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Original Research Article

Predictors of oocyte yield in controlled ovarian hyperstimulation IVF/ICSI cycles: a retrospective analysis in a tertiary care centre

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ABSTRACT

Background: The most important predictor of oocyte yield in ART cycles is female age, but other biochemical and ultrasonographic markers done before controlled ovarian stimulation may predict the oocyte yield in women undergoing COS in IVF cycles.
Methods: The main aim of the study was to evaluate ovarian reserve markers before COS which can help to individualise treatment protocols to achieve optimal response and minimise risk of complications. It is retrospective observational study, 1924 women undergoing COS in IVF/ICSI cycles in tertiary care centre in India, from January 2010 to June 2017 were included.
Results: Univariate analysis showed that age, D2FSH, AMH, D2AFC and E2 on the day of trigger were significant predictors of oocyte yield ($p<0.05$). E2 on day of trigger with ROC (0.81), indicating good discriminating potential for predicting poor ovarian response, followed by age and D2 FSH. The formula to calculate, number of oocytes retrieved=18.46+(0.174×AFC)+(0.092×AMH)-(0.123×age)-(1.19×FSH), FSH was formulated, with $r^2=0.2486$ ($p<0.001$). ROC curve analysis shows that FSH has statistically significant discriminability to detect poor response than age [AUC (95% CI) FSH 0.77 (0.74, 0.81), age 0.56 (0.52, 0.60), ($p<0.05$)]. FSH >7.82 IU/ml was predictor of poor response (sensitivity 78.13%, specificity 79.53%).
Conclusions: A combination of predictors demonstrated superior ability of predicting oocyte yield after controlled ovarian stimulation than compared with any single endocrine marker. D2 FSH though thought to be obsolete, but we found significant predictive ability in terms of oocyte yield in the Indian population.

Keywords: Ovarian reserve, AMH, AFC, FSH, Oocyte yield, E2 levels

INTRODUCTION

The success of IVF and embryo transfer depends upon many variables of which first parameter is adequate response of the ovaries to controlled ovarian stimulation (COS).[1] Ovarian reserve is traditionally defined as woman's reproductive potential in terms of quantity and quality of oocytes. The most important predictor of oocyte yield especially quality of oocytes is woman's age. Age related decline in ovarian response to exogenous gonadotrophins is mainly attributed to decreasing ovarian reserve (DOR).[2] Poor ovarian response indicates women of reproductive age having normal menstrual cycles but poorer response to controlled ovarian stimulation as compared to women of comparable age.[3] IVF cycle cancellation because of diminished ovarian reserve is an important problem seen in 12-30% of all stimulated cycles.[4] On other hand, patients with polycystic ovarian syndrome (PCOS) present with hyper response to COS in IVF cycles thus

leading to ovarian hyper stimulation syndrome (OHSS) and cycle cancellation. During COS, ovarian response to gonadotrophins is the key factor for IVF success. Ovarian reserve markers help to predict the response and to individualise the stimulation protocol so that patients with poor response or hyper response can be predicted and management can be tailored and couple can be counselled accordingly. Ovarian reserve markers have evolved over last 4-5 decades and include biochemical markers and ultrasound (USG) markers. Tests include static tests-serum follicle stimulation hormone (FSH), inhibin, serum estradiol, antral follicle count (AFC) and lately serum anti-mullerian hormone (AMH). The dynamic tests such as clomiphene citrate challenge test (CCCT) are obsolete as they are expensive and inconvenient as they require more than one patient visit and inconclusive results.

Most traditional and time tested ovarian reserve test, being used over the decades is day 2-3 FSH. Anti-mullerian hormone (AMH) indicates FSH independent pool of oocytes but there are no international cut-off of AMH as different assays have been used since its invention and there is wide variation in AMH levels in different ethnic populations. An ideal ovarian reserve test should be cheap, easily available, rapidly interpretable and reproducible and should have minimal variability within the menstrual cycle and between the cycles. Also, it should have good sensitivity and specificity and should be able to interpret the DOR at an early age so that timely intervention can be done.[3]

No ideal ovarian reserve marker has been devised till date. There have been many studies published to identify the best and ideal ovarian reserve marker. We are presenting a retrospective analysis of 1924 IVF cycles where ovarian reserve markers are being compared with oocyte yield in women undergoing COS in Indian population. The main aim of this retrospective study was to correlate different ovarian reserve markers with oocyte yield in Indian women undergoing IVF/ICSI in ART centre. The study also evaluated sensitivity and specificity of different ovarian reserve markers to predict oocyte yield and suggested a multimarker assessment formula for calculating the approximate oocyte yield based on multiple predictors of ovarian reserve before starting the stimulation cycle.

METHODS

Study population

In this retrospective cohort study, all patients (n=1924) who had undergone controlled ovarian hyper stimulation and IVF±ICSI cycles from January 2010 to June 2017 in IVF unit were included and analysed. The following data was collected for all patients from the unit database: Age (years), BMI (Kg/m^2), D2 FSH, S.AMH, D2-5 antral follicle count (AFC), amount of gonadotrophins used for stimulation, E2 on the day of ovulation trigger and number of oocytes retrieved. The biochemical and ultra-sonographic markers were compared with oocyte yield. Cases of donor oocyte IVF cycles and empty follicle syndrome were excluded from the study. The main objective of the study was to correlate these predictors of ovarian reserve with oocyte yield, in Indian population undergoing controlled ovarian stimulation in IVF/ICSI cycles.

Study protocol

The variables recorded from the database were hormone analysis (FSH, LH, AMH) and antral follicle count (day 2-5). All the USGs were done consistently by three consultants in IVF unit with same ultrasound machine (5-9MHz, GE Voluson S6). The patients underwent either GnRH agonist or GnRH antagonist ovarian stimulation protocol. Controlled ovarian hyper stimulation (COH) protocols are decided according to patient characteristics and clinician discretion. Ovarian stimulation was started with recombinant FSH (Gonal-F, Merck Serono, Switzerland) and/or human menopausal gonadotropin dose (range 150-300 IU). Dose adjustments were done according to serial USG monitoring for follicular response. Ovulation trigger was given with either recombinant hCG (Inj. Ovitrelle, Merk Serono, Switzerland) or GnRH analogue (Inj Leupride 2mg, Bharat Serums) when there were at least three lead follicles measuring ≥18 mm. All follicles >/ or equal to 14mm were aspirated transvaginally under ultrasound guidance 34-36 hours after ovulation trigger. Oocyte number and quality were assessed by the embryologist and fertilization was done with IVF or ICSI according to cause of infertility and male and female factors. For data analysis, patients with <4 oocytes were considered as poor responders, 4-15 as normal responders and >15 oocytes as high responders/excessive response, the oocyte yield was compared with female age, FSH, AMH, AFC, amount of gonadotrophins used for stimulation and E2 on the day of ovulation trigger.

Hormone assays

FSH was measured with automated multi-analysis system with chemo luminescence technique (ARCHITECT), detection in terms of IU/l. S.AMH was measured using various assays over the last 7 years, as methods are evolving over time, and various assays have been introduced for AMH measurement. The various assays available are Immunotech I generation kit, Beckmann Coulter II generation kit RUO and Beckmann Coulter II generation kit.

Statistical analysis

We performed all statistical analyses using Stata 14. Quantitative variables were presented as mean±SD and qualitative variables as number (%). Spearman correlation was used to assess the association between two quantitative variables. The univariate logistic

regression analysis were used to estimate the risk of each independent variable on the dependent variable and multivariable logistic regression analyses were used to assess the independent effect of these variables after controlling confounding between them. The area under the receiver operating characteristic curve (ROC_{AUC}) was computed to assess the predictive accuracy of each independent variable. Quantitative variables applied among the group were compared by one way ANOVA (following normality), Kruskal-Walis test (non-normal data), followed by multiple comparisons using Bonferroni test/Dunn test with Bonferroni test. A formula for calculating ovarian response/yield using variables that were found significant on multivariate analysis was devised. A p value of <0.05 was considered statistically significant.

RESULTS

Total 2093 patients underwent Controlled ovarian hyper stimulation for IVF/ICSI cycles from January 2010 to June 2017. Patients who had undergone IVF with donor oocyte cycles and empty follicle syndrome were excluded from the study. So, 1924 patients were analysed for the present study.

Depending on the number of oocytes retrieved, 256 patients were characterised as poor responders (<4 oocytes), 1412 were normal responders (4-15 oocytes), whereas 256 patients were hyper responders (>15 oocytes). The mean age of patients (n=1924) was 31.32±4.03 (range 22-41) years. The mean S.AMH, mean D2 FSH and mean AFC was 3.89±2.6 ng/ml (range 1.1-25), 7.11±1.56 IU/ml (2.53-12.8) and 13.71±6.49 (range 3-36) respectively. The mean and median E2 on the day of trigger was 3713.33±3584.98 and 3306 pg/ml respectively.

Table 1: Predictors of oocyte yield using multivariate logistic regression before starting controlled ovarian stimulation.

Variable	Adjusted regression coefficient "ß" (95% CI)	P value
Age (years)	-0.12 (-0.18, -0.07)	<0.001
AMH (ng/ml)	0.09 (0.003, 0.18)	0.043
D2 FSH (IU/ml)	-1.19 (-1.33, -1.05)	<0.001
AFC	0.17 (0.14,0.21)	<0.001

Number of oocytes retrieved=$18.46 + (0.174 \times AFC) + (0.092 \times AMH) - (0.123 \times age) - (1.19 \times FSH)$.

Logistic regression analyses were done and analysed before and after ovarian stimulation predictors. Univariate analyses showed that age, AMH, FSH, AFC, gonadotrophins used for stimulation and E2 on the day of trigger were significant predictors for oocyte yield. In the multivariate analysis for predictors during ovarian stimulation, age, FSH, AFC and E2 on the day of trigger were found to be significant predictors of oocyte yield (p<0.05), AMH was not found to be significant (p=0.068). When logistic regression analyses was done using only before ovarian stimulation predictors i.e. age, AFC, FSH and AMH, then all were found to have statistically significant correlation with oocyte yield (p<0.05). The following model with r^2=0.2486 (p<0.001), number of oocytes retrieved= 18.46+(0.174×AFC) + (0.092×AMH)-(0.123×age)-(1.19×FSH) was formulated. This can help in prognosticating the patient based on age, FSH, AMH and AFC. Predictors for oocyte yield using Spearman correlation, univariate and multivariate logistic regression analysis before and after controlled ovarian stimulation is shown in (Table 1-2).

Table 2: Predictors of oocyte yield using spearman correlation, univariate and multivariate logistic regression after starting controlled ovarian stimulation.

Predictors	Spearman correlation coefficient (r)	P value	Unadjusted Regression coefficient "ß" (95% CI)	P value	Adjusted Regression coefficient "ß" (95% CI)	P value
Age (years)	-0.21	<0.001	-0.27 (-0.33, -0.21)	<0.001	-0.13 (-0.17, -0.07)	<0.001
BMI (kg/m^2)	-0.01	0.768	-0.03 (-0.09, 0.04)	0.437	-	-
AMH (ng/ml)	0.25	<0.001	0.45 (0.36, 0.53)	<0.001	0.08 (-0.01, 0 .16)	0.068
D2 FSH (IU/ml)	-0.62	<0.001	-1.43 (-1.57, -1.29)	<0.001	-1.08 (-1.20, -0.94)	<0.001
AFC	0.36	<0.001	0.28 (0.25, 0.32)	<0.001	0.16 (0.13, 0.20)	<0.001
Total dose of gonadotrophins (IU)	-0.15	<0.001	-0.001(-0.00078, -0.00038)	<0.001	-	-
E2 on the day of trigger (pg/ml)	0.56	<0.001	0.00047 (0.00041, 0.00054)	<0.001	0.00035 (0.0003, 0.0004)	<0.001

ROC curve analysis shows that FSH has statistically significant discriminability to detect poor response than age [AUC (95% CI) FSH 0.77 (0.74, 0.81), age 0.56 (0.52, 0.60), (p<0.05)]. FSH >7.82 IU/ml was predictor of poor response (sensitivity 78.13%, specificity 79.53%). Among the AMH, AFC and E2 on the days of trigger, E2 was found to have statistically significant discriminability to detect normal response than AFC and AMH, with E2 on the day of trigger AUC (95% CI), 0.81 (0.77-0.84). AUC (95%CI) for AMH and AFC was 0.58 (0.54-0.62) and 0.63 (0.59-0.67) respectively, which was statistically significant, p<0.05. E2 cut off levels >2202 pg/ml (sensitivity 73.94%, specificity 73.98%), AFC cut off levels >12 (sensitivity 57.72%, specificity 60.16%), AMH cut off levels >2.95 ng/ml (sensitivity 56.60%, specificity 56.25%) were associated with normal response in terms of oocyte yield. ROC curve analysis shows that E2 on the day of trigger has statistically significant discriminability to detect hyper response than AFC and AMH with AUC (95% CI) for E2 being 0.74(0.71-0.77), for AMH and AFC, 0.64 (0.60-0.67) and 0.68 (0.65-0.72) respectively, p<0.001. The E2 on the day of trigger cut off >4533 pg/ml (sensitivity 67.72%, specificity 68.22%), AMH cut off >3.5 ng/ml (sensitivity 60.55%, specificity 58.09%), AFC cut off >15 (sensitivity 58.98%, specificity 65.58%), will predict hyper response to controlled ovarian hyper stimulation. ROC curves to predict poor, normal and hyper response are shown in (Figure 1A-D).

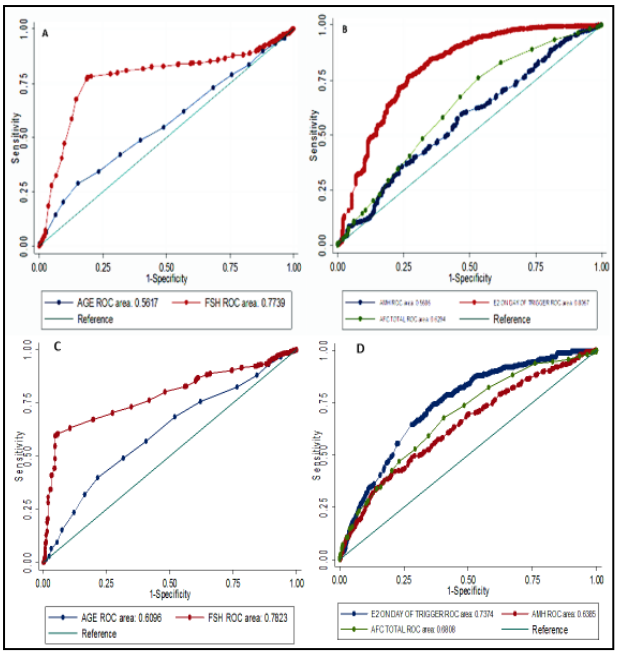

Figure 1: Receiver operating characteristic curves for oocyte yield in patients with different parameters before and after ovarian stimulation. In addition to age and FSH, AMH levels, AFC and E2 on day of ovulation trigger, predict ovarian response and oocyte yield in normo responders and hyper-responders, A) poor response <4 oocytes, B-C) normal response 4-15 oocytes, D) hyper response >15 oocytes.

The oocyte yield was divided into three groups: poor response <4 oocytes, normal response (4-15 oocytes) and hyper response (>15 oocytes) and was correlated with age, BMI, AMH, FSH, AFC and E2 on the day of trigger. Age and FSH were negatively correlated with oocyte yield (p<0.001), as it increased, the oocyte yield decreased. AMH, AFC, E2 on day of trigger were positively correlated with oocyte yield, as it increases, the yield also increases (p<0.001), as shown in (Table 3).

DISCUSSION

The present retrospective study was done to evaluate different ovarian reserve markers in terms of oocyte yield in women undergoing IVF/ICSI cycles. The study found that after age, FSH is the best ovarian reserve marker to predict oocyte yield in ART cycles. Although AMH and AFC have almost completely replaced FSH, still our study shows that women with FSH >7.8 IU/ml may be used as a predictor of poor response and COS protocol may be individualised. The study also concluded that multi-marker assessment can better predict the ovarian response better than any specific single marker and can be used to individualise stimulation protocols and counsel couples. No single ovarian reserve marker can predict oocyte quality and quantity, so a test based on a combination of markers might provide better identification of diminished ovarian reserve and act as a more sensitive predictor of response to ovarian stimulation in patients undergoing IVF/ICSI treatment. Kline et al produced predictive models based on chronological age, ovarian volume, FSH and inhibin B. Combinations of various markers (AFC, AMH and inhibin B) have also been used to predict poor response to stimulation, with up to 87% sensitivity, 80% specificity and a positive likelihood ratio of 4.36%.[5] Tsakos et al conducted a prospective study to compare the efficacy of AMH, AFC and FSH for prediction of oocyte yield and embryos generated in women undergoing IVF ICSI cycles using GnRH antagonist protocols. The study concluded good predictability of oocyte yield using all these three markers but the best predictive parameter was AFC.[6] In present study, all the patients irrespective of stimulation protocol used (agonist, antagonist or micro-dose flare protocol) were analysed. Cases with empty follicle syndrome and donor recipient cycles were excluded. In contrast to Tsakos et al study, showing maximum predictability of AFC, our study showed maximum sensitivity of FSH, the less sensitivity of AFC may be due to subjective variation, as AFC was done by three different consultants. Also there has been wide variation in assays used in AMH levels over the last decade. In contrast, FSH is an age old traditional test with same assays used for years. In our study, ROC curve analysis shows that FSH has statistically significant discriminability to detect poor response than age [AUC (95% CI) FSH 0.77 (0.74, 0.81), age 0.56 (0.52, 0.60), (p<0.05)]. FSH >7.82 IU/ml was predictor of poor response (sensitivity 78.13%, specificity 79.53%) and indicated decreased ovarian reserve and predicted poor

ovarian response in COH. This is a significant finding as FSH is an old traditional test and is done in all infertility patients and can be used to predict ovarian response in our population where affordability is an issue and AMH being an expensive test and not all patients can afford to get AMH done. FSH though thought to be obsolete, still is a significant marker to predict ovarian response, especially poor ovarian response. FSH is routinely done in fixed post menstrual phase in all study subjects. But AMH was done irrespective of menstrual cycle phase. There has been lot of debate going on the best time to get AMH.[7] To study the menstrual cycle variation of AMH levels, Tsakos et al in their study, measured AMH levels at two times one in post menstrual phase and then on day 5. Authors did not find any significant difference in levels and mid follicular phase AMH did not provide better predictability of AMH to oocyte yield.

Table 3: Baseline characteristics (Mean ± SD, Median) of the normal response (4-15 oocytes), poor response (<4 oocytes) and hyper response (>15 oocytes).

Parameter	Poor response (N=256)		Normal response (N=1412)		Hyper response (N=256)		Overall p value	Multiple comparison p value	
	Mean± SD	Median (range)	Mean± SD	Median (range)	Mean± SD	Median (min-max)		Normal vs. poor	Normal vs. hyper-response
Age	32.33± 4.54	32 (22-45)	31.38± 3.93	31 (21-44)	29.98± 3.74	30 (21-32)	<0.001	<0.001	<0.001
AMH*	3.24± 2.20	2.8 (0.3-18)	3.77± 2.52	3.12 (0.5-25)	5.15± 3.4	4.15 (3.2-8.5)	<0.001	<0.001	<0.001
D2FSH	8.11± 1.39	8.4 (2.5-14.5)	7.11± 1.33	7.2 (1.5-12)	6.13± 2.18	6.3 (1.8-7.8)	<0.001	<0.001	<0.001
AFC	11.11±5 .68	10 (2-14)	13.44 ±5. 96	12 (5-20)	17.80± 8.01	16 (12-32)	<0.001	<0.001	<0.001
E2 on day of trigger*	1830.69± 1818.57	1302 (352-2250)	3679.51± 2586.37	3313 (670-5980)	5722.7± 6978	4858 (2258-6870)	<0.001	<0.001	<0.001

*Kruskal Walis test applied and in others one way ANOVA test applied

Al-Azemi et al conducted a prospective trial to evaluate multi marker assessment of ovarian reserve tests to predict oocyte yield and ongoing pregnancy rate. They reported that the age, FSH and AMH were important predictors for poor oocyte yield; AMH had ROC_{AUC} of 0.827 followed by FSH with an ROC_{AUC} of 0.721 indicating their good discriminating potential for predicting poor ovarian response. In the multivariate analysis, the variables age, FSH and AMH remained significant and the resulting model provided a high ROC_{AUC} of 0.819.[8] In contrast, our results showed FSH to have the highest ROC_{AUC} of 0.77 indicating a good discriminating potential to predict poor oocyte yield. This is an important finding especially in developing countries, FSH is significant predictor of oocyte yield and it is affordable as compared to AMH, it can be used as prognostic marker to counsel the patients regarding their ovarian response prior to ovarian stimulation. Among normo-responders, E2 was found to have statistically significant discriminability to detect normal response. E2 cut off levels >2202pg/ml (sensitivity 73.94%, specificity 73.98), indicate normal response according to our study.

Siddhartha N et al reported that elevated E2 levels on the day of ovulation trigger can predict higher oocyte yield after ovarian stimulation. E2 levels in the ranging from3000 to 4000 pg/ml can predict increased fertilization rate and pregnancies in ICSI cycles (9). In our study, ROC curve analysis shows that E2 on the day of trigger has statistically significant discriminability to detect hyper response than AFC and AMH, AUC (95% CI) for E2 was 0.74 (0.71-0.77), p<0.001. The E2 on the day of trigger cut off >4533 pg/ml (sensitivity 67.72%, specificity 68.22%). AMH is the latest among the ovarian reserve markers and extensive research has already been done on the normal range, ideal assay and its potential to predict ovarian response. The main advantage of AMH is that it has minimal intra-cycle and inter cycle variation and it is not altered with situations like pregnancy and oral contraceptive pills use.[10] In contrast to study published by Li R et al, who conducted a prospective trial to evaluate AMH levels to predict ovarian response, ROC curve with AUC (95% CI) being 0.75 (0.71-0.79). The optimal AMH cut-off value predicting high and normal ovarian response was 2.6 ng/ml (sensitivity: 81.28%, specificity: 59.51%).[11] Lesser ROC_{AUC} in our study can be explained due to different assays used at different times for AMH analysis and lab variations. As compared to any single marker, multiple markers can be combined to predict ovarian response in women undergoing controlled ovarian stimulation in IVF cycle. This multi marker assessment can help to individualise treatment protocols

and counselling the couples regarding predicted ovarian response with given stimulation protocols. Ovarian reserve markers can help to identify patients prior to stimulation, which might not have a good ovarian response, ovarian response can be predicted and couples can be counselled accordingly. Age and FSH can be used as prognostic markers for patients undergoing IVF, especially in developing countries where getting AMH test done is not cost effective and affordability, accessibility is an issue. The combination of predictors of oocyte yield can also be used to predict the oocyte yield in infertile patients undergoing ovarian stimulation (OVI±IUI) or patients with advanced age planning pregnancy or young patients planning to delay childbearing, patients can be counselled about their ovarian reserve, outcome and early referral to IVF unit if poor ovarian response is expected.

CONCLUSION

A combination of predictors demonstrated superior ability of predicting oocyte yield in women undergoing COS in IVF cycles than compared with any single endocrine marker. D2 FSH though thought to be outdated, but in our study, age, D2 FSH and E2 on the day of trigger had significant predictive ability in terms of oocyte yield in the Indian population. There is a need to have age specific cut off of AMH levels for Indian population. The following model with r^2=0.2486 ($p<0.001$), number of oocytes retrieved= 18.46+(0.174×AFC)+(0.092×AMH)-(0.123×age)-(1.19×FSH) was formulated. This can help in prognosticating the patient based on age, FSH, AMH and AFC. Larger study is required to validate this equation.

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