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Assessment of Ovarian Volume and Antral Follicular Count among Normal and Infertile Women

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ABSTRACT

Background: Infertility is well-defined as the inability to conceive after one year of unprotected intercourse. Primary infertility refers to women unable to accomplish a clinical pregnancy, and secondary infertility applies to those who have previously conceived. Globally, over 186 million people are affected, with varying prevalence rates across regions. The Western Pacific has the highest rate at 23.2%, while the Eastern Mediterranean has the lowest at 10.7%. The study aimed to assess the significance of Antral Follicle Count (AFC) as a marker of ovarian reserve in fertile and infertile women.

Methods: This prospective observational study, conducted at Narayana Medical College, Andhra Pradesh, over two years, involved 100 participants: 50 women with primary infertility and 50 fertile controls. The primary objective was to evaluate the role of antral follicle count (AFC) as an ovarian reserve marker.

Results: The mean age was similar (29.44±2.18 vs. 28.82±2.23 years, p=0.43), and no significant differences were found in BMI (p values ranging from 0.63 to 1) or menstrual cycle regularity (p=0.69). However, the infertile group had a significantly longer duration of marriage (7.20±2.89 vs. 5.70±2.21 years, p=0.0001). The infertile group also had a significantly lower antral follicle count (7.18±1.57 vs. 11.92±2.28, p<0.0001), but no significant difference in ovarian volume (p=0.465).

Conclusion: This study concluded that AFC is a reliable indicator of fertility in women and serves as an effective marker of ovarian reserve in Indian women of childbearing age.

Key-words: Infertility, Ovarian volume, Antral follicle count, Primary infertility, Fertility marker

INTRODUCTION

Infertility is the inability of a couple to conceive naturally after one year of unprotected intercourse (Abdelazim). Fertility is the natural ability to conceive and achieve a clinical pregnancy. Infertility occurs when a couple is unable to establish a clinical pregnancy despite having regular, unprotected intercourse for 12 months.

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This inability to conceive within one year defines infertility. The term sub-fertility is often used interchangeably with infertility, while sterility refers to a permanent state of infertility [1]. Infertility is categorized as primary or secondary in both males and females. In women, primary infertility means they have not been able to achieve a clinical pregnancy even after meeting the necessary diagnostic criteria. Secondary infertility refers to women who have had at least one prior pregnancy but are now unable to conceive. A similar classification applies to men based on their role in initiating pregnancy.

Globally, over 186 million people experience infertility, with the majority residing in developing countries [2]. One

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of the most significant negative predictors of fertility is increasing maternal age at conception [3]. Reproductive aging results from a decline in both the quantity and quality of the ovarian follicle pool [4].

Fig. 1 shows the primary infertility prevalence across various regions reveals notable variations. The Western Pacific region exhibits the highest prevalence at 23.2%, followed closely by the Region of the Americas at 20%. In comparison, the European region has a moderate prevalence rate of 16.5%. The African region shows a relatively lower rate of 13.1%, while the Eastern Mediterranean region has the lowest prevalence at 10.7%. The global average for primary infertility is estimated to be 9.6%, suggesting that while infertility affects a significant portion of the population globally, the impact varies by region, with certain regions experiencing notably higher rates. These variations could reflect differences in healthcare access, societal factors, and regional health conditions.

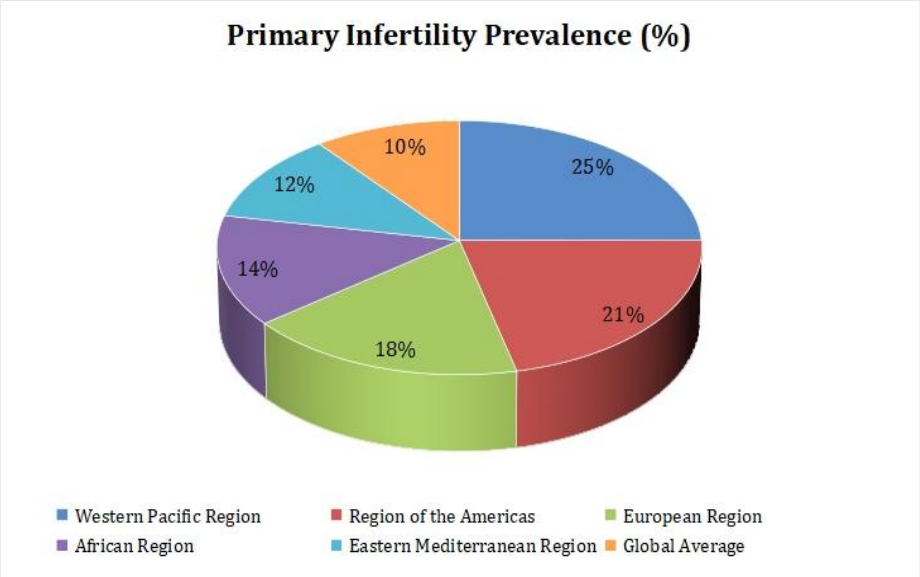

Fig. 1: Prevalence of Primary Infertility [Source: WHO, 2025]

Autopsy studies of human ovaries indicated a rapid decline in follicle numbers with age that begins from the fetal stages and continues beyond menopause. Therefore, ovarian health is a key factor in reproductive well-being and should be routinely evaluated using markers like follicle-stimulating hormone (FSH), anti-Mullerian hormone (AMH), antral follicle count (AFC), and ovarian volume [5]. Real-time transvaginal ultrasonography is a widely used, accurate, and reliable method for determining antral follicle count and total ovarian volume [6]. AFC is measured by counting the number of ovarian follicles between 2 mm and 10 mm in both ovaries. This count reflects the remaining primordial follicular pool and correlates with chronological age in healthy women [9]. Different studies have proposed different approaches, however Chang *et al.,* suggested that instead of counting follicles measuring 2-5 mm or 4-6 mm in diameter that strongly correlate only with the total number of follicles measuring 2–10 mm, all follicles within the 2–10 mm range should be preferred and is considered the most practical approach in clinical settings. The size and activity of the human ovary fluctuate throughout life. Ovarian antral follicles can be visualized and counted using transvaginal ultrasound (US) [10]. Since no definitive tests are available to evaluate the true ovarian reserve, AFC is widely accepted as a reliable surrogate marker[11]. AFC is commonly assessed in women of reproductive age for various reasons, including infertility assessment, success of assisted reproduction technology (ART), work-ups, prediction of menopause risk Martins WP 2014) and the identification of ovulatory dysfunction secondary to hyperandrogenism anovulation [12]. Fig. 2 summarizes the potential applications of AFC in reproductive health. Although AFC is a non-invasive and easily performed technique of ovarian reserve, transvaginal ultrasound-based follicle counting lacks complete standardization. Therefore, the outcomes may vary between observers due to differences in ultrasound equipment and operator technique [13].

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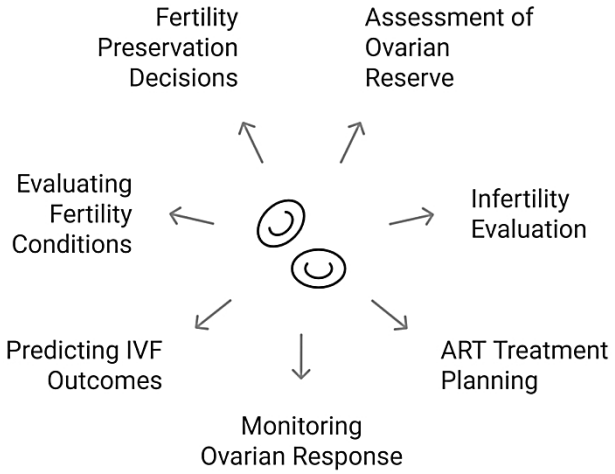

Fig. 2: Role of AFC assessment in different aspects of reproductive health

MATERIALS AND METHODS

Study Design and Setting- The prospective observational study was conducted in the Department of Obstetrics & Gynecology at Narayana Medical College and Hospital, Chintareddypalem, Nellore, Andhra Pradesh, over two years. The study included 100 participants, comprising 50 women diagnosed with primary infertility and 50 fertile controls. Institutional Ethical clearance was obtained before the study commenced, and informed consent was obtained from all participants. Fig. 3 provides an outline of the methodology followed in this study. A study was conducted in the Department of Obstetrics & Gynecology, Narayana Medical College, and Hospital, Chintareddypalem, Nellore, Andhra Pradesh, among 50 primary infertility cases and 50 Controls (normal), over two years.

Inclusion and exclusion criteria- The inclusion criteria for cases that were women diagnosed with primary infertility included aged of 26-35 years, who underwent transvaginal ultrasonography (TVUS) for primary infertility assessment. Cases with uterine malformations or uterine pathology or any history of ovarian surgery were excluded. For controls, that is, fertile women, the inclusion criteria for age were 26-35 years with proven natural fertility, followed regular menstrual cycles, without any evidence of endocrine disorders or history of ovarian surgery. Women under hormonal contraception within three months before study enrollment or who had a history of ovulation induction in the past three months were excluded from the study.

Data Collection- Fifty infertile women were compared with an equal number of fertile controls. Before participation, all subjects provided written informed consent. A comprehensive medical history was obtained, and demographic and clinical information was gathered using a structured questionnaire. A comprehensive general and clinical examination was performed for each participant. Endovaginal measurements of AFC and basal ovarian volume were taken. AFC was assessed on day 3 of the menstrual cycle using TVUS. The number of small antral follicles (2-10 mm in diameter) in both ovaries was recorded. Ovarian size and volume were also measured using ultrasound, with ovarian volume calculated using the ellipsoid formula.

$$Ovarian\ Volume = D1 \times D2 \times D3 \times 0.52$$

where D1, D2, and D3 represent the three axial measurements. The mean ovarian volume for non-menopausal women is typically 6-7 mL (Cohen). Ultrasound findings and relevant clinical data were documented for each patient.

Statistical Analysis- The data were recorded in Microsoft Excel and analyzed with SPSS Version 22 (IBM SPSS Statistics, Somers, NY, USA). Categorical data were summarized as frequencies and percentages, and the Chi-square test was used to determine statistical significance. Continuous data were reported as mean±standard deviation (SD). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess the normality of continuous variables. To compare group means, an independent t-test was conducted. A p-value less than 0.05 was considered statistically significant, assuming all test assumptions were met.

Ethical Consideration- Ethical approval from the institution was secured before the study began. All participants provided informed consent before their enrollment. Throughout the study and follow-up period, patients received standard care in compliance with ethical standards.

RESULTS

A comparison was made between the demographic characteristics of the infertile and fertile groups. The mean age of the infertile group was 29.44±2.18 years, while that of the fertile group was 28.82±2.23 years. The

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difference between the groups was not statistically significant, as indicated by a p-value of 0.43. In terms of age distribution, 70% of the infertile group and 72% of the normal group were aged 26-30 years, and 30% of the infertile group and 28% of the normal group were aged 31-35 years. The p-value for both age categories was 0.826, showing no significant difference. BMI classification revealed that the majority of both groups had a normal BMI (88% in the infertile group and 90% in the normal group), with only minor differences in underweight and overweight individuals. The p-values for BMI categories were 0.63 for underweight, 0.63 for normal, and 1 for overweight, indicating no significant differences. However, the duration of marriage was significantly longer in the infertile group (7.20±2.89 years) compared to the normal group (5.70±2.21 years), with a p-value of 0.0001. Both groups had a majority of individuals with regular menstrual cycles (90% infertile, 94% normal), and no significant difference was observed (p=0.69). The duration of infertility was recorded as 5.74±3.01 years in the infertile group, but this measure did not apply to the normal group. Lastly, the infertile group had no previous pregnancies (0%), while all members of the normal group had at least one previous pregnancy (100%), with a highly significant p-value of <0.0001 (Table 1).

Table 1: Demographic characteristics of the patients between the primary infertility and fertile groups

Parameter	Infertile Group (n=50)	Normal Group (n=50)	p-value
Age (mean±SD)	29.44±2.18	28.82±2.23	0.43
Age Group (Years)			
- 26-30 years	35 (70%)	36 (72%)	0.826
- 31-35 years	15 (30%)	14 (28%)	0.826
BMI Classification			
- Underweight (BMI <18.5)	1 (2%)	0 (0%)	0.63
- Normal BMI (18.5–24.9)	44 (88%)	45 (90%)	0.63
- Overweight (BMI ≥25)	5 (10%)	5 (10%)	1
Duration of Marriage (Years)	7.20±2.89	5.70±2.21	0.0001
Menstrual Cycle Regularity			
- Regular	45 (90%)	47 (94%)	0.69
- Irregular	5 (10%)	3 (6%)	0.69
Duration of Infertility (Years)	5.74±3.01	N/A	N/A
Parity (No. of Previous Pregnancies)	0 (0%)	50 (100%)	<0.0001

While taking the duration of marriage into context, in 22% of the infertility group subjects, the duration of marriage <5 years, in 68% it was 6 to 10 years, whereas in the rest 10% subjects it was >10 years. However, in the fertile group, 48% subjects had <5 years, another 48% had 6 to 10 years 48% and in the rest 4% a duration of marriage was >10 years. In addition to the observed significant difference in duration of marriage between the two groups, the mean duration of marriage was significantly higher in the infertile group (7.20±2.89 years) compared to the normal group (5.70±2.21 years). Regarding the BMI classification, 2% of the participants in the infertile group were underweight, 88% had normal BMI and 10% were overweight. In the fertile group, 90% of individuals had a normal BMI, while 10% were classified as overweight. The distribution of BMI did not show a significant difference between the two groups.

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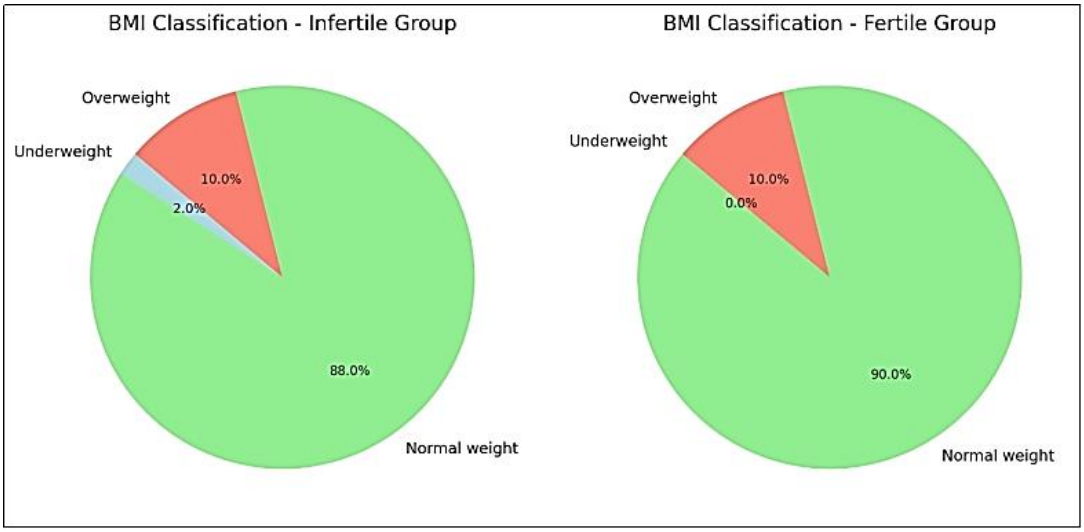

Fig. 3: BMI classification of the participants between the fertile and infertile groups

The mean AFC was significantly lower (p<0.0001) in the infertile group (7.18±1.57) compared to the fertile group (11.92±2.28). However, the mean ovarian volume did not differ significantly between the two groups (infertile: 11.13±1.45 ml, normal: 11.33±1.22 ml, p=0.465The mean duration of infertility in the infertile group was 5.74±3.01years, with 54% of cases having infertility for <5 years, 40% for 6–10 years, and 6% for >10 years.

We found that the infertile group had significantly lower mean AFC (7.18±1.57) compared to the normal group (11.92±2.28), with a highly significant p-value (<0.0001). This is highly suggestive of the fact that women with infertility have a reduced ovarian reserve, which may be contributing to their difficulty in conceiving. In the case of the infertile group, the mean ovarian volume was slightly lower (11.13±1.45 ml) than in the fertile group (11.33±1.22 ml). Still, this difference was not statistically significant (p=0.465) (Fig. 4). This suggests that ovarian volume alone might not be a definitive marker for infertility in this population (Table 2).

Table 2: Comparison of ovarian volume and AFC between the fertile and infertile groups

Parameter	Infertile (n=50) (Mean±SD)	Normal (n=50) (Mean±SD)	p-value
Ovarian Volume (ml)	11.13±1.45	11.33±1.22	0.465
Antral Follicle Count	7.18±1.57	11.92±2.28	<0.0001

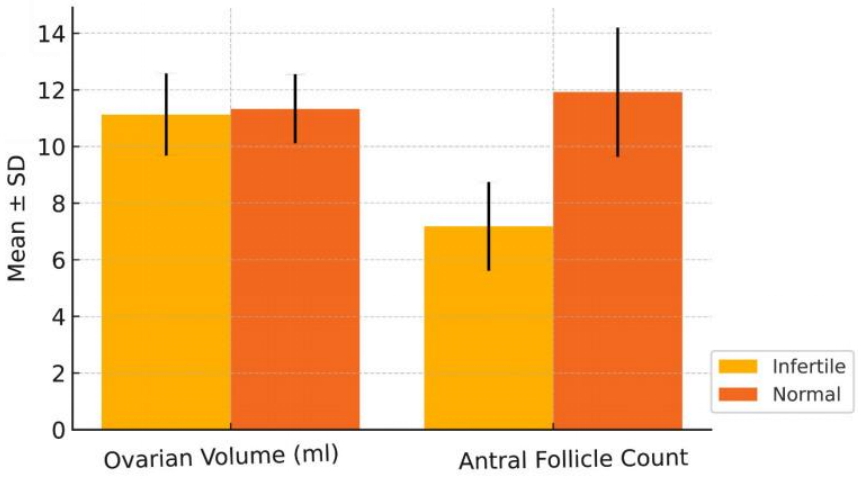

Fig. 4: Differences between the mean values of Ovarian volume and AFC among fertile and infertile groups

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DISCUSSION

The primary objective of this study was to establish the role of AFC as a marker of ovarian reserve function in fertile and infertile women, determine baseline AFC cutoff values, and compare ovarian volume between these groups. AFC is widely assessed via transvaginal ultrasound US and has been accepted as a reliable surrogate marker of ovarian reserve [14]. Since no test can directly assess ovarian reserve, Cremains a key parameter in evaluating the reproductive potential [15]. Despite the advantages of AFCs in determining reproductive health, AFC measurements can vary due to differences in observer experience, ultrasound equipment, and lack of standardization [16].

In addition to AFC, AMH that is secreted by granulosa cells of antral and preantral follicles has also been used as an ovarian reserve marker [17]. Although AMH and AFC show comparable accuracy in predicting ovarian reserve and response to stimulation. But AMH has limitations, such as a lack of international standardization and higher costs [18]. While AMH has proved useful in detecting the diminished ovarian reserve, it does not provide additional information about ovarian morphology, such as the presence of endometriomas or tubal pathologies like hydrosalpinx [19].

In this study, we observed that the mean age of participants was similar between the infertile (29.44±2.19 years) and normal (28.82±2.23 years) groups, with no statistically significant difference. This aligns with the previous published studies [20-23]. However, we observed significant differences in the duration of marriage, with infertile women having longer durations (7.20±2.89 years) compared to fertile women (5.70±2.21 years. In consistency with some of the previous studies, we also found no significant difference in BMI between the two groups, where the infertile group had a mean BMI of 22.38±2.45 compared to 22.19±2.29 in fertile women [24-26]. Meanwhile, the mean ovarian volume was also similar between groups, suggesting that ovarian volume alone might not be a strong predictor of infertility [25].

However, we observed a significant difference in mean AFC, where the infertile women had a mean AFC of 7.18±1.57, and fertile women had 11.92±2.28. This confirms findings from previous research, where lower AFC was associated with infertility [26]. An AFC threshold of ≤9 showed high sensitivity (100%) and specificity (86%) in differentiating infertile from fertile women, which supports its use as a diagnostic marker [27]. This difference is significant, particularly because variation in AFC between Indian and Western populations may be attributed to racial, socioeconomic, and environmental factors. There is ample research evidence suggesting that ovarian reserve parameters differ across ethnic groups, which warrants emphasis on the need for region-specific AFC reference values [28]. Additionally, we found a significant negative correlation between AFC and age which indicates an age-associated decline in the ovarian reserves. A similar trend has been reported in previous studies that reinforces the role of AFC as a reliable marker of reproductive aging [29].

CONCLUSIONS

This study confirms that AFC is a reliable indicator of fertility in women and serves as an effective marker of ovarian reserve in Indian women of childbearing age. Indian women exhibit a lower mean AFC value compared to Western populations, which necessitates the standardization of region-specific cutoff values. Standardizing AFC measurements and improving ultrasound techniques will enhance diagnostic accuracy and clinical utility. An AFC of ≤9 demonstrated high validity in distinguishing the infertile group from fertile women by exhibiting the highest sensitivity, specificity, positive predictive value, and negative predictive value. Given its diagnostic accuracy, AFC can be effectively utilized as a screening tool to differentiate fertile and infertile women.

CONTRIBUTION OF AUTHORS

Research concept – Dr. P. Tejeswini, Dr. M. Abhigna
Research design – Dr. P. Tejeswini, Dr. Sindhu Bhargavi
Supervision – Dr. P. Tejeswini
Materials – Dr. M. Abhigna, Dr. Sindhu Bhargavi
Data collection – Dr. M. Abhigna, Dr. Sindhu Bhargavi
Data analysis and interpretation – Dr. P. Tejeswini
Literature search – Dr. M. Abhigna, Dr. Sindhu Bhargavi
Writing article – Dr. M. Abhigna, Dr. Sindhu Bhargavi
Critical review – Dr. P. Tejeswini
Article editing – Dr. Sindhu Bhargavi, Dr. M. Abhigna
Final approval – Dr. P. Tejeswini

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