[b]	163 (4.08 ± 4.51)[b]	117 (2.66±3.65)[c]	p<0.05

The values expressed as mean±SD, SD = Standard deviation,
Different superscript shows significantly different within row, the level of significance $p<0.05$.

Correlation analysis

The correlation analysis of all factor were presented in [Table 2].The correlation analysis of patients age group were significantly (<0.05) negatively correlated with AMH level (r= -0.568), AFC (r = -0.622) and final follicles count (r= -0.591) respectively.

The correlation analysis of M2 oocyte were found to be moderate positive (r = 0.445) with AMH level and strong positive with AFC (r = 0.646) and final follicle count (r = 0.721) respectively. Similarly, moderate positive association with AMH leveland strong positive with AFC and final follicle count were observed for total oocyte retrieval.

Table: 2 Correlation Analysis

Correlation Analysis							
	Age	**Duration of infertility**	**AMH**	**AFC**	**Expected Follicle**	**Total oocyte**	**M2 oocyte**
Age	1						
Duration of infertility	0.449	1					
AMH	-0.568	-0.291	1				
AFC	-0.622	-0.279	0.573	1			
Final Follicles Count (on day of hCG)	-0.591	-0.307	0.494	0.745	1		
Total Oocyte	-0.497	-0.298	0.492	0.633	0.773	1	
M2 Oocyte	-0.438	-0.266	0.445	0.646	0.721	0.926	1

The significant (<0.005) moderate positive (r = 0.573) correlation were found between AMH and AFC. Which means there is a tendency for high AMH value indicated high AFC (and vice versa).

The correlation analysis of duration of infertility were found to be negative with AMH level (r= -0.291), AFC (r = -0.279, final follicle count (r = -0.307), total Oocyte (r = -0.298) and M2 oocyte (r = -0.266) respectively.

IV. Discussion

There are many parameters which predictthe presence of oocytes in ovary (functional ovarian reserve). The most commonly used markers are Serum AMH and AFC,by which presence of oocyte can be identified before the ovum pick-up and IVF plan can be decided for self-oocytepick-upor by donor oocyte recipient cycle. Many studies have been conducted to predict the number of oocytes yieldsprior to ovum pick-up in IVF/ICSI cycle however, most of the studies have reported AMH and AFC count but very few studies have reported final follicle count on day of hCG trigger.Previous study reported that AMH level predicted more precisely the quantity of retrieved oocyte than other parameter.[13] Similarly in another study also suggested that AMH offer as a specific diagnostic marker of oocyte reserve in different age.[17]

In the present study AMH level were found significantly lower in older age group (D)as compare to younger groups (A,B andC). This may be due to fact that AMH is barely detectable in the serum at birth[18] later increases after puberty [19] and then declines withadvancing female age, to become undetectable again at the time ofthe menopause.[20]Previous study reported that reduced baseline serum AMH concentration may be indicative of poor response in *in vitro* fertilization in women with a diminished ovarian reserve[21]. In the many current literature, the AMH cut-off value for predicting poor ovarian response is between 0.30 and 1.40.[22,23,24,25,13] There is a wide variation of cut-off values in the literature for ovarian response prediction, probably resulting from the use of different assays and different poor responder prevalence between studies. In our study the AMH level in group A (24-27year) 2.82±0.95ng/µland group B(28-31year)2.68±1.06ng/µl did not found any significant difference.However, the significant differences were observed with group C (1.99±1.21ng/µl)and D (1.01±0.82 ng/µl) as compare to group A.

The higher number of AFC (18.03±7.35) and final follicles count(14.19±5.35) total number of retrieved oocyte (11.19±5.10) and M2 oocytes (8.88 ± 5.32) were found in younger age groupA(22-27 year).It may due the ageing and ovarian response, the fertility decline with age corresponds to the natural biological attrition of the ovarian reserve[26]. AFC is known to have a high specificity (73%–100%) for predicting a poor response predicting a failure to achieve pregnancy.[27]A Recentstudy Abbara*et al.*determined that follicles of 12–19mm on the day of hCGtrigger administration had the greatest contribution to the number of oocytes retrieved.[28]In another study by Revelli*et al.* suggested that follicles of sizes 16-22mm on the day of oocyte retrieval (measured 2 days later) contribute the most to the number of oocytes retrieved.[29]

After correlation analysis it was observed that AFC, AMH, final follicle count on the day of hCG, total retrieved oocytes and M2 oocytes were negatively correlated with age. Similarly to our study Edson *et al. and*Elizebeth*et al.*found negative correlation between serum AMH levels with age,[30,31]which confirm that our study is in line with their study. Agarwal *et al. and* Haadsma*et al.* found negative relationship between AFC and the age of female [32,33], which is also similar to our study.However, in our study AFC,AMH,final follicle count, total oocyte and M2 oocyte were correlated significantly positive. The more numbers of oocytes were collected when the patient had high levels of AMH and AFCs. In previous literatures a strong positive correlation between AMH levels and the number of oocytes has been reported.[34, 35]Similarly an another recent study found significant positive correlations between serum AMH levels and number of aspirated follicles, number of retrieved oocytesand number of mature oocytes.[30]Frattarelli*et al.*also correlated the AFC in the early follicular phase with age, and observed that decreased AFC were sign of ovarian aging[27], which is a feature observed prior to an increase level of FSH.[36] It was also previously demonstrated by many workers that an AFC cut-off value of 3 to 7 indicates significant decline in ovarian reserve and subsequently poor ovarian response in IVF cycles.[37, 38, 39, 27,40,41, 42]Serum AMH has an important advantage as a biomarker, itdecreases with age as a sign of follicular reserve exhaustionand its level exhibits no intra-cycle fluctuation.[34]AFC is also a predictive of ovarian response but the combination of two methods could give additionalinformation and a better picture of ovarian reserve.[43] In our study, we also observed that final follicles count on day of hCG trigger give better prediction of oocyte retrieval, which is an advantage with the AMH level and AFC.

Furthermore, this study also foundnegative correlation forduration of infertilitywith the level of AMH, AFC,final follicles count,total oocytesand M2 oocytes, but there was positive correlation between age and duration of infertility were observed.Previous study reported that the fertility declines with increasing maternal ageas early as 32 year, and especially after the mid-30's, and beyond a certain age, cannot be overcome by IVF treatment.[44]The poorer IVF outcomes in women over the age of 40 years are due to the lower number of oocytes collected as well as poorer oocyte quality, giving rise to lower fertilization and implantation rates. Fecundity decreases due to oocyte atresia which occurs continually throughout a woman's life, as she is born with millions of follicles but will only ovulate around 400 times in her lifetime [45] and compromised prior to the onset of peri-menopausal menstrual irregularities. The decline in fertility is also accompanied by an increased risk of aneuploidy and spontaneous abortions.[46]One of the genetic reasons is that the genes encoded on the X chromosome and autosomes.[47]The mechanisms postulated to be responsible for such loss include decreasing ovarian reserve[48]and poor oocyte quality.[49]

V. Conclusions

Our study concluded thatoocyte recovery positively correlated with AMH, AFC andfinal follicle count, and negatively correlated with age. The quantity of total retrieved oocytes and M2 oocytes declined with AMH level, AFC and final follicle count in older age group of patients.This study confirmed the usefulness of AMH and AFC as a biomarker of ovarian function reserve along with number of final follicle count on day of hCGprovide actual idea of total oocytes recovery prior to oocyte retrieval. This study also helps to decide the self-oocyte retrieval cycle or donor oocyte cycle IVF plan forpatients.

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