ORIGINAL ARTICLE

The Value of Different Ovarian Reserve Tests in the Prediction of Ovarian Response in Infertility

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ABSTRACT

Background: Ovarian reserve tests are done among the infertility patients before providing management either by ovulation induction or by the IVF program. These tests are worthy as it saves and prevent unnecessary procedures, canceled cycles, wasting of resources, induction complications, and emotional stress to the couple in case of the low estimate. **Aim of the study:** The aim of the study was to study the values of antral follicle count (AFC), maternal age, follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B, anti-Müllerian hormone (AMH), and mean ovarian volume in the infertile patients. The primary outcome is to predict the best parameter of ovarian reserve. Design: This is a perspective cross-sectional study comprising 120 infertile patients in the study who received treatment, for example, induction of ovulation and timed intercourse/IUI or IVF. They were investigated in the form of basal FSH, LH, AFC, and mean ovarian volume assessment, inhibin B, AMH, and response of all patients was then evaluated according to the number of follicles. **Results and conclusion:** The total AFC and AMH are found to correlate significantly with the ovarian response with $P < 0.001$ and <0.001, respectively, indicating that they are good predictors of ovarian reserve. The basal FSH and ovarian volume do not correlate with the ovarian response indicating their poor value as predictors of ovarian reserve.

Key words: Infertility, Ovarian reserve, Antral follicle count

INTRODUCTION

The human ovary establishes several hundred thousand non-growing follicles (NGFs) during the second half of intrauterine life, which is followed by a decline toward the menopause when approximately 1000 follicles remain at an average age of 50–51 years.[1] With the potential for only approximately 450 ovulatory monthly cycles in the normal human reproductive lifespan, this progressive decline in NGF numbers is attributed to the loss of growing follicles by atresia. The ovary, a female gonad, is of great importance with respect to the reproductive as well as the endocrine status of a female. The ovary ensures the production of the sex steroidal hormones and differentiation of mature oocytes for fertilization. Sex steroidal hormones help in developing female sexual characters and even support a pregnancy. They also have effects beyond the reproductive system. During reproductive life ovarian aging results in the decreased fertility status and eventually leads to the cessation of ovarian function. The ovarian aging constitutes both the quality and quantity of the oocytes comprising the ovary.[2]

Ovarian reserve refers to the "residual oocyte-granulosa cell repertoire" that, at any given age, is available for procreation. Ovarian reserve tests and prognostic markers are an indirect measurement of women remaining follicular pool and give an estimate of her sensitivity to ovarian stimulation.[2] Decreased ovarian reserve constitutes both quantitative and

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qualitative deterioration in the oocyte complement, a known phenomenon associated with advancing age.

Ovarian reserve collectively refers to the ovarian follicle pool and the quality of the ovarian follicles recruited. It is also defined as an estimate of oocytes remaining in the ovary that is capable of fertilization resulting in a healthy and successful pregnancy.[2] Ovarian follicles are recruited as a result of the pituitary-ovarian axis. The follicle-stimulating hormone (FSH) acts on the ovarian pool as a result of which follicles grow resulting in secretion of inhibin A and estradiol (E2) by the early antral follicles.[3] With the decline of the follicle pool, the inhibin A and the E2 decreases with increase in the FSH levels.[3] Several mathematical models have been proposed relating the decline in follicle number (based on histological analysis) to age but an accurate and non-invasive method to assess follicle number, i.e., ovarian reserve for an individual woman remains elusive.

Being part of the classical feedback loop of pituitary-gonadal axis, composite testing and evaluation of serum levels of FSH, E2, and inhibin A are required to be performed, thus concluding that the serum levels of FSH, E2, and inhibin A are not independent of each other.[4] Moreover, the levels are cycle-dependent and show great variability at different phases of menstrual cycles.[1,2] Independent evaluation of serum levels of FSH, E2, and inhibin A is poor predictors of ovarian reserve as shows variability in assay, laboratory, population, and reproductive groups.[2] Furthermore, changes in serum levels of FSH, inhibin B, and E2 occur relatively late in the reproductive aging process.[3] Their serum level changes are evident only when the ovarian reserve is critical and chances of pregnancy are significantly reduced. The purpose of the testing is, thus, not fulfilled satisfactorily.[2-5]

The ultrasonographic parameter for measurement of ovarian reserve is antral follicle count (AFC) (total number of 2 mm–10 mm antral follicles in both ovaries are measured) and ovarian volume.[2] AFC, till date, is considered as the best predictor of ovarian reserve in quantitative aspect.[6] In recent years, accumulated data indicate that serum anti-Müllerian hormone (AMH) may fulfill the requirements to be the best test to predict ovarian reserve, but due to interspecies, interracial and intercommunal, geographical variations, and various external factors also affecting infertility, the findings need to evaluated in various groups of communities for generalization.

Table 1: Role of AFC in ovarian reserve

AFC group	*n* (%)
<4	7 (5.83)
4–7	15 (12.50)
8–12	76 (63.33)
>12	22 (18.33)
Total	120 (100.00)

AFC: Antral follicle count

Table 2: Association between age and fertility

Age group	*n* (%)	Mean±SD
<20	17 (14.17)	18.82±0.64
21–30	77 (64.17)	25.19±2.27
31–40	15 (12.50)	36.07±1.91
>40	11 (9.17)	42.64±1.12
Total	120 (100.00)	27.25±6.96

SD: Standard deviation

Table 3: Relation between age and serum FSH

Age group	*n*	Mean±SD	*P* value*
<20	17	4.88±0.62	<0.001
21–30	77	6.03±0.47	
31–40	15	6.31±0.63	
>40	11	7.71±0.84	

SD: Standard deviation, FSH: Follicle stimulating hormone

Table 4: Variations in serum LH value with age

Age group	*n*	Mean±SD	*P* value*
<20	17	4.00±0.50	<0.001
21–30	77	5.46±0.44	
31–40	15	5.81±0.46	
>40	11	5.82±0.91	

SD: Standard deviation, LH: Luteinizing hormone

Table 5: Role of serum E2 in predicting ovarian reserve

Age group	*n*	Mean±SD	*P* value*
<20	17	23.56±8.34	<0.001
21–30	77	52.81±13.81	
31–40	15	67.18±9.98	
>40	11	55.46±8.64	

SD: Standard deviation, E2: Estradiol

Table 6: Role of serum inhibin B in prediction of ovarian reserve

Age group	*n*	Mean±SD	*P* Value*
<20	17	82.85±12.90	<0.001
21–30	77	58.23±18.76	
31–40	15	48.61±22.57	
>40	11	72.19±31.82	

SD: Standard deviation

Aims and objectives

The aims are as follows:

1. To determine the values of serum AMH, AFC, day 3 Serum FSH, luteinizing hormone (LH), E2, inhibin A, and ovarian volume to assess ovarian function.
2. To determine the strength of correlation of the AFC and the hormonal parameters.
3. To evaluate the relationship of the individual hormonal parameters to the day 3 ovarian follicle status.

MATERIALS AND METHODS

This is a perspective cross-sectional study comprising 120 infertile patients in the study who received treatment for infertility, for example, induction of ovulation and timed intercourse/IUI or IVF. The demographic and clinical details were taken as per set performa after getting the valid consent of the patients. They were investigated in form of basal FSH, LH, AFC and mean ovarian volume assessment, Inhibin B, AMH, and response of all patients was then evaluated according to the number of follicles. The data were then compiled, and statistical evaluation was done with the SPSS software, depicted as charts, tables, graphs, etc.

RESULTS AND DISCUSSION

The grouping done on the basis of the AFC is <4, 4–7, 8–12, and >12 and contains 7 out of 120 cases (5.83%), 15 out of 120 cases (12.50%), 76 out of 120 cases (63.33%), and 22 out of 120 cases (18.33%) in respective groups. The <4 demonstrated the poor ovarian reserve and 4–12 AFC shows optimal one and >12 shows good ovarian reserve.

The observations were evaluated after dividing them on the basis of the age with 17 cases out of 120 (14.17%) in <20 years age Group 1, Group 2 (21–30 years) with 77 cases out of 120 (64.17%), Group 3 (31–40 years) with 15 cases out of 120 (12.0%), and Group 4 (>40 years) with cases out of 120 (9.17%). The mean age (in years) of presentation in Groups 1, 2, 3, and 4 was 18.82, 25.19, 36.07, and 42.64, respectively [Table 1].

The majority of infertile women are seen in age Group 3, 31–40 years, i.e., 15 out of 120 whereas lesser cases were seen in Group 4 (>40 years), i.e., 11 out of 120 cases [Table 2]. The age-dependent decrease in fertility was demonstrated by the decreasing follicle count as the age increases [Table 8].

The mean serum FSH values in Group 1 are 4.88 ± 0.62, Group 2 are 6.03 ± 0.47, Group 3 are 6.31 ± 0.63, and Group 4 are 7.71 ± 0.84. $P < 0.001$ suggesting that the serum FSH values in the respective age groups shows significant relation to the age groups. The serum FSH values show a significant increase in the values with age ($P < 0.001$) [Table 3].

Table 7: Serum AMH variation with age

Age group	*n*	Mean±SD	*P* value*
<20	17	3.35±0.55	<0.001
21–30	77	4.61±1.61	
31–40	15	2.31±1.06	
>40	11	1.00±0.78	

AMH: Anti-Müllerian hormone, SD: Standard deviation

Table 8: Association between Ovarian volume and age

Age group	*n*	Mean±SD	*P* value*
<20	17	7.96±0.61	<0.001
21–30	77	7.41±0.89	
31–40	15	6.34±1.45	
>40	11	6.12±1.94	

SD: Standard deviation

Table 9: Relationship between serum AMH and fertility

Serum AMH	*n* (%)
Very low/undetectable (0.0–0.3)	3 (2.50)
Low fertility (0.3–2.2)	14 (11.67)
Satisfactory fertility (2.2–4.0)	53 (44.17)
Optimal fertility (4–6.8)	40 (33.33)
High level (>6.8)	10 (8.33)
Total	120 (100.00)

AMH: Anti-Müllerian hormone

Table 10: Serum FSH and AFC value relation

AFC group	*n*	Mean±SD	*P* value*
<4	7	6.68±0.07	<0.001
4–7	15	7.04±1.31	
8–12	76	6.06±0.50	
>12	22	5.17±0.77	

FSH: Follicle-stimulating hormone, AFC: Antral follicle count, SD: Standard deviation

The mean serum LH values in Group 1 are 4.00 ± 0.50, Group 2 are 5.46 ± 0.44, Group 3 are 5.81 ± 0.46, Group 4 are 5.82 ± 0.91. $P < 0.001$ suggests that the serum LH values in the respective age groups show a significant relation to the age groups [Table 4].

The mean serum E2 values in Group 1 are 23.56 ± 8.34, Group 2 are 52.81 ± 13.81, Group 3 are 67.18 ± 9.98, and Group 4 are 55.46 ± 8.64. $P < 0.001$ suggests that the serum E2 values in the respective age groups show a significant relation to the age groups. The serum E2 values show

Table 11: Serum E2 and AFC relation

AFC group	*n*	Mean±SD	*P* value*
<4	7	59.34±9.66	<0.001
4–7	15	57.20±8.55	
8–12	76	55.56±14.56	
>12	22	26.76±9.48	

AFC: Antral follicle count, E2: Estradiol, SD: Standard deviation

Table 12: Serum AMH and AFC relation

AFC group	n	Mean±SD	*P* value*
<4	7	1.27±0.83	<0.001
4–7	15	1.37±0.87	
8–12	76	4.73±1.49	
>12	22	3.14±0.63	

AFC: Antral follicle count, AMH: Anti-Müllerian hormone, SD: Standard deviation

Table 13: Ovarian volume and AFC counts

AFC group	*n*	Mean±SD	*P* value*
<4	7	5.92±2.12	<0.001
4–7	15	6.28±1.68	
8–12	76	7.33±0.88	
>12	22	7.97±0.60	

AFC: Antral follicle count, SD: Standard deviation

significant increase in the values with age ($P < 0.001$) [Table 5].

The mean serum inhibin B values in Group 1 are 82.85 ± 12.90, Group 2 are 58.23 ± 18.76, Group 3 are 48.61 ± 22.57, and Group 4 are 72.19 ± 31.82. $P < 0.001$ suggests that the serum inhibin B values in the respective age groups show a significant relation to the age groups. The inhibin B values increase with the increasing age [Table 6].

The mean serum AMH values in Group 1 are 3.35 ± 0.55, Group 2 are 4.61 ± 1.61, Group 3 are 2.31 ± 1.06, and Group 4 are 1.00 ± 0.78. $P < 0.001$ suggests that the serum AMH values in the respective age groups show a significant relation to the age groups. The serum AMH value goes on decreasing with the increasing age significantly ($P < 0.001$) [Table 7].

The mean ovarian volume values in Group 1 are 7.96 ± 0.61, Group 2 are 7.41 ± 0.89, Group 3 are 6.34 ± 1.45, and Group 4 are 6.12 ± 1.94. $P < 0.001$ suggests that the ovarian volume values in the respective age groups show a significant relation to the age groups. The ovarian volume decreases significantly with increasing age [Table 8].

According to the data obtained maximum fertility was seen in cases serum AMH levels 2.2–6.8. Three cases with very high levels of serum AMH were seen, but the ultrasonographic picture revealed polycystic ovarian disease. Despite very high levels of serum AMH, the cases seem to have very low fertility probably due to the disturbed hormonal status. This was the need for ovarian ultrasonographic status in exclusion criteria [Table 9].

The serum FSH values in the AFC groups as <4, 4–7, 8–12, and >12 are 6.68 ± 0.07, 7.04 ± 1.31, 6.06 ± 0.50, and 5.17 ± 0.77, respectively. *P* value being <0.001 indicates the significance of the relationship between serum FSH values and AFC values. As the number of AFC decreases, the serum FSH values go on decreasing with $P < 0.001$ [Table10].

The mean serum E2 values in the AFC groups <4, 4–7, 8–12, and >12 are 59.34 ± 9.66, 57.20 ± 8.55, 55.56 ± 14.56, and 26.76 ± 9.48. Thus as the AFC decreases, the rise in serum E2 values is seen with $P < 0.001$ [Table 11].

The AFC groups <4, 4–7, 8–12, and >12 show the mean serum AMH values as 1.27 ± 0.83, 1.37 ± 0.87, 4.73 ± 1.49, and 3.14 ± 0.63, respectively. Thus as the AFC decreases, a decrease in mean serum AMH values is seen with $P < 0.001$ [Table 12].

The mean ovarian volumes in the AFC groups as <4, 4–7, 8–12, and >12 are 5.92 ± 2.12, 6.28 ± 1.68, 7.33 ± 0.88, and 7.97 ± 0.60, respectively. With the decrease in the AFC, a decrease in mean ovarian volume values is seen with $P < 0.001$ [Table 13].

DISCUSSION

In search of more promising markers, anti-mullerian hormone emerged out to be more appealing on the basis of the data recruited. AMH or Mullerian inhibiting substance is a glycoprotein hormone, with a molecular weight of 140 kDa, and produced by granulosa cells in ovarian follicles from 36 weeks of gestation until menopause. AMH plasma levels reflect the continuous non-cyclic growth of small follicles, thereby mirroring the size of the resting primordial follicle pool and thus acting as a useful marker of ovarian reserve. AMH seems to be the best endocrine marker for assessing the age-related decline of the ovarian pool in healthy women; thus, it has a potential ability to predict future reproductive lifespan.[5] The most established role for AMH measurements is before initiation of *in vitro* fertilization because AMH can be predictive of the ovarian response, namely poor and hyper-responses. However, recent research has also highlighted the use of AMH in a variety of ovarian pathological conditions, including polycystic ovary syndrome, granulosa cell tumors, and premature ovarian failure. A new commercial enzyme-linked immunosorbent

assay for measuring AMH levels has been developed, making results from different studies more comparable. Nevertheless, widespread clinical application awaits an international standard for AMH so that results using future assays can be reliably compared.[3,5,6]

In human ovary, AMH expression is flanked by two major regulatory steps of folliculogenesis, i.e., initial follicle recruitment and cyclic selection for dominance.[7,4] AMH is expressed in granulosa cells of primary follicles and being strongest in preantral and small antral follicles. AMH expression disappears in follicles of increasing size and is lost in large antral follicles, where weak staining only remains present in the granulosa cells of the cumulus.[1] Production of AMH gradually decreases as the follicle grows and then finally stops once the follicle reaches 8-mm diameter. AMH levels do not change significantly throughout the menstrual cycle.[2] This specific expression pattern of AMH in growing non-selected follicles has led us and others to study whether serum AMH levels are indicative for the number of growing follicles indeed, in women, and serum AMH levels decline with increasing age and changes in serum AMH levels were apparent before changes in other serum markers of ovarian aging, such as FSH and inhibin B, were present. In contrast with other serum markers, AMH levels remain relatively constant during the menstrual cycle.[4,8] Furthermore, studies suggest that serum AMH levels are not influenced by the gonadotropic status, and only reflect the follicle population. The decline in AMH levels correlates with the decrease in the number of growing follicles with aging, and most importantly, with the size of the primordial follicle pool.[8,9] These findings show that serum AMH levels reflect the quantitative aspect of ovarian reserve. Normal serum AMH level range is 2–6.8 ng/ml (14.28–48.55 pmol/l) in any phase of the cycle.

CONCLUSION

Ovarian reserve evaluation helps to identify patients with no response or hyper-response to ovarian stimulation in the ART, hence, individualizing the treatment protocols to achieve optimal response. Therefore, ovarian reserve testing could be considered as a screening method. At present no perfect ovarian reserve test is available, but AFC and AMH level have good predictive value. The total AFC and AMH are found to correlate significantly with the ovarian response with $P < 0.001$ and <0.001, respectively, indicating that they are good predictors of ovarian reserve. The basal FSH and ovarian volume do not correlate with the ovarian response indicating their poor value as predictors of ovarian reserve.

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