Role of Antral Follicle Count as a Marker of Ovarian Reserve and Its Correlation with Antimullerian Hormone and Follicle Stimulating Hormone

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ABSTRACT

Introduction: In recent time there has been a significant increase in subfertile couples in India. Few studies labeled Antral Follicle Count (AFC) as more accurate biomarker in comparison to Anti-Mullerian Hormone (AMH) for assessing female subfertility. However limited Indian data is available and we continue to follow western literature irrespective of difference in genetic make-up and different socio-economic status.

Aims: To study age related trends in markers of ovarian reserve and their correlation with each other.

Material and Methods: This was a prospective study where 50 patients with complaints of infertility were included. Subjects underwent transvaginal sonography on day 3 of menstrual cycle for estimation of AFC and Total ovarian volume (TOV). Blood sample was taken on the same day to measure hormonal profile related to infertility work-up. The data was subjected to statistical analysis and correlation coefficients were determined. p-value <0.05 was considered statistically significant for all analysis.

Results: There was statistically significant negative correlation between age as compared to mean AFC (p < .05) and mean AMH (p <.05). However, Follicle stimulating hormone (FSH) showed positive correlation with age. AFC was found to be more statistically sensitive marker as compared to AMH and FSH.

Conclusion: AFC is a reliable marker of ovarian reserve assessment and more sensitive to age related decline in ovarian reserve.

Keywords: Anti-Mullerian Hormone, Follicle Stimulating Hormone, Antral Follicle Count, Total Ovarian Volume.

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INTRODUCTION

Subfertility is defined as the inability to conceive after one year of unprotected intercourse of reasonable frequency. Primary infertility refers to couples who have never conceived, whereas secondary infertility refers to couples failed to conceive after first pregnancy.¹ Subfertility can be due to male (20%) or female (80%) factors. Female factors can be divided into tubal (40%), ovulatory (40%), uterine (10%) and cervical (10%).² There are about 20 million infertile couples in India in whom ovulatory disorders are the most common reasons where women are unable to conceive and account for 30 % of women infertility.² Fecundity of women gradually decreases after the age of 30 years and more rapidly in the mid to late thirties and is almost negligible almost a decade before menopause. This age-related decline in fertility is manifested in form of reduced quality and quantity of ova and a gradual increase in circulating FSH and with decreasing anti-Müllerian hormone (AMH) and inhibin B concentrations.³,⁴ To assess the quantitative functioning of ovaries various tests are done including: (1) Total ovarian volume (TOV), (2) Antral follicle count (AFC), (3) Anti-mullerian hormone (AMH), (4) Luteinisng hormone (LH), (5) Follicle stimulating hormone (FSH). Antral follicle count, serum inhibin B levels, ovarian volume and vascular resistance have also been studied as markers of ovarian reserve.² AMH is also called Mullerian Inhibiting Substance (MIS). It is a member of transforming growth factor beta (TGF-beta) which is a peptide homo-dimeric consisting of two identical glycoprotein subunits, connected by disulfide bridges.⁵ In females AMH has been suggested as a representative of the ovarian reserve because it is produced by granulosa cells of preantral (primary and secondary) and small antral follicles.⁷ The number of the small antral follicles directly depends on the size of the primordial follicle pool. There is increasing evidence that AMH in contrast to other markers can be used as a cycle independent marker.²
A statistically significant reduction in ovarian volume is seen in females older than 30 years which has been proved by large scale studies.\textsuperscript{10,11} The usefulness of ovarian volume to predict ovarian responsiveness is limited due to clinically relevant changes.\textsuperscript{10,12} The antral follicle count (AFC) is a direct quantitative marker of ovarian responsiveness.\textsuperscript{9} As the AFC declines progressively over time (annual losses of 0.35–0.95 antral follicles/year) it provides a more useful clinical marker of ovarian responsiveness than ovarian volume.\textsuperscript{12} AFC has been widely used as the ovarian reserve test, due to convenience of the ultrasonography and ease of availability. There are well-known difficulties to obtain correct AFC due to higher inter-observer differences, however; follicle count can be performed easily with the help of high resolution of sonographic systems.

Development of follicles is dependent on relationship of FSH and AMH which are secreted by ovaries. Abnormally low levels of these hormones signify low ovarian reserves hence low AFC count. Women’s age and FSH assay were among the earlier and most used parameters for evaluation of ovarian reserves.\textsuperscript{13–15} With evolution of sonographic systems, AFC and TOV were included in assessing ovarian reserves.

Various sonographic parameters used for evaluation of ovarian reserve have variable reliability including ovarian volume and AFC.\textsuperscript{16} FSH levels vary from being low during follicle development and maximum during ovulation.\textsuperscript{6} Day 3 FSH level has been the most commonly used test of ovarian reserve and has been the standard practice of determining ovarian reserve, providing maximum accuracy.\textsuperscript{17} La Marca et al in their study showed that serum AMH level remains same throughout the menstrual cycle unlike other parameters.\textsuperscript{18} It has been suggested that AMH is more effective than other hormonal parameters in prediction of ovarian reserve.\textsuperscript{19}

**Material & Methods**

**Subjects**

The study was carried out over a period of one and half years (from January 2016 to June 2017) in Radio-diagnosis department. Females from 20 to 45 years of age, presenting to the gynecology out-patient department with complaints of difficulty in conceiving were included in the study. Cases which were excluded from the study were: (1) Ovarian cysts (simple or complex), (2) Endometriomas, (3) Endocrinological abnormality (polycystic ovarian syndrome, abnormal thyroid levels), (4) Uterine fibroids, (5) Fallopian tube diseases and (6) Previous ovarian surgeries. Total of 87 patients were included in the study (37 patients were excluded as they did not fulfill the inclusion criteria).

**Statistical Analysis**

The study was approved by the Institutional Review Board and a written consent was obtained from all patients in study after explaining them the nature and type of examinations that would be carried. Patient’s detailed history and biometric parameters were taken i.e. age, height, weight and Body Mass Index (BMI).

**Sonographic assessment**

Transvaginal sonography of all the patients was done on day 3 of their menstrual cycle, using Siemens Acuson S2000 system (Siemens Healthcare, Erlangen, Germany) with 4-9 MHz transducer. Both ovaries were assessed and complete sweep of ovaries was done. The number of anechoic follicles between 2-10 mm diameter (in short axis) were counted.\textsuperscript{20} Follicles of both the ovaries were added to obtain AFC. Ovarian volumes were also calculated by taking dimensions in three orthogonal planes and applying ellipsoid formula (Ellipsoid Volume = \(AP \times TR \times CC \times 0.523\)). Volumes of individual ovaries was taken and summed up to obtain TOV as shown in figure 1.

**Hormonal Analysis**

On day 3 of menstrual cycle, 5 ml of blood was drawn under all aseptic conditions by venopuncture and samples were sent for estimation of serum AMH & FSH. The serum AMH and FSH were determined by sandwich Enzyme linked Immuno-Sorbent Assay (ELISA) in Biochemistry department of our institution.

**Fig. 1:** In the ‘A’ and ‘B’ TVS images showed TOV of both ovaries are measured and in ‘C’ images a follicle is measured. All the follicles are summed up to obtain AFC.
Table 1: Descriptive statistics of biophysical, sonographic and biochemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29.26</td>
<td>+/- 5.7</td>
<td>20-45</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>26.8</td>
<td>+/- 4.17</td>
<td>18.1 -35.1</td>
</tr>
<tr>
<td>Total ovarian volume (cc)</td>
<td>13.6</td>
<td>+/- 4.35</td>
<td>5- 27</td>
</tr>
<tr>
<td>Antral Follicle Count (n)</td>
<td>13.8</td>
<td>+/- 5.3</td>
<td>3 –27</td>
</tr>
<tr>
<td>Anti mullerian hormone (ng/ml)</td>
<td>2.9</td>
<td>+/- 1.20</td>
<td>1.2 – 5.9</td>
</tr>
<tr>
<td>Follicle stimulating Hormone (ng/ml)</td>
<td>5.8</td>
<td>+/- 1.3</td>
<td>3.4 – 8.5</td>
</tr>
</tbody>
</table>

Table 2: Distribution of mean values of the parameters according to the three age groups.

<table>
<thead>
<tr>
<th>Age Groups (yrs.)</th>
<th>Mean of AFC (n)</th>
<th>Mean of AMH (ng/ml)</th>
<th>Mean of TOV (cc)</th>
<th>Mean of FSH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>15.8</td>
<td>3.2</td>
<td>15.8</td>
<td>5.4</td>
</tr>
<tr>
<td>30 to 40</td>
<td>11.8</td>
<td>2.4</td>
<td>11.0</td>
<td>6.2</td>
</tr>
<tr>
<td>&gt;40</td>
<td>9.6</td>
<td>2.2</td>
<td>10.8</td>
<td>6.7</td>
</tr>
</tbody>
</table>

Table 3: AFC was divided into two categories i.e. more than 10 and less than 10 and the mean values of other variables were tabulated for comparison

<table>
<thead>
<tr>
<th>AFC Category</th>
<th>Mean BMI (kg/m²)</th>
<th>Mean of TOV (cc)</th>
<th>Mean of FSH (ng/ml)</th>
<th>Average of AMH (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10</td>
<td>26.8</td>
<td>9.1</td>
<td>6.5</td>
<td>1.4</td>
</tr>
<tr>
<td>&gt;10</td>
<td>26.9</td>
<td>14.9</td>
<td>5.6</td>
<td>3.3</td>
</tr>
</tbody>
</table>

RESULTS

50 sub-fertile women were evaluated, the age group ranged from 20 to 45 years. Mean values of the biophysical, hormonal and sonographic parameters of subjects are given in Table 1. AFC is negatively correlated with age and FSH while there is significant positive correlation between AFC & TOV. While age and AFC have significant negative correlation at 5% level of significance, as its p-value is less than 0.05. AMH is negatively correlated with age and positively correlated with AFC. There is no significant correlation between AMH & TOV. AFC and AMH are significantly positively correlated at 1% as their p-value is <0.01. The subjects were divided into three age groups - below 30 years, 30 to 40 years and more than 40 years. Significant reduction in AFC and AMH was noted with increasing age. Both mean AFC and mean AMH showed statistically significant decline in their values with increasing patient’s age. Mean TOV also showed negative correlation with increasing age whereas, FSH showed statistically significant positive trend with age as seen in Table 2. Line diagram between ovarian reserve markers and age groups is shown in Fig.2. AFC was divided into two categories i.e. more than 10 and less than 10 and the mean values of other variables were tabulated for comparison (Table 3). Mean BMI was similar in both the categories. AMH and TOV was higher in the group in which AFC was greater than 10 as shown in clustered column chart (Fig.3).
DISCUSSION
In this study there has been a direct relationship between values of AFC when compared to AMH i.e. mean AMH was lower in patients with Mean AFC <10 and mean AMH was higher in patients with mean AFC more than 10. Mean AMH, mean AFC and mean TOV shows statistically significant negative correlation with increasing age hence, establishing that fertility decreases with age. Due to ease of convenience and inexpensiveness of ultrasonography AFC can be widely used after proper training (even on USG machines without automatic software).

The limitations of this study were: (1) The total number of patients was less; (2) There was no control group.

In a study by Barbakadze et al, they stated that AMH should be considered as the more reliable marker of the ovarian reserve as compared to FSH. In our study AFC was found to be superior than AMH as well as FSH for ovarian reserve assessment. They also demonstrated a strong positive correlation between serum AMH level and AFC which is in concordance with this study. The use of AMH combined with AFC may improve ovarian reserve evaluation. Similarly in study conducted by Jayprakasen et al, they suggested that the number of antral follicle measuring 2-6 mm was most closely related to serum AMH levels. Which suggest that follicular cohort is most reflective of quantitative status of ovarian reserve. They also stated that FSH levels have increased with age but they don’t show a sensitive relationship with increasing age and with AFC and AMH. In this study antral follicles measuring 2-10 mm were included and they also show that antral follicle count is directly proportional to AMH and shows increase in FSH with age.

There was negative correlation between AFC and AMH as compared to FSH which is in concordance with a study conducted by Bala et al in subfertile females. They also showed that AMH and FSH were independent indicators of ovarian reserve. AMH and AFC showed a positive correlation (r=0.641 and p<.001) but not between FSH and AFC. Goksef et al stated strong positive correlation between serum AMH levels and AFC, and also this correlation is stronger than the other ovarian reserve parameters.

Syeda et al carried out a study in Karachi which also stated that there is a statistically significant difference in AMH and AFC values as the age increases which is in concordance with this study.

This study shows that AFC has better sensitivity as compared to AMH, FSH and TOV. Due to wide availability of sonographic systems, AFC can be performed easily after proper training. However there is possibility of inter-observer differences and errors which depends upon the operator. In earlier studies AMH was stated as more reliable marker as it remained same throughout the menstrual cycle but parameters included in our study were obtained on Day 3. AMH has a narrow range and the decline is not as steep in our study as compared to AFC. FSH shows inverse relationship to both AFC and AMH, and is directly proportional to FSH.

CONCLUSION
In conclusion, AFC and AMH are reliable markers of ovarian reserve and superior to other markers like FSH and TOV. AFC appears to be better than AMH due to its steep curve of age related reduction than AMH. In view of its easy availability, cost effectiveness and non-invasive nature, AFC can be considered as a one stop-shop criterion for ovarian reserve analysis and for further treatment planning.
REFERENCES

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