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Age-Specific Nomograms for Antral Follicle Count in Fertile and Infertile Indian Women: A Comparative Study

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Abstract

Objectives The aim of this study was to develop age-specific nomograms for antral follicle count (AFC) in fertile and infertile Indian women and (2) to compare the influence of age on AFC in both groups.

Setting and Design It is a prospective cross-sectional study in a tertiary-care hospital in north-central India.

Methods and Material One-thousand four-hundred seventy-eight fertile and 1,447 infertile women (primary infertility) of reproductive age (18–49 years) were recruited. One-thousand one-hundred eighty-one fertile and 1,083 infertile women fulfilled the selection criteria for the study. Transvaginal ultrasonography was done on the second or third day of the menstrual cycle.

Statistical Analysis Age-specific nomograms for AFC were built for the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles in both groups. Correlation and regression analysis was done to estimate the relationship between the study variables. Statistical analysis was done by using IBM SPSS Statistics for Windows, version 20.

Results At every age, each percentile value of AFC was lower in infertile than in fertile women. The decline of AFC with increasing age was linear in both fertile ($r = -0.431$, $p < 0.001$) and infertile ($r = -0.520$, $p < 0.001$) women; however, the rate was higher in the latter (0.50 follicle/year) than in former (0.44 follicle/year) group. The variation in AFC explained by age was 16.3% in fertile and 22.7% in infertile women.

Conclusion AFC decreased linearly with advancing age in both fertile and infertile women, but more rapidly in the latter. The age only modestly explained the decline of AFC. The age-specific percentile thresholds for AFC should be used instead of age-independent constant thresholds in infertility counselling.

Keywords
- antral follicle count
- age-specific nomograms
- infertile
- fertile

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Introduction

Antral follicle count (AFC), ovarian volume, and Doppler studies of ovarian blood flow are the ultrasound methods used to detect ovarian reserve. Out of these, AFC has been used as a major predictor of ovarian reserve as there is a continuous decline of the follicle pool with age.[1,2] Moreover, due to delayed childbearing nowadays, it becomes more relevant to know the current AFC percentile and identify its normal range in a woman for the detection of decline in her ovarian reserve. This necessitates age-specific nomograms for AFC that can help to tailor the treatment strategy and act as a reference guide for infertility specialists. Till now, no specific and normative data are available to find out the AFC in the Indian population. Thus, the primary objective of this study was to establish separate age-specific nomograms for AFC in fertile ($n = 1181$) and infertile ($n = 1083$) Indian women. The secondary objective was to find out and compare the influence of age on AFC in fertile and infertile Indian women.

Materials and Methods

This cross-sectional study was performed at the Department of Radiodiagnosis & Imaging, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, for a duration of 8 years (March 2014 to February 2022). The study was done after approval of the institutional ethical committee and was in accordance with the 1964 Declaration of Helsinki and its later amendments. The females ($n = 1478$) with proven natural fertility (at least one term pregnancy); that is, the "fertile" group, were recruited from those attending the outpatient department (OPD) of the Department of Obstetrics and Gynecology of the same institute for unrelated problems like preconceptional counseling ($n = 351$), contraception ($n = 523$), family planning ($n = 227$), tubal sterilization ($n = 334$), or routine health checkup ($n = 43$). The females with primary infertility ($n = 1447$), that is, the "infertile" group, were prospectively recruited from the patients attending the aforesaid OPD.

Sample Size Calculation

The sample size was computed based on the mean and standard deviation of AFC of fertile and infertile women of a pilot study. For an equal sample size in each group of women, the formula used for two independent sample means was as follows:

$$n = \frac{\left(Z_{\alpha/2} + Z_{1-\beta}\right)^2 s^2}{d^2}$$

Where n is the sample size in each group, $Z_{\alpha/2} = 1.96$ at 5% level of significance, $z_{1\text{-}\beta} = 1.282$ at 90% power, s is the pooled variance of the variances of AFC of fertile and infertile women, and d is the anticipated difference to be detected.

The pilot study on 100 fertile and infertile women each indicated mean and standard deviation of AFC as 12.68 ± 3.587 and 12.14 ± 4.144, respectively. By taking pooled variance, $s = (3.587 + 4.144)/2 = 3.8655$ and anticipated difference $d = 12.68–12.14 = 0.54$; the required sample size computed was 1,083 each for fertile and infertile women.

The required sample was selected based on the following inclusion and exclusion criteria.

Inclusion and Exclusion Criteria for "Fertile" and "Infertile" Groups

The inclusion criteria for both the fertile and infertile groups were regular menstrual cycle (length: 25–35 days) with less than 5 days' difference between cycles, reproductive age (18–49 years), no hirsutism, serum luteinizing hormone (LH)/follicle-stimulating hormone (FSH) ratio less than 2, and presence of both ovaries (3). The exclusion criteria for both the groups were history of hormone administration in the previous 6 months ($n_{\text{fertile}} = 41$, $n_{\text{infertile}} = 34$), pelvic inflammatory disease ($n_{\text{fertile}} = 17$, $n_{\text{infertile}} = 21$) or ovarian surgery ($n_{\text{fertile}} = 8$, $n_{\text{infertile}} = 29$), ovarian endometrioma ($n_{\text{fertile}} = 11$, $n_{\text{infertile}} = 41$), premature ovarian failure ($n_{\text{fertile}} = 9$, $n_{\text{infertile}} = 33$), uterine malformations or uterine pathology ($n_{\text{fertile}} = 35$, $n_{\text{infertile}} = 47$), known systemic, metabolic, and endocrine disease including hyperandrogenism ($n_{\text{fertile}} = 149$, $n_{\text{infertile}} = 124$; ►**Fig. 1**). Although women with the polycystic ovarian syndrome (PCOS) were excluded based on clinical and biochemical criteria, those having only polycystic ovary (PCO) morphology on ultrasound (presence of more than or equal to 12 follicles, 2–9 mm in diameter, and/or increased ovarian volume [> 10 mL]) were included.[3] Women with poor ultrasound visualization of ovaries, because of retrouterine or abnormal position ($n_{\text{fertile}} = 16$, $n_{\text{infertile}} = 19$) and the presence of at least one of cysts more than or equal to 20 mm ($n_{\text{fertile}} = 4$, $n_{\text{infertile}} = 7$), were also excluded subsequently. Finally, 1,188 fertile and 1,092 infertile females were enrolled in the study. Out of these, the number of women having age less than 20 or more than 40 years was only 7 in fertile and 9 in infertile groups. This number was too small to construct valid nomograms for that specific age, and hence, statistical analysis and development of age-specific nomograms were finally done only in 1,181 fertile and 1,083 infertile females, aged between 20 and 40 years.

Materials

All sonographic measurements were performed by using a 5 to 9 MHz transvaginal transducer (Philips C9–5ec curved array transducer with 5 to 9 MHz extended frequency range) of diagnostic ultrasound, iU22, Philips Medical System, California, United States.

Data Collection and Procedure

All the relevant clinical data of the study subjects including biometry were noted. Among the biochemical parameters, LH, FSH, triiodothyronine (T3), thyroxine (T4), thyroid stimulating hormone, total testosterone, prolactin, and anti-Mullerian hormone were documented. The transvaginal ultrasound and hormonal assays were done on the second or third day of the menstrual cycle. All sonographic measurements were performed by the same investigator. A thorough

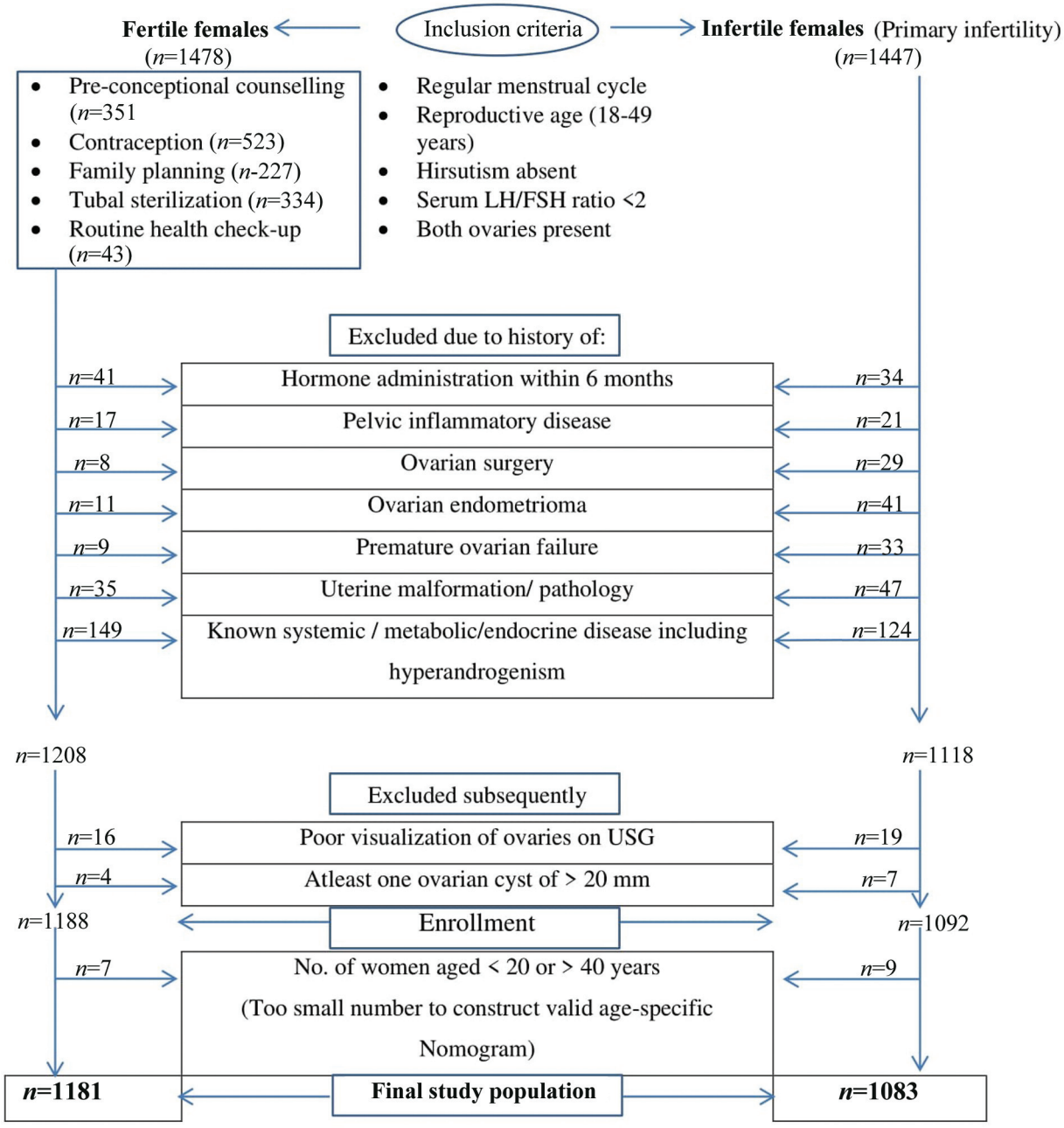

Fig. 1 Flowchart of the study population. FSH, follicle stimulating hormone; LH, luteinizing hormone.

survey of each ovary was done by scanning from the outer to the inner margin. 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) were measured and counted in each ovary.[4] The sum of both counts was labeled as the AFC. The follicular size was measured using the internal diameters of the area. The mean of two perpendicular measurements was assumed to be the follicular size.

Statistical Analysis

Descriptive statistics were calculated in form of mean ± standard deviation or median with interquartile range as per the suitability of numerical values. Student's *t*-test / Mann–Whitney U test was applied to find out the significant difference in the mean values between the groups. The chi-squared test was used to find out the significant difference between the proportions. The nomograms were built for the 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles in both the fertile and infertile groups. The Pearson correlation coefficient was calculated and regression analysis was done to find out the relationship between the study variables. All statistical analysis was done by using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, New York, United States). A *p*-value of less than 0.05 was considered statistically significant at two-tailed test.

Table 1 Clinical and biochemical parameters of fertile and infertile women

Parameters	Fertile ($n = 1,181$) Mean ± SD [a]Median (IQR)	Infertile ($n = 1,083$) Mean ± SD [a]Median (IQR)	*p*-Value
Age (years)	29.28 ± 5.47	29.72 ± 5.73	0.060
Weight (kg)	57.38 ± 4.85	57.60 ± 4.61	0.266
Height (m)	1.55 ± 0.04	1.55 ± 0.04	0.533
BMI (kg/m^2)	23.93 ± 2.48	24.05 ± 2.32	0.268
T3 (ng/dL)	111.65 ± 14.35	112.15 ± 14.23	0.405
T4 (µg/dL)	7.98 ± 1.29	7.94 ± 1.25	0.559
TSH (µIU/mL)	2.82 ± 0.82	2.82 ± 0.79	0.813
FSH (mIU/mL)[a]	5.15 ± 1.97 4.51 (3.63–6.18)	5.58 ± 2.10 4.73 (4.01–6.66)	<0.001
LH (mIU/mL)[a]	6.05 ± 1.97 5.69 (4.59–7.59)	6.41 ± 1.90 6.45 (5.26–7.89)	<0.001
LH/FSH ratio[a]	1.26 ± 0.42 1.29 (0.94–1.62)	1.25 ± 0.42 1.29 (0.93–1.59)	0.429
PRL (ng/dL)	15.26 ± 2.86	15.11 ± 2.92	0.205
AMH (ng/mL)[a]	2.78 ± 1.19 3.12 (1.95–3.81)	2.51 ± 1.25 2.81 (1.57–3.68)	<0.001
Total testosterone (ng/dL)	16.21 ± 2.81	16.28 ± 2.93	0.563
Hb (gm/dL)	12.92 ± 1.05	12.94 ± 1.05	0.728
Blood sugar fasting (mg/dL)	95.66 ± 3.04	95.41 ± 3.15	0.049
Blood sugar postprandial (mg/dL)	112.95 ± 5.43	114.15 ± 4.37	<0.001

Abbreviations: AMH, anti-Mullerian hormone; BMI, body mass index; FSH, follicle stimulating hormone; LH, luteinizing hormone; PRL, prolactin; T3, triiodothyronine; T4, thyroxine Hormone; TSH, thyroid-stimulating hormone.
[a]The standard deviations (SDs) of these parameters were quite high and did not follow the condition of normality. Therefore, each of these parameters is presented as its mean along with its median and interquartile range (IQR) within brackets. Instead of using an independent *t*-test applied for parameter satisfying normality, Mann–Whitney U test (nonparametric test) was used that tests median instead of mean.

Results

The fertile and infertile groups showed no significant difference in age (29.28 ± 5.47 vs. 29.72 ± 5.73 years, respectively; $p = 0.06$), weight (57.38 ± 4.85 vs. 57.60 ± 4.61 kg, respectively; $p = 0.266$), height (1.55 ± 0.04 vs. 1.55 ± 0.04 m, respectively; $p = 0.533$), and body mass index (23.93 ± 2.48 vs. 24.05 ± 2.32 kg/m^2, respectively; $p = 0.268$; ►**Table 1**). The mean and median (interquartile range) AFC of the fertile subjects were 13.66 ± 5.98 and 13.0 (10.0–17.0) follicles, respectively. The mean and median (interquartile range) AFC of the infertile subjects were 11.91 ± 6.02 and 11.0 (8.0–15.0) follicles, respectively, significantly lower than that of fertile women ($p < 0.001$).

The normality of data distribution was checked through the Kolmogorov–Smirnov normality test. The data analysis included log-transformed AFC values due to their skewed distribution. The trend of fall of AFC with age was linear in pattern in both fertile and infertile women. The age-wise mean AFC was lower in infertile women than in fertile women; and from the age of 28 years, the mean difference between the two groups was significant for most of the ages (►**Fig. 2**). For each

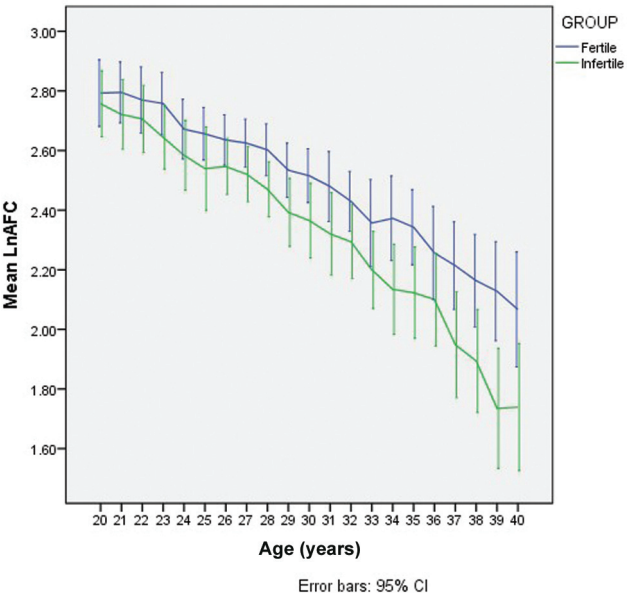

Fig. 2 Age-wise trend of mean LnAFC (log-transformed values of antral follicle count) in fertile and infertile women.

Table 2 Age-specific percentiles (3rd to 97th) for antral follicle count (AFC) in fertile women

Percentiles of AFC in fertile women								
Age (years)	No.	3rd	10th	25th	50th	75th	90th	97th
20	42	8.0	10.3	12.7	16.0	21.0	26.4	34.8
21	56	7.7	10.0	12.2	15.5	20.5	25.9	34.0
22	45	7.4	9.6	12.0	15.0	20.0	25.4	33.2
23	52	7.1	9.3	11.5	15.0	19.5	24.7	32.4
24	56	6.7	9.0	11.2	14.5	19.0	23.9	31.6
25	74	6.4	8.5	11.0	14.0	18.5	23.5	30.7
26	72	6.2	8.3	10.5	13.5	18.0	22.7	30.0
27	96	5.9	8.0	10.2	13.0	17.5	22.1	29.2
28	85	5.6	7.6	10.0	13.0	17.0	21.8	28.4
29	72	5.3	7.3	9.5	12.5	16.5	21.1	27.6
30	72	5.0	7.0	9.0	12.0	16.2	20.4	26.7
31	56	4.7	6.7	8.7	11.5	15.7	19.6	26.0
32	72	4.3	6.3	8.2	11.0	15.2	19.0	25.4
33	36	4.1	6.0	8.0	11.0	14.7	18.6	24.5
34	46	3.7	5.7	7.4	10.5	14.2	18.0	23.6
35	52	3.6	5.3	7.2	10.0	13.7	17.4	22.6
36	38	3.2	4.9	7.0	9.5	13.2	16.5	22.0
37	45	3.0	4.6	6.5	9.0	13.0	16.1	21.2
38	41	2.4	4.2	6.0	9.0	12.5	15.6	20.5
39	36	2.2	4.0	5.6	8.5	12.0	15.0	19.8
40	37	2.0	3.5	5.5	8.0	11.5	14.4	19.0

percentile, at any age, the AFC was lower in infertile women than in fertile women (►**Tables 2**, **3**; ►**Fig. 3A, B**). For all the percentiles, the AFC showed progressive linear decline with increasing age of both fertile and infertile women. The correlation between AFC and age was modest and negative in both fertile ($r = -0.431$, $p < 0.001$) and infertile ($r = -0.520$, $p < 0.001$) women. The AFC declined with age significantly ($p < 0.001$) in both the groups, but at a higher rate in infertile (0.50 follicles/year; 0.050 follicles/year using log-transformed values of AFC) than in fertile (0.44 follicles/year; 0.036 follicles/year using log-transformed values of AFC) group. Age alone explained only 16.3% variation in AFC in fertile and 22.7% variation in AFC in infertile groups.

Discussion

In this study, we developed age-specific nomograms against 3rd, 10th, 25th, 50th, 75th, 90th, and 97th percentiles for AFC, separately in fertile ($n = 1181$) and infertile ($n = 1083$) females, aged between 20 and 40 years. Almog et al[5] studied only 18 to 50 years old infertile females ($n = 1,866$) without PCO, while Loy et al[6] considered only 26 to 44 years old subfertile females with or without PCO; and both prepared nomograms against the same percentiles as ours. On the contrary, La Marca et al[7] included women from the general population ($n = 362$), excluded the infertile ones, and prepared nomograms with 5th, 25th, 50th, 75th, and 95th percentiles in the 16 to 49 years age group. Also, in the study by Bozdag et al,[1] women from the general population, aged between 20 and 50 years ($n = 381$), were included, but without considering their fertility status. They produced nomograms for the 10th, 25th, 50th, 75th, and 90th percentiles of AFC in women with regular menstrual cycles like us and also by Bozdag et al[1] and La Marca et al.[7] However, this was not taken into consideration by Almog et al.[5] and Khan et al.[8] Though the 25th, 50th, and 75th percentiles of AFC were common to all, the studies differed in the selection criteria of the study population and the sample size analyzed. This probably was guided by the variation in the prevalence and etiology of infertility and PCO across borders, justifying the need for region-specific nomograms.

Whether to include or exclude the patients with PCO morphology from such nomograms remains a matter of debate to date. Unlike us, Almog et al[5] preferred to exclude women with PCO morphology. However, much earlier, Murphy et al[9] in their longitudinal study had opined that women with regular menstrual cycles and PCO morphology were not at significant risk for the development of PCOS, and with increasing age, the PCO morphology might even become normal due to age-related decline of ovarian follicles. Moreover, Wiser et al[10] suggested that the previous threshold of more than 12 follicles for the definition of PCO might no

Table 3 Age-specific percentiles (3rd to 97th) for antral follicle count (AFC) in infertile women

Percentiles of AFC in infertile women								
Age (years)	No.	3rd	10th	25th	50th	75th	90th	97th
20	45	7.7	9.6	12.0	15.0	19.5	25.4	33.6
21	42	7.3	9.3	11.7	14.5	19.0	24.7	32.8
22	48	7.0	8.9	11.2	14.0	18.5	24.1	32.0
23	54	6.6	8.5	10.7	13.5	17.7	23.5	31.0
24	42	6.3	8.0	10.5	13.0	17.2	22.7	30.1
25	36	6.0	7.7	10.0	12.5	16.7	22.0	29.4
26	86	5.6	7.3	9.5	12.5	16.2	21.3	28.8
27	76	5.3	7.0	9.0	12.0	15.7	20.6	27.7
28	82	5.0	6.5	8.7	11.5	15.0	20.0	27.0
29	56	4.7	6.0	8.2	11.0	14.5	19.3	26.3
30	46	4.4	5.7	7.7	10.5	14.0	18.6	25.3
31	44	4.0	5.5	7.5	10.0	13.5	18.0	24.6
32	56	3.7	5.0	7.0	9.5	13.0	17.3	23.6
33	56	3.3	4.7	6.4	9.0	12.2	16.6	22.8
34	44	3.0	4.5	6.0	8.5	11.7	16.0	22.0
35	44	2.5	4.0	5.6	8.0	11.2	15.5	21.3
36	46	2.4	3.7	5.0	8.0	10.5	14.6	20.3
37	42	2.0	3.3	4.7	7.5	10.0	13.7	19.4
38	58	1.7	2.9	4.4	7.0	9.5	13.2	18.6
39	44	1.3	2.4	4.0	6.5	9.0	12.5	18.0
40	36	1.1	2.0	3.4	6.0	8.5	11.8	17.0

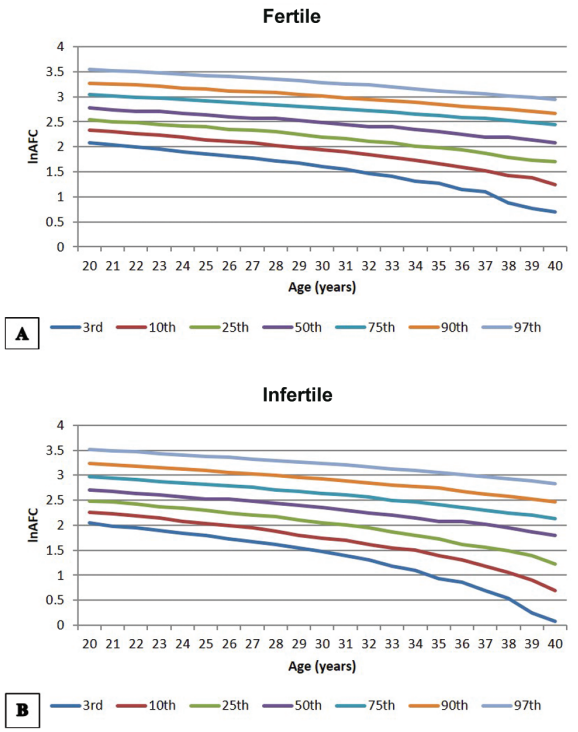

Fig. 3 Age-specific nomogram against 3rd to 97th percentiles for lnAFC (log-transformed values of antral follicle count) in fertile (**A**) and infertile (**B**) women.

longer be valid in the era of advanced ultrasound technology having higher sensitivity for follicular detection and suggested the need for a higher cutoff. Bozdag et al[1] also opined that for the definition of PCO morphology, AFC higher than the 95th percentile for the given age might be preferred over some constant threshold applicable to all ages. Therefore, the mere existence of PCO morphology in fertile or infertile women was not considered as the exclusion criterion in our study, as long as the clinical and biochemical parameters remained normal.[3]

Although AFC is used as a direct quantitative marker of ovarian reserve globally,[1] its pattern and rate of decline with increasing age are still controversial. Further, the decline in AFC whether linear or biphasic also remains debatable. In this study, the linear pattern of decline without any switching was noted against all the percentiles in both fertile and infertile women. Similarly, Loy et al[6] and La Marca et al[7] also found that the best fit to the AFC was given by the linear model, against the 3rd to 97th and 5th to 95th percentiles, respectively. Bozdag et al[1] found the decline to be obvious after 35 years of age. Previous studies, however, showed an accelerated decline in oocytes after 37[11] or 43 years.[12] Wiweko et al[13] and Almog et al.[5] also noticed the linear biphasic correlation between AFC and age. Nevertheless, there were differences even between them. While the former got a similar decreasing pattern against all the percentiles

(3rd, 10th, 25th, 50th, 75th, 90th, and 97th) with similar switching age of 33 years, the latter observed a different switching age for each percentile, with the lower percentiles (3rd, 10th, 25th) having high follicle loss rate preceding the slow loss rate, while the higher percentiles (75th, 90th, and 97th) having slow follicle loss rate preceding the high one. To add to this, Faddy et al[14] had earlier found that the AFC declined biexponentially rather than as a single exponential function of age. These differences in opinion and observations only highlighted the lack of complete knowledge regarding the factors influencing follicle loss.

In our study, the rate of loss of follicles occurred at a higher rate in infertile (0.50 follicles/year; 0.050 follicles/year using log-transformed values of AFC) than in fertile (0.44 follicles/year; 0.036 follicles/year using log transformed values of AFC) females. The latter decline rate was nearly similar to that of 0.4 follicles/year in the fertile population and 0.41 follicles/year in the general population, as observed by La Marca et al[7] and Bozdag et al,[1] respectively. However, Almog et al,[5] who studied only the infertile population, observed a lower decline rate of 0.4 follicles/year, while Loy et al[6] observed a much higher decline rate of 0.79 follicles/year in subfertile females. The latter also agreed that there was a constant loss of follicles with age and raised concern about any dramatic change in the treatment strategy of infertility in a woman, after a certain threshold of age.

Further, we found that AFC showed a modest negative correlation with age ($r = -0.431, p < 0.001$) in fertile women. However, in a longitudinal study conducted by van Rooij et al[15] on 81 healthy women at a mean time interval of 4 years, AFC was noted to have a strong negative correlation with age at both the visits ($r = -0.74$, $p \leq 0.001$ at visit 1 and $r = -0.79$, $p \leq 0.001$ at visit 2). This difference might be due to the smaller sample size studied by these authors and different criteria of selection, that is, also the exclusion of subjects with polycystic ovarian morphology on ultrasound. In our study, there was a modest negative correlation of AFC with age ($r = -0.520, p < 0.001$) in infertile women, similar to that observed by Barbakadze et al[16] ($r = -0.55$, $p < 0.0001$) and Göksedef et al[17] ($r = -0.40$, $p < 0.0001$). However, Scheffer et al[18] noted a relatively weaker negative correlation between the two parameters ($r = -0.34$, $p < 0.00001$). Nevertheless, these observations unanimously indicate the decline of AFC with increasing age in infertile women.

On multivariate analysis in this study, age was responsible for only 16.3% variation in AFC in the fertile group and 22.7% variation in the infertile group. However, it varied from 12 to 37% in fertile population in other studies.[7,19] Loy et al[6] claimed that age explained much lower, that is, only 10% decline in AFC in the subfertile population. The variation in these figures might be due to the technical limitations involved in counting the number of antral follicles. At the same time, two important inferences can be drawn from these observations. First, there might be an interplay of many known and unknown genetic, ethnic, nutritional, socioeconomic, and environmental factors with age, influencing the number and loss of follicle pool that need to be identified, included and analyzed in future studies. Iglesias et al[20] suggested ethnic differences in ovarian aging and noted the same to be 6 years earlier in Indian women than in Spanish women. Second, it reiterates the fact that the antral follicles are the markers of biological age, rather than the chronological age of the ovary,[8] and the two may not necessarily correspond to each other.[13,14] Applying a single cutoff of AFC less than 7 as proposed by others[21] to all the women in our study, irrespective of their age, we found that the sensitivity for the detection of the poor ovarian reserve was merely 17.1%, though specificity was 90.9%. This further reiterates the need for regionally developed age-specific nomograms for AFC in infertility counselling, rather than using age-independent cutoffs indiscriminately. Further, the presence of AFC less than 10th percentile for that age can be used to define "poor ovarian reserve," while AFC less than 3rd percentile for that age can be used to define "very poor ovarian reserve." At the same time, we strongly recommend that the AFC cutoff should not be used as a stand-alone criterion to start aggressive and costly treatment like assisted reproductive technique, due to fear of the rapid loss of follicles in infertile women.

Saxena et al[22] in their review remarked that out of all ovarian reserve tests, AFC should be used as a reliable marker to assess the sensitivity and success rate of the woman undergoing ovarian stimulation, although it could not predict future fertility or even the timing of the decline or cessation of the future fertility. However, many researchers[7,23] were of the opposite view and claimed that the marking of the current AFC of a woman across the nomogram would help in the prediction of her total fertility potential. This held true not only for assisted reproductive techniques but also for the chronologic age-related phenomena like the ease of natural conception, trisomy conception, pregnancy outcome, and time until menopause. However, they finally concluded that a comprehensive approach based on age-specific thresholds of AFC and clinical conditions could be used to predict the reproductive potential of a woman in the future.

Limitations of the Study

Although the power of our study was 90%, it had some limitations. *First*, it was a single-center, tertiary hospital-based study, done in a specific region of the country and so the results could not be generalized at the community level. *Second*, due to the cross-sectional study design, the subjects could not be followed up to document the actual trend of loss of follicles or decline of ovarian reserve with age. For this, a dedicated longitudinal study design will be required. *Third*, a distinction between obese and nonobese subjects was not done, although there was no significant difference in body mass index between the fertile and infertile population in the study. *Fourth*, the antral follicles were not divided based on size into small, intermediate, and large,[19,24] and their correlation with age was not analyzed separately. *Last*, no actual intervention in form of ovarian stimulation was performed to assess the ovarian response based on AFC in either the fertile or infertile group.

Conclusion

This study showed a steady fall in AFC in both fertile and infertile women, though more rapid in the latter group. These age-specific nomograms for AFC should be used as a reference guide in the current modern era of assisted reproductive technology and artificial intelligence, to find out the present status of ovarian reserve on an individual basis. Thus, the incorporation of a comprehensive approach based on age-specific AFC nomograms, along with relevant clinical and biochemical parameters into the infertility protocol, will not only reduce the unnecessary stress on the patient but also improve the standard of care to such patients. Further studies with longitudinal study designs and larger sample sizes are still required for the final validation of our age-specific nomograms for AFC.

Note
The authors would like to declare that they have published the manuscript, using part of the data of this paper. However, both studies are not entirely the same.

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Conflict of Interest
None declared.

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