Original Research Article

The relevance of antral follicle count (AFC) as a marker of ovarian reserve in normal and infertile women in a tertiary care centre in Central India

Prasanta K. Nayak[1]*, Chandrima Mukherjee[1], Subarna Mitra[2]

[1]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Raipur, Chhattisgarh, India
[2]Department of Obstetrics and Gynecology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

*Correspondence:
Dr. Prasanta Kumar Nayak,
E-mail: drprasant79@gmail.com

ABSTRACT

Background: The objective of the present study was to establish the role of AFC as a marker of ovarian reserve in fertility-proven and in sub-fertile Indian women, and to establish the baseline cut-off AFC values for Indian women.
Methods: This is an Observational Case-Control type of study. Test subjects (n=30, age range 20-35 years) were selected randomly from all the women coming to our Out-Patient Department with Primary Infertility. Healthy female volunteers (n=30, age range 20-35years) were recruited from the local population. Statistics: Student t test was applied to compare the mean Antral Follicle count between the case and control group. Pearson correlation test was applied to assess the correlation between age and AFC for case and control group.
Results: The results of the study show that there is a significant difference between the number of antral follicles between fertile and infertile women (p < 0.001). The baseline cut-off for successful pregnancy was established as an AFC of 12. There exists negative correlation (r = -0.249) between age and AFC case group indicating as the age increases AFC decreases.
Conclusions: The findings from this study help in strengthening the idea that AFC serves as a marker for 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).

Keywords: Antral follicle count, Infertility, Ovarian reserve

INTRODUCTION

Infertility is not merely a health problem, but it leads to distress, depression, discrimination and ostracism.[1] Infertility is the failure of a couple to conceive after 1 year of regular, unprotected intercourse. Infertility affects approximately 8%-10% of couples worldwide.[2] Ovulatory disorder is one of the most common cause of female factor infertility. Reproductive aging is considered to be the consequence of a decrease in the quantity and quality of the ovarian follicle pool. Many women these days defer childbearing to the later years, raising their risk of infertility associated with ovarian ageing. Demographic and clinical studies show that female fertility starts to decrease from the age of 30 years, and the ability to conceive becomes almost zero at a median age of 41 years.[3]

About 30% cases of female infertility are due to ovulatory disorders.[4] Ovarian reserve relates to both the quantity and the quality of the ovarian follicle pool. 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) are conducted, such as -Day-3 follicle stimulating hormone (FSH) assay, anti-Mullerian hormone (AMH) assay, antral follicle count (AFC) and doppler assessment of ovarian blood flow. Antral follicle count (AFC) has been labelled as the most accurate biomarker to assess female fecundity.[5] Because of lack of adequate and standard Indian data, we mostly depend on western literature for this. 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. Indeed, several studies have demonstrated that AFC declines with chronologic age in women.[6,7]

To what extent ovarian ageing per se contributes to the reproductive failure of an individual couple remains a matter of speculation. The number of antral follicles as measured by trans-vaginal ultrasonography (TVUS) have been mentioned in the literature to predict declining fertility related to reproductive aging.[8-11] Antral follicle count is easy to determine by TVUS, examination as the early phase follicles have small volume of antral fluid and has relatively low inter-cycle variability and has low to moderate inter-observer variability.[12] The AFC consists of counting all small follicles in the range of 2–10 mm as visualised by TVUS examination. The number of small follicles at the beginning of the cycle may well represent the actual functional ovarian reserve. So also, the number of small antral follicles are clearly related to age and could well reflect the size of the remaining primordial pool.[13]

METHODS

This is an observational case-control type of study carried out in the out-patient clinic of department of Obstetrics and Gynaecology in a tertiary care centre in central India. The study period was of 2 months (May and June 2017).

This study was approved by the Institute Ethics Committee and written informed consent was obtained from all participants. Healthy female volunteers (n =30, age range 20–35years) were recruited from the local population.

Inclusion criteria

- Proven natural fertility (by having at least one pregnancy carried to term)
- Regular menstrual cycles
- No evidence of endocrinological disease
- No evidence of ovarian surgery
- No ovarian abnormality as assessed by trans-vaginal USG
- Hormonal contraception stopped > 2 months before entering the study protocol.

Test subjects (n=30, age range 20-35 years) were selected randomly from all the women coming to our out-patient department with primary infertility.

Exclusion criteria

- Ovarian abnormality (polycystic ovary, ovarian endometriomas) as assessed by transvaginal USG.
- Evidence of uterine malformations or uterine pathology
- Evidence of endocrinological disease
- Evidence of previous ovarian surgery.

TVUS of the ovaries was carried out on cycle day 2 and 3. All sonography measurements were performed by the same observer using Mindray DP-7, digital ultrasonic diagnostic imaging system.

Examination of the ovary was established by scanning from the outer to the inner margin. All follicles 2–10 mm in size were measured and counted in each ovary. The sum of both counts was the antral follicle count.

Statistical analysis

Statistical analyses were performed by SPSS version 16. Student t test was applied to compare the mean Antral Follicle count between the case and control group. Pearson correlation test was applied to assess the correlation between age and AFC for case and control group. For all statistical analyses, $P < 0.05$ was considered as significant and $p<0.001$ as highly significant.

RESULTS

Mean values of the biophysical parameters and AFCs of sub-fertile females and healthy female volunteers are given in Table 1.

Table 1: Comparative analysis of age and antral follicle count in infertile and fertile women (according to student's t test).

Variables	Cases (n=30) Mean±SD	Controls (n=30) Mean±SD	P value
Age (years)	27.86±3.33	28.8±3.58	0.3
AFC	11.2±4.36	16.36±3.95	<0.001**
BMI	23.41±2.09	24.25±1.86	0.105

**p<0.001 highly significant difference

Significant difference was noted only for AFC, confirming hereby an adequate matching of both groups and exclusion of selection bias. The difference in the AFC count between the fertile and infertile patients is highly significant(p<0.001). Figure 1 shows the following data in a diagrammatic format. No significant differences between the mean age and BMI of cases and controls

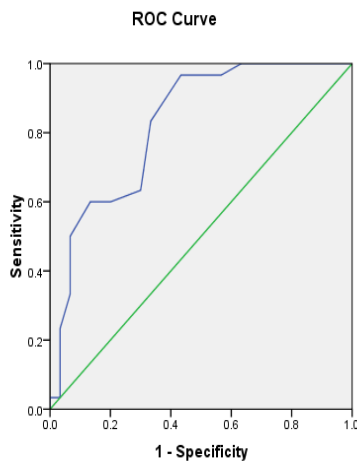indicate that there is a good match between the two groups.

Figure 1: ROC curve of AFC in infertile and fertile women.

Table 2 shows that there was negative correlation (-0.249) between age and AFC case group, indicating as the age increases AFC decreases but the relationship is not statistically significant.

Table 2: Correlation of age and antral follicle count in subfertile and fertile women (according to Pearson's correlation test).

Variable		AFC Cases	AFC Controls
Age (years)	r value	-0.249	0.093
	p value	0.185	0.625

In control there was weak positive correlation (r= 0.093) between the age and AFC which is not statistically significant. An analysis was carried out with n = 60 (30 cases and 30 controls) to find if AFC can help in predicting fertility in women. The best cut-off that maximizes (sensitivity + specificity) is 12. At this count, the sensitivity is 0.833 and specificity is 0.77 (1 – specificity = 0.333). The chi –square test (Table 3) showed highly significant difference (p<0.001) between the two groups. It seems from the ROC that Antral Follicle count is a good indicator to detect fertility.

Table 3: Distribution of study group according to the AFC cut-off point of 12(Chi square test).

	Infertile (n=30)	Fertile (n=30)	P value
AFC 12 or less	20 (66.67)	5 (16.67)	<0.001**
AFC >12	10 (33.33)	25 (83.33)	

DISCUSSION

There are few Indian studies which actually explains the role of AFC as a marker of ovarian reserve.[5] The present study, therefore, evaluates the relationship of AFC with age in subfertile cases and healthy (fertility proven) controls. 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 AFC in women of the former group (P < 0.001). The range of total AFC in females presenting with complaints of infertility was 3-24 (median value of 11), while that in healthy females (with proven natural fertility) was 10-26 (median value of 16.5). Similar trends have been noted by previous workers worldwide.[5]

In another study, 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.[14] 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.

The data from international database clearly supports the notion.[15] Reproductive ability (fecundity) of a woman is directly related to the remaining pool of primordial follicles at a particular point in time.[16] 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. Present study shows that there was negative correlation (-0.249) between age and AFC in case group indicating as the age increases AFC decreases, but the relationship is not statistically significant. In control there was weak positive correation (r= 0.093) between the age and AFC which is not statistically significant. Similar findings have been noted by earlier workers but with a greater strength of correlation (r = −0.298) compared to our population of subfertile patients.[5,17]

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 (27 years). As in the case of AFC, the trend of decline of follicle pool coincides well with most previous studies. Comparable data in another similar study, however, shows a stronger correlation (r = −0.68) in healthy women compared to present study.[17] 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 present study population (29 years). 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.[18] A cut-

off value of 12 follicles (aggregate of both ovaries) may be taken as a standard for successful pregnancy outcome which has been shown by ROC plotting. The major limitation of present study is its cross-sectional nature. Hence, we could not conclusively establish the fact that lower AFC actually results in infertility. 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. 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. AFC may be helpful in determining stimulation protocol, as it is the most reliable determinant of retrievable oocytes.

CONCLUSION

From the above study it is reaffirmed that AFC is a reliable predictor of fertility. A mean AFC count of 12 can be considered as a cut off of successful treatment for infertile Indian women. This cut off can be used while selecting patients for artificial reproductive techniques and predicting the success.

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