ORTs in an Infertile Woman – A Comparative Study

Authors
Virendra Prasad Pandey*1, Devatwal Kasturi Gabrulal2, Deepa Masand Lokwani3, Surabhi Tomar (Sharma)4
1,2,3,4Department of Obstetrics & Gynaecology
National Institute of Medical Science and Research, NIMS University Rajasthan, Jaipur 303121 India
*Corresponding Author
Virendra Prasad Pandey
Email: virendrapandey1958@gmail.com

Abstract
Background: There exists a lacuna in the assessment of ovarian reserve in an aged infertile woman, which reflects their reproductive potential.
Objective: To compare and evaluate the role of serum FSH, LH, E2, Inhibin B and AMH with the ovarian follicle status in an infertile woman.
Material and Methods: A comparative observational and cross-sectional study was done in 120 females aged 18 to 43 years fulfilling the inclusion and exclusion criteria. Venous blood (5 ml) collected from subjects for measurement of serum FSH, LH, E2, Inhibin B and AMH on day 3rd of menses. Serum FSH, LH, E2 and AMH levels were measured by chemiluminescent immunometric assay. Serum Inhibin B estimated by ELISA method. Antral Follicle count (AFC) and Ovarian volume (OV) was measured by ultrasonography. The serum values of FSH, LH, E2, Inhibin B and AMH were correlated with AFC and OV. P value <0.05 was considered significant.
Results: Our study showed positive correlation exist between the serum levels of FSH, LH, E2 (p< 0.001). Inhibin B values raised in <20yrs and >40yrs age groups. Serum AMH, AFC and OV values showed significant decrease with increasing age(p<0.001). The serum AMH and OV was positively related to AFC status while serum FSH, LH, E2 values was negatively related to the AFC status. Age was negatively related to AFC status.
Conclusion: AMH test predicts the reproductive potential of an aged infertile women (>35 yrs.) and reduce the use of Assisted Reproductive Techniques (ART).
Keywords: Count, ART-Assisted Reproductive Technique, FSH-follicle stimulating hormone, LH-luteinizing hormone, E2-Estradiol, OV-Ovarian volume. ORTs-ovarian reserve tests, AMH-Anti Mullerian Hormone, AFC-Antral Follicle.

Introduction
The trend towards later motherhood, increasing age of marriage and the increasing reliance on assisted reproduction technique, has emerged the need for establishment of more reliable tests to dictate the ovarian reserve (OR). OR is nothing but functional potential of ovary, which constitutes the size of the ovarian follicle pool and
reflects the number and quality of oocytes, within it. Thus, an assessment of OR helps in reflecting the reproductive potential of women.

Various markers like serum FSH, Serum LH, Serum E2, Serum Inhibin B, Serum AMH, AFC and ovarian volume are available for assessing the OR. Then hormonal tests show intercycle dependent and interdependent values, while ultrasonic markers cause interobserver variation. AMH and AFC are considered to be equally predictive of poor ovarian response, however AMH is considered advantageous over AFC because AMH concentration can be measured at any time during menstrual cycle. Age specific AMH levels have been found to be better predictor of oocyte yield than FSH women aged between 34 and 42 years. Overall AMH has been found to be the most sensitive predictor of over and under response to controlled ovarian stimulation.

Thus, the aim of this study was to measure the values of various hormonal and ultrasonographic markers in an infertile woman. Furthermore, determining the relation and the strength of correlation of the various variables to the Antral Follicle Count.

**Materials and Methods**

A comparative observational and cross-sectional study was conducted in the IVF centre, department of Obstetrics and Gynaecology, National Institute of Medical Science and Research, Jaipur on 120 infertile female aged 18-43 years between July 2015- July 2016. All the patients fulfilled the inclusion criteria like regular menstrual cycles of 21-35 days, no current or past diseases, not underwent any hormonal treatment, body mass index (BMI): 18-27 kg/m² and no evidence of any endocrine disorders. Patients with any endocrine disorders, abnormal liver functions, abnormal kidney functions and genital tuberculosis were excluded. After taking an informed consent and obtaining a detailed history, physical examination and laboratory examination, patients were subjected to the measurement of day 3 serum values of serum FSH, LH, E2, Inhibin B, AMH, AFC and ovarian volume. After compiling the data, statistical analysis was done, and results were computed and evaluated.

Adequate venous blood samples were obtained from the subjects for the measurement of Serum FSH, LH, E2, Inhibin B and AMH on the day 3 of menses at around 10.11 hrs. Serum FSH, LH, E2 levels were measured by solid phase, two sites chemiluminescent immunometric assay, reagent kit being Abbott/ Siemens. Instrument used was Architect/ Avida Centaur. Inhibin B levels were measured by Enzyme linked Immunosorbert Assay (ELISA) method. Serum AMH levels were measured in human serum by two sites chemiluminescent immunometric assay, reagent kit being of BECKMAN COUTLER, Instrument used was BECKMAN COUTLER for in vitro quantitative measurement. Antral follicle count and Ovarian volume was measured by ultrasonography. Follicles measuring 2-10mm were measured by scanning from outer to inner margins of ovaries individually. All the scan was done by a single operator on Voluson E8 (GE Health care), with 5-9 MHz transvaginal volume probe on day 3 of menses. The addition of the follicles measured in both the ovaries was obtained. The sum was designated as the “Antral Follicle Count”. The measurement of ovarian volume is done along with the measurement of the AFC. The diameter of the ovarian contour is measured in 3 perpendicular direction as D1, D2, D3 and the ovarian volume of individuals ovaries is calculated by using the formula D1 x D2 x D3 x 0.52. The ovarian volume of the right and the left ovary was summed up to obtain the total ovarian volume.

**Statistical analysis**

The cases were divided in four groups on the basis of age as <20 as group 1, group 2 as 21-30, group 3 as 31-40, group 4>40 and also on basis of AFC as <4, 4-7, 8-12, >12. After compiling the data obtained in a tabulated form, the values of the individual variables were compared and the correlation between the various serum values i.e.
serum AMH, FSH, LH, E2, Inhibin B with the antral follicle count and the ovarian volume was found out. The correlation was evaluated for positive and negative. The correlation as termed significant only if the obtained P value was <0.05 and the rest were termed as not significant. Thus, subsequently the results were obtained.

Results
Graph 1: Age group

In our study, patients age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs showed 14.17%, 64.17%, 12.5% and 9.17% respectively.

Table 1: FSH, LH, E2, Inhibin B, AMH, AFC and OV Mean values

| Age   | FSH  | LH    | E2    | Inhibin B | AMH  | AFC  | OV   |
|-------|------|-------|-------|-----------|------|------|------|
| <20   | 4.88 | 4     | 23.56 | 82.85     | 5.35 | 16.65| 7.96 |
| 21-30 | 6.03 | 5.46  | 52.81 | 58.23     | 4.61 | 9.68 | 7.41 |
| 31-40 | 6.31 | 5.81  | 67.18 | 48.61     | 2.31 | 6.80 | 6.34 |
| >40   | 7.71 | 5.82  | 55.46 | 72.19     | 1.00 | 4.82 | 5.12 |

Chart 2: FSH, LH, E2, Inhibin B, AMH, AFC and OV Mean values

In our study, patients FSH mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 4.88, 6.03, 6.31, and 7.71 respectively. Patients LH mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 23.56, 52.81, 67.18 and 55.46 respectively. Patients E2 mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 82.85, 58.23, 48.61 and 72.19 respectively.
respectively. Patients Inhibin B mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 82.85, 58.23, 48.61 and 72.19 respectively. Patients AMH mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 5.35, 4.61, 2.31 and 1.00 respectively. Patients AFC mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 16.65, 9.68, 6.80 and 4.82 respectively. Patients OV mean values in age group < 20yrs, 21 -30yrs, 31-40yrs and > 40yrs was 7.96, 7.41, 6.34 and 5.12 respectively.

Our study showed positive correlation exist between the serum levels of FSH, LH, E2 (p< 0.001). Inhibin B values raised in <20yrs and >40yrs age groups. Serum AMH, AFC and OV values showed significant decrease with increasing age (p<0.001). The serum AMH and OV was positively related to AFC status while serum FSH, LH, E2 values was negatively related to the AFC status. Age was negatively related to AFC status.

Correlation between Serum Inhibin B & AFC

There exists positive correlation between Inhibin B and AFC

Correlation between Serum AMH & AFC

There exists positive correlation between Serum AMH and AFC.
**Discussion**

Our study demonstrated that the Serum AMH, AFC, Inhibin B decreases with increase in Age. Serum FSH, LH values increase with increase in Age. Serum E2 values increase with increase in Age but beyond 40 yrs age serum E2 values seem to be decreasing significantly. A study by Dayal Meena et al 2013, demonstrated that with advancing age, Serum AMH levels (P <0.0001), AFC (P<0.05). Ovarian volume (>0.05) and Inhibin B (>0.05) were decreased and serum FSH (P<0.05), LH (>0.05) and also E2 (p<0.05) were increased.

Ernest Hung Yu Ng et al 2003, conducted a study on Chinese women with proven fertility with respect to effects of age on hormonal and ultrasound markers of ovarian reserve, AFC showed best correlation with women’s age and declined linearly at a rate of 3.8 % per year as compared to other hormonal parameters. This was similar to our results. Our results suggested that Serum AMH also shows a correlation to AFC along with the correlation to Serum FSH, LH, Inhibin B, E2 levels. Serum FSH, LH, E2 is negatively correlated to AFC and Serum AMH is positively correlated with AFC.

In a study conducted by Dayal Meena et al 2013, which demonstrated that serum AMH levels were correlated to the AFC (P<0.0001) and ovarian volume (P<0.001). Serum AMH values were found to be robustly correlated with AFC than Serum FSH, LH, E2 and Inhibin B on day 3 of cycle. This suggested that Serum AMH should be taken as a single to reflect ovarian reserve. This study did not suggest the degree of correlation in terms of Pearsons correlation.

Renato Fanchin et al 2003 studied the relation of ovarian follicular status with Serum AMH, Serum Inhibin B, Estradiol, FSH and LH. Serum AMH levels were more strongly correlated (P<0.001) with follicular count (r=0.74, P<0.00001), E2 (r= - 0.08, p =NS) and LH (r= 0.05, p= NS) suggesting that the serum AMH reflect ovarian follicular status better than the usual hormonal parameters.

In a prospective study, conducted by Shanti Muttukrishna et al 2004, demonstrated that AMH has greater association (r= 0.69, P<0.001) with number of eggs collected as compared to Serum FSH, Inhibin B levels. Thus concluded that Serum AMH is the best single marker of ovarian response to gonadotrophin stimulation. These results were similar to those of ours. The study also suggested that the combined markers modestly improved prediction.

Tito Silvio Patrelli et al 2012, in their study, demonstrated a significant correlation between AMH in normal responders and AMH in both high and poor responders and thus confirmed the usefulness of AMH in ART cycles to customize treatment protocols and suggest of verifying an eventual permanent decrease in AMH levels after IVF.

Serum AMH is the only marker identified yet for ovarian reserve. It is the only showing no intercycle dependability and no interobserver variability with set standards according to the manufacturer. Other hormonal parameters show intercycle variability and are interrelated. AFC is the ultrasonographic marker with inter-observer variation.

**Conclusion**

Our study concludes that Serum AMH values can be considered for evaluation of ovarian reserve. Serum AMH levels are comparable to AFC values. The results thus concluded in making this marker being useful.

Outcome of the present study showed that the combined use of AMH and Ultrasonographic marker in screening the current status of ovarian function in general sub-fertile population as it has a role in the process of initial and cyclic recruitment. It can be used to identify those patients who are destined to fail induction and ART programs without incurring the financial burden of the interdependent several serum hormonal markers and ART programs.

Serum AMH values with the ultrasonographic markers like AFC can modestly increase the
predictability of diminished ovarian reserve (DOR) without increasing the cost of the screening process. An AMH test helps women to beat the biological clock by predicting how long they left to achieve motherhood with its relation to age and may also reduce the need for ART in these patients.

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