

To Establish Relationship between Biochemical and Ultrasonographic Markers of Ovarian Reserve with Age

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Abstract: *This study aims to understand the variation of Biochemical (S. FSH, S. AMH, S. ESTRADIOL) and Ultrasonographic (AFC and Ovarian volume) markers of Ovarian Reserve with increasing age of the healthy women with proven fertility. It will improve our understanding of reproductive aging for prediction of the ovarian reserve both in terms of reproductive prognosis and distance to menopause.*

Keywords: Ovarian reserve, serum FSH, AFC, Ovarian volume, serum Estradiol, serum AMH, Declining fecundity

1. Introduction

The fecundity and the age at which menopause occurs shows a wide variability among women. Which is profoundly influenced by ovarian function therefore also affecting women's hormonal milieu and their subsequent risk for the development of disease. Reproductive age women experience a decline in fecundity as a result of ovarian ageing which is correlated with their increasing chronological age. **Ovarian reserve** refers to the size of the non-growing, or resting, primordial follicle population in ovaries [1]. The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of the oocytes therein, declines with increasing age, resulting in the decrease of a woman's reproductive function [2]. Age is considered as the single most important factor in determining ovarian reserve. The size of the follicle pool is established during fetal life, maximum oocyte number is reached by 16-20 weeks that is 6-7 million in both ovaries. Then oocytes number starts decreasing, most rapid decline occurs before birth (2 million at birth) and to 300000 at puberty (Faddy et al.1992) [3], [4], [5]. The rate of decline of follicles during the reproductive years is steady at approximately 1000 follicles per month [6].

In last few decades a number of tests involving biochemical measures (S. AMH, S. FSH, S. ESTRADIOL, S. INHIBIN B, Clomiphene Citrate Challenge Test etc.) and ovarian imaging (ovarian volume, AFC etc.) have been proposed to help predict ovarian reserve and/or reproductive potential. Early follicles secrete AMH in a gonadotropin – independent state. Serum AMH levels are indicative of the size of the growing follicle pool [7], [8]. In case of normal ovarian function, a developing cohort of follicles secrete estradiol and Inhibin B which suppress FSH and keep it in the normal range [9]. As women and their follicles age, the amount of FSH secreted increases due to the lack of responsiveness of the ovary (30. Serum estradiol, released from the ovary during follicular development. It is usually low (<50 pg/ml) on day 2-4 of a cycle but shows some cycle to cycle

variability. An elevated value in the early follicular phase can indicate reproductive ageing and hastened oocyte development [9]. The AFC correlates very well with chronological age in normal fertile women and appears to reflect remains of the primordial follicular pool. Total AFC < 4 is predictive for poor response [1]. With age, changes in ovarian volume are concordant with the age-related decrease in ovarian follicles. The role of ovarian volume in the assessment of ovarian reserve remains uncertain. AFC and S. AMH have good predictive value and are superior to day-3 S. FSH. Basal estradiol on day 2, 3, or 4 of the menstrual cycle has poor inter- and intra-cycle reliability, individually as a test of ovarian reserve [10]. Ovarian volume has limited reliability as an ovarian reserve test [11]. However, these markers serve as a proxy for oocyte quantity but are considered poor predictors of oocyte quality.

It's critical to improve our understanding of reproductive aging for prediction of the ovarian reserve both in terms of reproductive prognosis and distance to menopause. The quantity and quality of oocytes (ovarian reserve) has been linked to ovarian function and so there is significant interest in developing noninvasive testing to characterize the rate and pattern of oocyte loss. Considering the literature available on ovarian testing and available resource setting in a country like India also the feasibility of tests, we have taken S. FSH, S. AMH, S. ESTRADIOL, AFC and Ovarian Volume to understand their variation with increasing age of the healthy women with proven fertility.

2. Materials and Methods

Study was carried out on 412 women between 30 - 45 yrs of age, who were relatively healthy with respect to ovarian function i.e. women attending OPD for discharge per vaginum, low backache, pain in abdomen, PID, UTI, postnatal women for routine visits, cervical screening, contraception counselling in Swaroop Rani Nehru Hospital and Kamla Nehru Memorial Hospital, department of obstetrics and gynecology affiliated to M.L.N. Medical

College, Prayagraj over a period of twelve months in the year 2019 to 2020. S. FSH and S. Estradiol were measured on day 2-3 of menses to achieve the basal level by ARCHITECT kits (a chemiluminescent micro particle immunoassay (CMIA)). S. AMH was measured by paramagnetic particle chemiluminescent immunoassay. Transvaginal sonography was carried out on day 2-3 of the menstrual cycle. All sonographic measurements were performed by using the 7.5 MHz transvaginal probe. Sonography findings were based on antral follicle count and total ovarian volume. All the follicles of size 2-8 mm were measured and counted in each ovary. The sum of both counts demonstrates AFC. The volume of the left and right ovary was assessed by measuring the diameter of the contour in three perpendicular directions and applying the equation of volume of an ellipsoid to calculate ovarian volume ($D1 \times D2 \times D3 \times \pi/6$). Total ovarian volume was then obtained by sum of the volume of the left and right ovary (mean ovarian volume). The volumes of both ovaries are added for the total basal ovarian volume (BOV).

2.1 Data Analysis

These 412 women were divided into 3 sub-groups on the

basis of age i.e. 30-35, 36-40 and 41-45. Descriptive statistics including calculation of frequency and percentage distribution of patients in sub-groups according to parameters studied (age, S. AMH, S. FSH, S. E2, AFC, Ovarian Volume). The calculation of mean and standard deviation of parameters S. AMH, S. FSH, S. E2, AFC, Ovarian Volume) in all 3 sub-groups. Comparison of means of different parameters in the subgroups was done by one-way ANOVA test. A p-Value <0.05 have been considered significant. It was done by using IBM SPSS v.25. Finally Scatter plots with centile lines were plotted for qualitative evaluation and spearman's coefficients were calculated for quantitative evaluation of correlation between chronological age and parameters (S. FSH, S. E2, S. AMH, AFC, Ovarian volume) by using R version 4.0.0.

3. Result

All the parameters taken in the study showed statistically significant variation with in the three sub groups according to age, summarized in **Table- 1**.

Table 1

Parameters	30-35	36-40	41-45	total	p-value
No of women [n,(%)]	183 (44.42)	128 (31.07)	101 (24.51)	412	--
Age(years) [mean±SD]	32.40±1.65	37.86±1.38	42.88±1.40	36.67±4.51	--
S. AMH (ng/ml) [mean±SD]	2.07±1.33	1.08±0.73	0.5±0.33	1.38±1.19	<.001
S. FSH (mIU/ml) [mean±SD]	5.6±1.66	6.2±1.76	7.23±2.92	6.19±2.16	<.001
S. E ₂ (pg/ml)[mean±SD]	41.39±18.01	41.91±15.31	35.35±16.42	40.07±17	<.05
Ovarian volume (unit?) [mean±SD]	6.94±1.43	6.01±0.99	5.58±0.93	6.32±1.32	<.001
AFC [mean±SD]	14.59±4.62	11.3±3.11	8.26±2.78	12.01±4.57	<.001

Means of S. AMH, AFC and Ovarian volume showed significant decrease across the sub groups with increasing age and it was statistically significant (p-Value= <0.001) whereas a subtle decrease was also marked in mean values of S. estradiol (p-Value=<0.05). S. FSH showed an increase with increasing age (p-Value=<0.001).

As distribution and reference range of every parameter for assessment of ovarian reserve depends on age of the women. Scatter plots with centile curves were plotted to obtain the actual pattern with respect to age. The 50th percentile line is like the median that can be used as a reference, and the 2nd and 98th percentile lines can be taken to define the lower and upper limits for healthy individuals which can be neglected to observe the trends (**Figure-1**). Centile curves were also in accordance with the trends observed above. S. AMH (2% centile value of S. AMH is 0.05 and 98 % centile value of S. AMH is 5.11.), AFC (2% centile value of AFC is 4 and 98 % centile value of AFC is 24), Ovarian volume

(2% centile value of ovarian volume is 3.15 cm³ and 98% centile value of ovarian volume is 9.6 cm³) and S. estradiol (2% centile value of S. E₂ is 16.15pg/ml and 98 % centile value of S.E₂ is 84.9pg/ml) showed decline in their values with age. S. FSH (2% centile value of S FSH is 3.33mIU/ml and 98 % centile value of S FSH is 12.37mIU/ml) showed an increase with increasing age.

In the above centile plots correlation of the parameters with chronological age has been observed, but the relationship was not necessarily linear. Therefore, to measure the strength of this type of relationship Spearman's correlation coefficients [12] were calculated with their level of significance. Correlation Coefficients of S.FSH=0.301 (p-Value= <0.001) , of S. estradiol= -0.163 301 (p-Value= 0.001), of S. AMH= -0.702 301 (p-Value= <0.001), of AFC= -0.616 301 (p-Value= <0.001), of ovarian volume= -0.552 301 (p-Value= <0.001). Correlation coefficients also showed similar results as observed above.

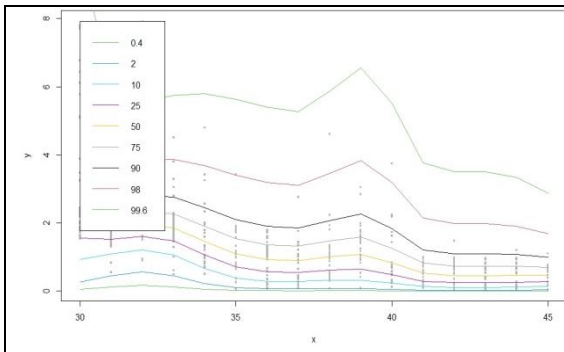


Figure-1.1: Scatter plot of S. AMH against age with centile lines. Where $y = S. AMH(ng/ml)$, $x = AGE(years)$.

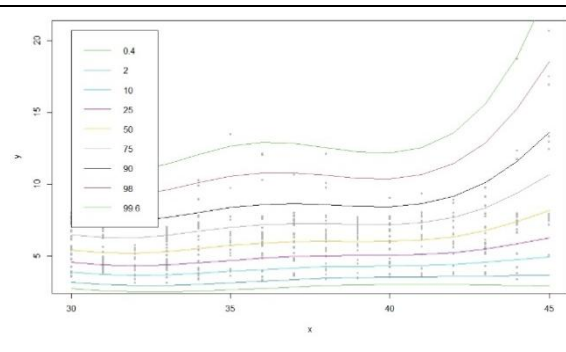


Figure-1.2: Scatter plot of S. FSH against age with centile curves. Where $y = S. FSH (mIU/ml)$, $x = AGE(years)$

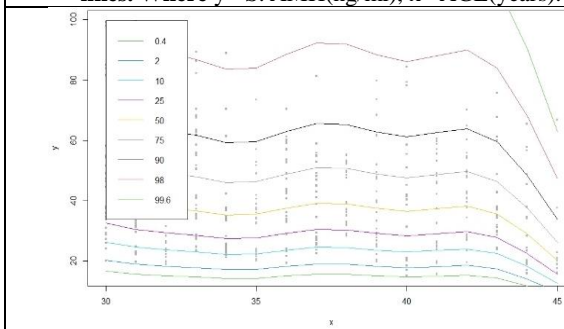


Figure-1.3: Scatter plot of S. E2 against age with centile curves. Where $y = S. E2 (pg/ml)$, $x = AGE(years)$.

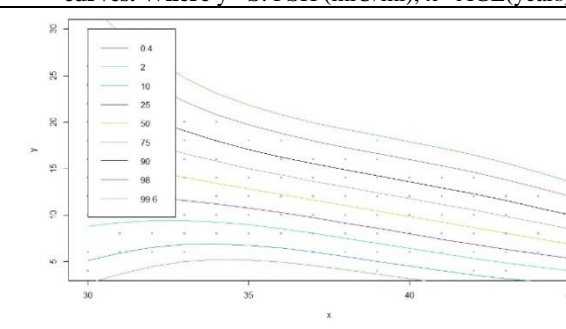


Figure-1.4: Scatter plot of AFC against age with centile curves. Where $y = AFC$, $x = AGE (years)$

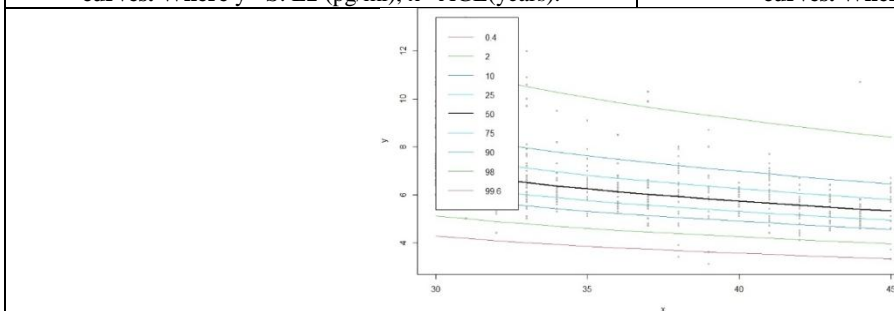


Figure-1.5: Scatter plot of volume against age with centile curves. Where $y = volume$, $x = AGE (years)$

Figure 1

4. Discussion

As ovarian aging is highly correlated to chronological aging of women and abnormal values of the markers shows accelerated aging of ovary. In healthy women scatter plot with centile lines has been drawn for every parameter with respect to age which showed a specific trend of these parameters i.e. S.FSH showed an increasing trend, S. E2 showed a decreasing trend, S.AMH showed a decreasing trend, AFC showed a decreasing trend and ovarian volume also showed a decreasing trend. To calculate the statistical dependence between these parameters and age or to find out the correlation between age and these parameters spearman's correlation coefficient has been calculated.

These were for AMH (0.702), FSH (0.301), E2(-0.163), AFC (0.616) and OVARIAN VOLUME (-0.553). All of these showed a very high statistical significance (p value <0.05 wald chi square test). Which is in accordance with a similar study done by **Roberta Venturella et al (2015)[13]** in which correlation coefficients for these parameters were - for AMH (-0.8090), FSH (- 0.6742),E2 (- 0.2289), AFC (-0.7304) and for OVARIAN VOLUME (- 0.5519).

5. Conclusion

Ovarian reserve depletion is a complex clinical phenomenon influenced by age, genetics, and environmental variables. Although it's challenging to predict the rate of an individual's ovarian reserve decline, clinicians are often asked for advice about fertility potential and/or recommendations regarding the pursuit of fertility treatment options. The purpose of this study is to summarize the state-of-the-art of ovarian reserve testing. As ovarian aging is highly correlated to chronological aging of women and abnormal values of the markers shows accelerated aging of ovary. Which is important in guiding patients' reproductive attempts and in reducing the rates of unnecessary surgeries for benign pathologies for which menopause may present the best therapy. Further research for validation and prospective evaluation is required.

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