Original Article

Predictive Accuracy of Antral Follicle Count (AFC) in the Assessment of Ovarian Reserve by Comparing it with Serum Basal FSH level in Women with Subfertility

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Abstract

Objective: This study aimed to assess the predictive accuracy of Antral Follicle Count (AFC) in evaluating ovarian reserve compared to serum basal FSH levels in women with subfertility.

Methodology: A cross-sectional study conducted at the Benazir Bhutto Hospital's Out-patient department of Obstetrics and Gynaecology from October 1, 2019, to March 30, 2020, included 93 women aged 18-48 with subfertility. Exclusions were made for those with a history of ovarian surgery, chemo or radiotherapy, and unwillingness to provide consent. Patient evaluation considered age, subfertility duration, menstrual cycle length, and BMI. Basal serum FSH levels were measured on day 3 of the menstrual cycle, and transvaginal ultrasound was used to determine antral follicle counts.

Results: Among participants, 42.1% were ≤ 30 years old, and 57.9% were > 30 years old. 71% had a BMI ≤ 27, and 29% had a BMI > 27. Cycle lengths of >21 days were observed in 9.7% of patients, while 49.7% had cycle lengths of 21-38 days, and 40% had > 38 days. High FSH levels were found in 64.8% of patients, and 35.2% had low FSH levels. In terms of AFC, 81.4% had low counts, while 18.6% had high counts.

Conclusion: Diminished ovarian reserve (DOR) prediction should consider multiple factors, including age, BMI, menstrual cycle length, and biomarkers such as AFC and basal FSH levels. A comprehensive assessment of these factors is essential for accurate ovarian reserve prediction in subfertile women.

Keywords: Basal FSH, antral follicle count, subfertility

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Introduction

Ovarian reserve measures the remaining number of oocytes in the ovaries, which declines due to factors such as age, genetics, and environmental influences.[1]

The maximum number of oocytes, around 6-7 million, is present at 20 weeks of gestation in the female fetus. This number decreases to about 1-2 million at birth, further dropping to approximately 300,000-500,000 at puberty and eventually to around 1000 at the age of 51. Female fertility begins to gradually decline after the age of 30 and accelerates in the mid- to late-30s. This decline is marked by both a decrease in egg quality and quantity.[2]

The success of ovulation induction and subsequent pregnancy relies on ovarian reserve. While there isn't a single best method for predicting ovarian reserve, Antral Follicle Count (AFC) and basal FSH are commonly used in fertility centers.[3] Early follicular phase FSH levels have often been used as a predictor of ovarian reserve. As the follicular pool diminishes with age, FSH production

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increases due to reduced inhibition from estradiol, indicating poor ovarian reserve. [4] However, FSH levels can vary significantly between cycles.

A 2020 study in Birmingham found that FSH levels on days 2, 3, and 4 of the menstrual cycle were elevated in women with decreased ovarian reserve. Elevated serum basal FSH levels are specific but not very sensitive for detecting decreased ovarian reserve.[5]

On the other hand, AFC is performed on day 3 of the menstrual cycle, is non-invasive, and can be easily conducted. A study in India showed that 60% of subfertile females had an AFC of ≤10, while 40% had an AFC > 10. However, AFC's reliability as an ovarian reserve marker can be affected by inter-observer variation.

Serum basal FSH is an invasive and expensive test that requires venipuncture, whereas AFC is non-invasive and easily performed with the necessary expertise. While the literature often compares FSH with Anti-Mullerian Hormone (AMH) as a predictor of ovarian reserve, limited data is available comparing FSH with AFC as predictive markers.

The aim of this study is to assess the accuracy of antral follicle count in predicting ovarian reserve by comparing it with serum basal FSH levels in women with subfertility.

Methodology

After obtaining permission from the Ethical Review Board of the institution and securing written informed consent, a total of 93 women aged 18-48, who presented with subfertility, were enrolled in the study. Those with a prior history of ovarian surgery, a history of chemotherapy or radiotherapy for any reason, and individuals unwilling to provide consent were excluded from the study.

All subfertile women underwent a comprehensive evaluation, with a particular focus on the patient's age, duration of subfertility, length of the menstrual cycle, and BMI. Basal serum FSH levels were measured on day 3 of the regular menstrual cycle by collecting 2 ml of blood in a serum vial, which was then sent to the hospital's pathology lab on the same day. FSH levels of ≤10mIU/ml were considered "normal," while levels >10mIU/ml were categorized as "high."

Transvaginal ultrasound was performed by a consultant gynecologist to determine the Antral Follicle Count (AFC) on day 3 of the menstrual cycle. The TVS for AFC was conducted with the patient in a dorsal position and an empty bladder. The operator scanned the ovary in both longitudinal and coronal planes. All follicles measuring 2–10 mm in diameter were identified and counted from one ovarian margin to another (Figure 1). For round follicles, only one measurement was required, while for oval follicles, the mean of two diameters was calculated (the greatest diameter and the greatest diameter perpendicular to it). The number of follicles measuring <2 mm or >10 mm was subtracted from the total number of identifiable follicles. In cases of uncertainty about the count, ultrasound measurements were repeated in the other scanning plane. A similar procedure was performed in the contralateral ovary. Antral follicle count (AFC) of ≤4 was considered "low," and >4 was categorized as "normal." Subsequently, the AFC was correlated with the FSH levels in the same patient.

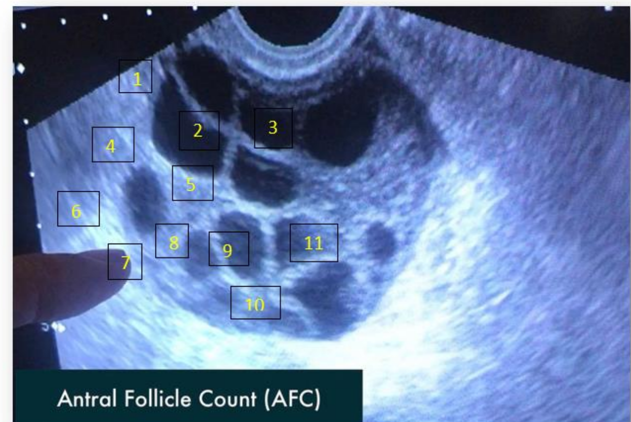

Figure 1: TVS image of ovary showing antral follicle count (AFC)

Data was analysed using SPSS version 26. Mean ± SD, was presented for quantitative variables like day 3 menstrual cycles serum basal FSH level, age, BMI, length of menstrual cycle, duration of subfertility. Frequency and percentage was calculated for both quantitative and qualitative variables like high FSH, low AFC. Post stratification chi-square test was applied, p value of ≤0.05 was considered statistically significant.

Results

In this study, total 93 patients were included the mean age of patients was 31.3 ±5.8 , mean BMI of patients was 24.7±3.1 kg/m2 (min 20 - max 30kg/m2), the mean length of menstrual cycle of patients was 32.6 ±8.4 days (min 18-max 45 days). Similarly, the mean subfertility duration of patients was 4.4 ± 2.6 (min 1.5-max 13 years), the mean FSH of patients was 12.9 ± 5.7 (min 6-

max 21) and the mean AFC of patients was 3.4 ± 1.3 (min 2 – max 10) in total 93 people.

Almost half of the patients were more than 30 years old. According to the world bank data 2017 the average age of marriage in Pakistan is 23.3 years. It means that it took almost 7 to 8 years before they presented for treatment of subfertility. It can been seen that almost 3/4 of the patients had subfertility of less than 5 years. Half of the patients had normal menstrual cycle length (21 - 35 days). It is evident that one third of the patients had BMI of >25kg/m2 while rest of them had normal BMI. 57 percent of the patients had AFC of more than 4. FSH was more than 10 mIU/ml in 2/3 of the patients.

Table I shows the positive correlation between serum basal FSH level and duration of subfertility. Table II shows significant negative correlation between AFC and serum basal FSH level. It can be observed that almost half of the patients with low AFC (n=33/70) had normal menstrual cycle length. It can be interpreted from Table III that level of serum basal FSH increases and the AFC decreases as female age advances reflecting the decline in the ovarian reserve with age. Table IV shows that patients with high BMI had FSH level of more than 10 mIU/ml which shows the decline in ovarian reserve with obesity but the results are not significant.

Discussion

Ovarian reserve plays a crucial role in assessing subfertility treatment outcomes. This study focuses on using Antral Follicle Count (AFC) as an indicator of ovarian reserve and compares it to serum basal FSH levels, examining its relationship with age, BMI, subfertility duration, and menstrual cycle length.

Table IV: Correlation of AFC and serum basal FSH with BMI

	BMI ≤25 kg/m2	BMI > 25kg/m2	p value
AFC≤4	51	19	0.484
AFC >4	15	08	
FSH >10mIU/ml	41	21	0.146
FSH ≤10mIU/ml	24	07	

Our study found that as age increases, AFC decreases, and FSH levels rise. A 2019 study in Pakistan demonstrated a 4.5 percent annual decline in AFC with increasing age, indicating reduced ovarian reserve as women grow older.[7] Similarly, a 2022 UK study revealed higher intra-follicular FSH levels and reduced oocyte quality with advancing maternal age, reinforcing the connection between AFC, FSH, and age.[8]

However, our study did not identify significant correlations between menstrual cycle length and BMI with AFC and FSH levels. In contrast, a 2023 study in India established a strong relationship between FSH levels, AMH, and physical factors like age and BMI.[9] Discrepancies in results could stem from differences in sample sizes and inclusion criteria.

Notably, our study showed that 29 percent of subfertile patients had a BMI >25 kg/m^2, linking obesity to female subfertility.[10] Obesity is considered an independent risk factor for subfertility, with overweight women experiencing subfertility three times more frequently than those with a normal weight. Follicular fluid contains high levels of oleic acid in patients with high BMI and this can be resulted in embryo fragmentation. When an embryo is in the blastomere stage, chemicals such as stearic acid can lead to poor blastomere score.[11]

Regarding menstrual cycle length, our findings indicated that AFC and FSH values for normal cycle lengths were similar to those with cycles longer than 35 days. A 2022 study in Brazil confirmed the associations between ovarian reserve, AFC, AMH, and menstrual cycle length, emphasizing AMH's significant role.[12]

Table I: Correlation of AFC and serum basal FSH with duration of subfertility

Correlation of AFC and duration of subfertility					
	≤ 2 yrs	2.5 – 5 yrs	5.5 – 10 yrs	>10 yrs	p-value
AFC<4	12	37	17	04	0.300
AFC>4	08	11	03	01	
Correlation of serum basal FSH levels with the duration of subfertility					
FSH ≤10mIU/ml	14	15	01	00	0.000
FSH >10mIU/ml	06	33	19	04	

Table II Correlation of AFC with serum basal FSH

	AFC >4	AFC <4	Total
FSH >10 mIU/ml	55	7	62
FSH ≤10 mIU/ml	14	17	21
Total	69	24	93
p- value	0.000		

Table III: Correlation of AFC and serum basal FSH with Age group.

Age Groups	AFC <4	AFC >4	
AFC ≤4	29	41	p value 0. 047
AFC>4	15	08	
FSH>10mIU/ml	21	41	p value 0.000
FSH≤10mIU/ml	23	08	

Taking into account the duration of subfertility it could be seen that 77 percent of patients presented had subfertility of less than 5 years. These results are comparable to a study done in Pakistan in 2022 that showed that the duration of subfertility was <5 years in 63 percent of patients and in 37 percent of cases, duration of subfertility was ≥5 years.[13]

When considering AFC as a predictive parameter for subfertility, our study suggests it is most reliable in patients older than 30 years. In younger patients, AFC did not significantly correlate with subfertility duration, consistent with the results of Barbakadze and colleagues. They also emphasized that AFC alone is not a dependable predictor of ovarian reserve; it should be used in conjunction with age and other markers like AMH.[14] A 2021 Chinese meta-analysis supported this, showing that combining AFC <5 with age >38 years had higher predictive accuracy than AFC alone.[15]

In summary, the results from various studies, including our own, highlight the significance of FSH levels and age as more reliable markers of ovarian reserve compared to AFC.[16] AFC's clinical predictability is limited due to its high variability and lack of sensitivity.[17]

Limitations:

This study was conducted in one hospital with limited sample size. Transvaginal ultrasound were performed by different consultant gynecologists so there may be inter observer variation in calculation of AFC. Being a tertiary care public sector hospital, majority of the patients who presented, already have taken multiple cycles of ovulation inductions without any monitoring in the private setups. These irregular and unmonitored treatments may affect AFC.

Future research:

The future study can be conducted in multi-centers (like public and private) with a better sample size. Involvement of one dedicated consultant radiologist can improve the inter observer variation in AFC.

Conclusion

Predictive accuracy of AFC for ovarian reserve in sub fertile women alone is not a reliable tool because of its lack of sensitivity and high inter and intra cycle variability. While serum basal FSH is still a reliable marker of ovarian reserve. There is no single parameter that can be used for prediction of decreased ovarian reserve. Multiple factors like Age, BMI, length of menstrual cycle affect AFC and basal FSH as marker of ovarian reserve.

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