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Antral Follicle Count in Normal (Fertility-Proven) and Infertile Indian Women

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Abstract
Antral follicle count (AFC) has been labeled as the most accurate biomarker to assess female fecundity. Unfortunately, no baseline Indian data exists, and we continue using surrogate values from the Western literature (inferred from studies on women, grossly different than Indian women in morphology and genetic makeup).
Aims: *To establish the role of AFC as a function of ovarian reserve in fertility-proven and in subfertile Indian women. To establish baseline cut-off AFC values for Indian women.*
Materials and Methods: *Thirty patients undergoing workup for infertility were included and compared to equal number of controls (women with proven fertility). The basal ovarian volume and AFC were measured by endovaginal. USG the relevant clinical data and hormonal assays were charted for every patient.*
Statistical Analysis Used: SPSS platform was used to perform the Student's t-test and Mann-Whitney U-test for intergroup comparisons. Correlations were determined by Pearson's ranked correlation coefficient.
Results: *Regression analysis revealed the highest correlation of AFC and age in fertile and infertile patients with difference in mean AFC of both the groups. Comparison of the data recorded for cases and controls showed no significant difference in the mean ovarian volume.*
Conclusions: *AFC has the closest association with chronological age in normal and infertile Indian women. The same is lower in infertile women than in matched controls. Baseline and cut-off values in Indian women are lower than that mentioned in the Western literature.*
Key words: *Antral follicle count; infertility; ovarian reserve.*

Introduction

Infertility is the failure of a couple to conceive after 1 year of regular, unprotected intercourse. Ovulatory disorder is one of the most common reasons of female factor infertility 30% of all cases).[1] Reproductive aging is considered to be the consequence of a decrease in the quantity and quality of the ovarian follicle pool.[1-3]

Autopsy studies of human ovaries show that the number of follicles decreases rapidly with female age, starting in fetal life and continuing until after menopause. However, between women of the same chronological age, the quantitative ovarian reserve may vary substantially.

To assess the individual quantitative ovarian reserve, various ovarian reserve tests (ORTs) have

been developed, viz. (1) day-3 follicle stimulating hormone (FSH), (2) anti-Mullerian hormone (AMH), and (3) antral follicle count (AFC). The number of antral follicles and the total ovarian volume as measured by transvaginal USG have been mentioned in the literature to predict declining fertility related to reproductive aging.[4-7]

Studies concerning physiological ovarian aging in women with and without fertility problems are very limited and most of them are done in Western countries. It, therefore, seems warranted to evaluate the aforementioned sonographic test parameters in women of different ages in Indian population. Since such women are likely to represent the age-related decline of the reproductive potential in the normal population, the assumption that their chronological age approximates reproductive age seems justified. The study also aims at predicting the fertility pattern in Indian women and to correlate AFC with age.

Materials and Methods

Infertile females (N = 30, age range 20-35 years) from those attending investigation of subfertility or undergoing assisted conception treatment were enrolled for the study. Healthy females of the same age group were enrolled from those being referred for unrelated problem (taking care of confounding factors) or routine health check-up, serving as controls.

Cases were enrolled in the study protocol if they met all of the following criteria: (i) primary infertility, (ii) no ovarian abnormality (polycystic ovary, ovarian endometriomas) as assessed by transvaginal USG, (iii) no evidence of uterine malformations or uterine pathology, (iv) no evidence of endocrinological disease, and (v) no evidence of previous ovarian surgery.

Healthy female volunteers were enrolled in the study if they met all of the following criteria: (i) proven natural fertility by having at least one pregnancy carried to term, (ii) regular menstrual cycles, (iii) no evidence of endocrinological disease, (iv) no evidence of ovarian surgery, (v) no ovarian abnormality as assessed by transvaginal USG, and (vi) hormonal contraception stopped > 2 months before entering the study protocol. All the relevant clinical data with patient biometry was acquired, along with the hormonal assays of the infertile group [day-3 FSH, T3, T4, and thyroid stimulating hormone (TSH)]. This study was approved by the institutional review board and written informed consent obtained from all participants.

Transvaginal USG measurements

Transvaginal USG was carried out on the second or third day of the menstrual cycle. All sonographic measurements were performed by the same observer using a 7.5-MHz transvaginal transducer (iU22; Philips Medical System, Andover, MA, USA). Thorough survey of each ovary was done by scanning from the outer to the inner margin.[8,9]

All follicles having adequate morphology as described for a healthy follicle (i.e., 2-10 mm size range of well-defined anechoic cysts with smooth margins and absence of internal septations or nodularity)[10] were measured and counted in each ovary. The sum of both counts was labeled as the AFC. 3-D volume calculations were performed independently, with no access to 2-D results. Virtual organ computer-aided analysis (VOCAL) was used to conduct 3-D rotational measurements of the ovarian volume using incremental rotations in both the longitudinal and coronal planes. The volumes of both ovaries were added to obtain the total ovarian volume (TOV) [10] [Figure 1].

Statistical methods

Statistical analysis was performed by using IBM SPSS Statistics for Windows (version16; SPSS, Inc., Chicago, IL, USA). For the study group as a whole, cases and controls were distinguished into two age groups: a young group of women aged< 0.05 was considered as significant. Comparison between subgroup variables was performed by Student's t-test and Mann-Whitney U-test.

The correlations between age and the various endocrine and sonographic parameters in fertile as well as infertile patients were presented as a correlation matrix. Correlations were determined by Pearson ranked correlation coefficient. Chi-square test was used for non-parametric variables. For all statistical analyses, $P < 0.05$ was considered as significant.

Results

Mean values of the biophysical and sonographic parameters of subfertile patients and healthy female volunteers are given in Table 1. Significant difference was noted only for AFC, confirming hereby an adequate matching of both groups and exclusion of selection bias. Further, the correlation matrices showed close correlation between various variables in the case group [Table 2 in conjunction with Figure 2] and in the control group [Table 3].

Significance could be ascribed to both the AFC and day-3 FSH results. Both cases and controls showed decline in the AFC with increasing age ($P < 0.003$ for cases and $P < 0.008$ for controls) [Figures 3 and 4]. However, no significance could be assigned to TOV in the context of ovarian reserve in the present study. The correlation of AFC with age was strong ($r = 0.527$) as was the correlation was of day-3 FSH with age, suggesting their role as the best markers of ovarian reserve [Table 2].

In this study, the mean age of cases as well as controls was almost the same (26.77 vs. 26.73 years) with no significant difference in the biophysical profile (height, weight, and body mass index) of both the groups, suggesting that both cases and controls were age and weight matched, which is crucial for comparative analysis of different parameters between the groups. Approximately 30-40% of both groups (cases and controls) were under 25 years of age, while 60-70% of them were above 25 years.

Table 1: Comparative analysis of biophysical and sonographic parameters in infertile and fertile patients

Variables	Cases (n=30) (Mean±SD)	Controls (n=30) (Mean±SD)	P value
Age (years)	26.77±2.837	26.73±2.164	0.959
BMI (kg/m^2)	20.71±1.53	21.15±1.35	0.237
Antral follicle count (AFC)	9.60±4.082	12.53±2.623	0.002
Total ovarian volume (cc)	12.95±7.279	11.384±4.9398	0.334

BMI: Body mass index, SD: Standard deviation. Antral Follicle count in fertile versus infertile patients, P-0.002; total ovarian volume in fertile versus infertile patients, P>0.005

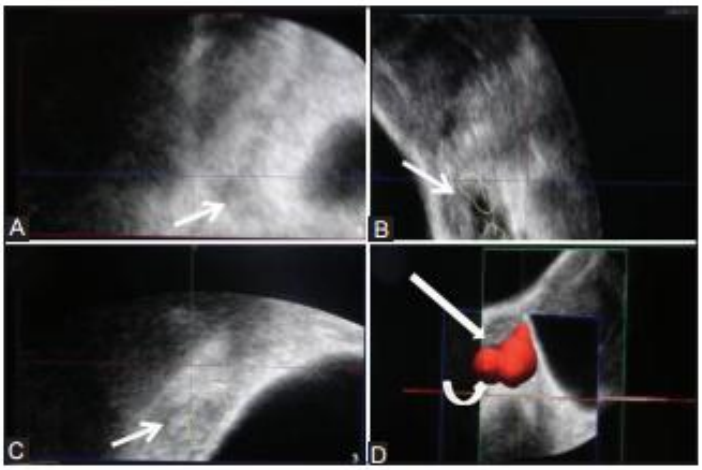

Figure 1 (A-D): 3D-volumetry showing reconstructed true planar images of right ovary (straight arrows) in three orthogonal planes, (A) axial, (B) sagittal, and (C) coronal. The images were obtained using the volumetric data acquisition (by a volume probe) and reconstructing true planes by "VOCAL" software. (D) Surface-rendered image of the right ovary (solid arrow), reconstructed using the same software, was used to assess the gross total volume. Note the healthy antral follicle (curved arrow) as seen in the surface-rendered image

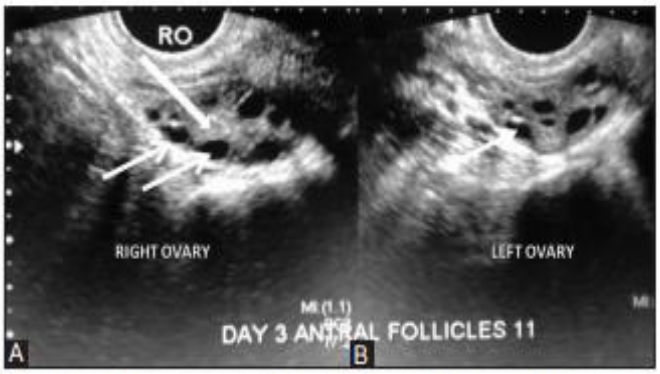

Figure 2 (A and B): Transvaginal USG of bilateral ovaries, (A) right and (B) left, showing healthy representative antral follicles (straight arrows), counted to assess the antral follicle count. Note the intervening healthy-appearing stroma as well (solid arrow)

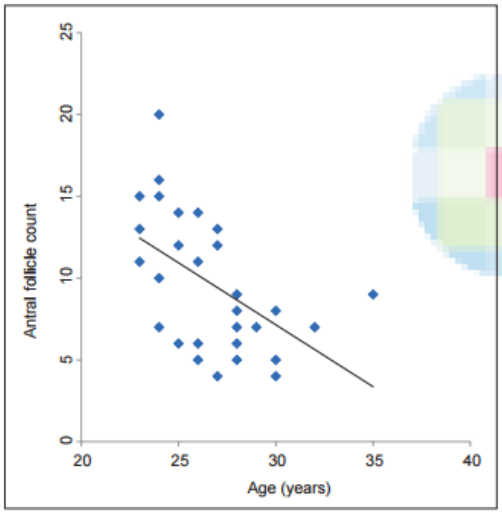

Figure 3: Scatter diagram showing age versus AFC in infertile group

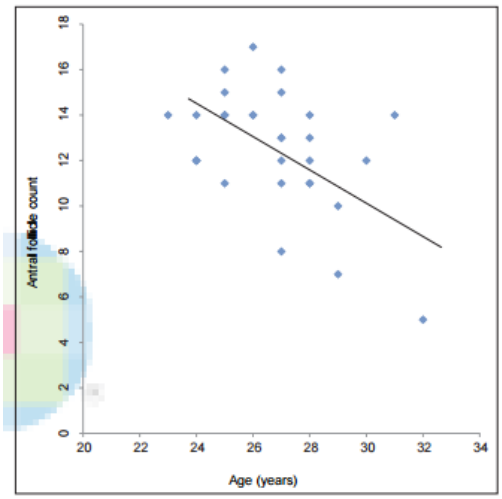

Figure 4: Scatter diagram showing age versus AFC in fertile group

Discussion

Limited data is available on ovarian aging in the subfertile and healthy population and the role of sonographic biomarkers (AFC, ovarian volume) of ovarian reserve. Most of the available data is based on studies outside the Indian context.[11]

The present study, therefore, evaluates the relationship of AFC with age and hormonal parameters in subfertile cases and with healthy (fertility proven) controls. Role of ovarian volume is also evaluated and compared with other markers of the ovarian reserve. Our observation indicates that the number of antral follicles is lower in the subfertile patients as compared to the fertile group (in all age groups), in view of the significantly lower median AFC in women of the former group ($P < 0.001$).

The range of AFC in females presenting with complaints of infertility was 5-20 (median value of 9), while that in healthy females (with proven natural fertility) was 5-17 (median value of 13). Similar trends have been noted by previous workers worldwide,[12,13] but this remains different in magnitude from that noted in our study (median values of AFC in subfertile and fertile groups being 16 and 20, respectively, in females of the age group 25-30 years).

In another study,[2] the median AFC value in fertile women of the same age group was 15; no comparison, however, was done between the subfertile and fertile groups. Hence, the ovarian reserve as depicted by AFC coincides well with the trends seen worldwide. It should, however, be noted that the cut-off value in Indian women is set at a lower baseline than that noted in the Western literature. This variability in the value of AFC is most probably due to the differences in the racial, socioeconomic, and geographic background of Indian and Western populations.

Table 2: Correlation matrix of physical, endocrinal and sonographic parameters of infertile patients ($n=30$)

Parameters	Age	BMI	TOV	AFC	Day 3 FSH
Age					
r-value	1				
P-value					
BMI					
r-value	0.142	1			
P-value	0.455				
Total ovarian volume (TOV)					
r-value	−0.107	−0.211	1		
P-value	0.575	0.263			
Antral follicle count (AFC)					
r-value	−0.527	−0.185	0.395	1	
P-value	0.003	0.327	0.031		
Days 3 FSH (4-14 mIU/ml)					
r-value	0.601	0.079	0.069	−0.489	1
P-value	<0.001	0.680	0.716	0.006	

AFC: Antral follicle count, BMI: Body mass index, FSH: Follicle stimulating hormone

Table 3: Correlation matrix of physical and sonographic parameters of fertile patients ($n=30$)

Parameters	Age	BMI	AFC	TOV
Age				
r-value	1			
P-value				
BMI				
r-value	0.007	1		
P-value	0.972			
Antral follicle count (AFC)				
r-value	−0.478	0.056	1	
P-value	0.008	0.768		
Total ovarian volume (TOV)				
r-value	−0.400	0.183	0.406	1
P-value	−0.28	0.333	−026	
Endometrial thickness				
r-value	0.073	−110	−0.037	−053
P-value	0.701	0.565	0.848	0.781

AFC: Antral follicle count, BMI: Body mass index

Though in the present study we did not systematically record the above mentioned variables, the data from international database clearly supports the notion.[21] Further, everyday clinical experience in our center as well as in the other high-volume centers in India should be sufficient to potentiate the fact. Reproductive ability (fecundity) of a woman is directly related to the remaining pool of primordial follicles at a particular point in time.[15] This stock depletes as the age progresses and is completely exhausted at the menopause. Hence, it may be reasonable to assume that the number of antral follicles reflects the ovarian pool and indirectly the reproductive age. Our data shows that there is an inverse relationship between AFC and the age of female (a negative correlation value; r = −0.528 with P value of 0.03).

Similar findings have been noted by earlier workers,[19] but with a lesser strength of correlation (r = −0.298) compared to our population of subfertile patients. This may be due to the fact that the median age in the above-cited study was higher (32.5 years), as opposed to that in the present study (26.5 years). As in the case of AFC, the trend of decline of follicle pool coincides well with most previous studies. Also, a similar correlation curve was noted in the present study between age and AFC of the control group (r = −0.427).

Comparable data in another similar study, however, shows a stronger correlation (r = −0.68) in healthy women compared to our study.[3] This difference arises due to the fact that the said study had a larger number of recruits and a higher median age (38 years) than our study population (27 years). Though most notifiable confounding factors have been excluded in the present study for both cases and controls, it may be noted that the control group in our study population comprised females being referred for abdominal USG for unrelated diseases.

Hence, a component to the above noted differences between our study and the cited comparable studies may be due to the remote systematic effects of various "unrelated" pathologies in the control group. It may be worthwhile to recruit healthy volunteers as controls in future studies. An experimental evaluation of various predictors of ovarian aging (such as E2, inhibin B, and FSH, and ovarian volume) detected superiority of AFC over all these put together.[19]

A strong correlation was, however, established between these parameters. The present study also evaluated the inter-relationship between AFC with other biophysical, USG, and hormonal parameters, and showed a strong correlation in both groups (for age, TOV, and day-3 FSH) and in cases alone (with day-3 FSH). The sensitivity of AFC to identify "poor responders" before induction of ovulation with exogenous gonadotropins has been found to be around 89% in previous studies.[16]

We, however, did not endeavor to establish any such correlation in our population, as the same was out of scope of this study. We, however, submit that the good correlation shown by our data between the above mentioned parameters may be used in future by other Indian groups evaluating metrics for patient selection during planning of ovulation induction. Further, as in few recent studies,[17,18,20] on evaluating antral follicles

up to 10 mm in diameter, significant difference in numbers (10.1 ± 3.0 in controls vs. 5.7 ± 1.0 in cases) was noted in our study population (9.60 ± 4.0 in cases vs. 12.53 ± 6.2 in controls; P value 0.002).

A cut-off value of 10 follicles (aggregate of both ovaries) may be taken as a standard for successful pregnancy outcome. Intergroup comparison of median values of TOV showed no significant difference in our study groups. This parameter, however, can be routinely measured without any added effort, along with AFC. Though our data reflects that TOV has no role as a biomarker of ovarian reserve, we would suggest a routine recording and further evaluation of the role of this parameter in population-based datasets.

The major limitation of our study is its cross-sectional nature. Hence, we could not conclusively establish the fact that lower AFC actually results in infertility. This, however, prima facie has not been included within the scope of the study. In addition, while lower AFCs are seen among subfertile women at the time of presentation, it could be ascertained from our data whether this results from a smaller initial oocyte pool or an accelerated rate of loss. Longitudinal studies of AFC in both fertile and subfertile women will be necessary to determine the predictive value of AFC for future fertility.

Threshold values that predict a very low likelihood of spontaneous conception may be identified, and thus, the nonspecific term "diminished ovarian reserve," currently overused in the infertility literature, could gain clinical relevance among the general population. Pre-ART (artificial reproductive technique) ultrasonographic AFC has been shown to be an excellent predictor of ovarian reserve and response, with significant superiority in relation to other markers. Results from literature seem to converge for recognition of the importance of AFC as a predictor of ovarian response.

AFC may be helpful in determining stimulation protocol, as it is the most reliable determinant of retrievable oocytes[18] AFC nomograms should, hence, be proposed according to age, ethnicity, and socioeconomic status, and also for individualizing the protocol.[14]

Conclusion

The results of this study indicate that AFC is a viable predictor of fecundity in Indian women of child-bearing age in terms of capability to conceive on a two-point scale (i.e., positive or negative). The mean AFC in Indian women is significantly different from that noted in Western literature, mainly due to racial, geographic, and socioeconomic reasons. A cut-off value of 10 may be used to prognosticate patients undergoing assessment for female factor infertility. On the other hand, same data can be utilized for optimum patient selection for ART; this would in turn lead to a higher success rate of the technique.

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